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Biomedical Application of Biosurfactant In Medical Sector

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CHAPTER

1 Application of low molecular weight and high molecular weight biosurfactant in medicine/ biomedical/pharmaceutical industries

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1.1 Introduction

Tensio-active materials produced by microorganisms are well-known as biosurfactants. Biosurfactants are a group of structurally varied molecules and they are classified using their chemical structure and microbial origin [1]. Biosurfactants are a type of surface-active biomolecules, which are produced by a kind of microorganisms, containing yeast, fungi, and bacteria from many substrates, like agricultural waste, hydrocarbons, oils, glycerol, and sugars [2–5]. Microbial surfactants have some benefits compared to synthetic surfactants, counting low toxicity, high biodegradability, and efficiency at multiple pH levels, salinities, and temperatures [6–8]. Biosurfactants are the most valuable and important products of biotechnology for medical and industrial applications. They are easy-produced from renewable and cheaper feedstock and they can be modified using genetic engineering, biochemical and biological techniques. However, there are many limitations to applying biosurfactants in industrial applications [6]. Because of the biosurfactants commercial potential, it is required to characterize and screen new biosurfactants. Due to biosurfactants potential benefits, they have various applications in laundry detergents, cosmetics and dermatology industry, food and pharmaceuticals industry, bioprocessing, and agriculture [8–11]. For example, in laundry detergents, agriculture, and the food industry, biosurfactants are can be used instead of surfactants, which are synthesized chemically, to avoid water pollution and soil toxicity [3–7]. In addition, in pharmacological, the biosurfactants behave as gene therapy and vaccines, simulative dermal fibroblasts, antimicrobial, antioxidants, antiadhesive, immunomodulatory, anticancer, antiviral activity, antifungal, and antibacterial [10]. Biosurfactants produced from microorganisms are amphipathic surface-active molecules including hydrophobic and hydrophilic moieties, which act using emulsifying hydrocarbons, increasing biosurfactants solubilization and consequently rendering them obtainable for microbial degradation [9]. Biosurfactant accumulates at the interface between a fluid and a solid or two immiscible fluids. Reducing the surface tension between liquid and air and interfacial tension between two immiscible liquid biosurfactants decrease the repulsive forces between two different phases and let two phases interact and mix more

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easily [12–14]. A characteristic property of biosurfactant is lipophilic–hydrophilic balance that specifies the portion of hydrophobic and hydrophilic constituents in surface-active materials. The lipophilic–hydrophilic balance quantity is an amount to show whether biosurfactant is associated with oil-in-water or water-in-oil emulsion [15–17]. Biosurfactants increase the surface of hydrophobic water-insoluble materials, increase water bioavailability of substances and change bacterial cell surface properties. Surface activity creates surfactants as excellent foaming dispersing agents and emulsifiers [11]. Consequently, it can be said that biosurfactant efficiency is determined by measuring its capability to vary surface tension and interfacial tension, emulsions stabilization, and investigating its lipophilic–hydrophilic balance [18]. Biosurfactants are amphiphilic molecules and made of (1) mono-, di- or polysaccharides, peptide, anions or cations and hydrophilic moiety viz. acid, peptide cations, or anions, mono-, di- or polysaccharides (2) hydrophobic moiety viz. unsaturated or saturated hydrocarbon chains or fatty acids. Various microbials *Stenotrophomonas*, *Serratia*, *Rhodococcus*, *Oceanobacillus*, *Enterobacter*, *Candida*, *Pseudomonas*, *Bacillus*, *Azotobacter*, and *Acinetobacter* are well-known to produce biosurfactants [13,19–21]. Biosurfactants are classified referring to their microbial origin and chemical structure and they fall into two categories: (1) low molecular weight that contains neutral lipids, fatty acids, peptides, lipopeptides, and glycolipids and (2) high molecular weight include lipoproteins, lipopolysaccharides, proteins, polysaccharides and mixtures of these biopolymers [11,22–26]. Polymeric biosurfactants are synthesized using several bacteria that are made of lipopolysaccharides, polysaccharides, and proteins [23].

Biosurfactants have various applications in the pharmaceutical industry. Some of them are given below:

- *Antimicrobial activity*: The various biosurfactants structures consult them the ability to show versatile efficiency. Some biosurfactants have strong antivirus, antifungal, and antibacterial activity; these types of surfactants act as the antiadhesive agent's role to pathogens preparing surfactants valuable for treating many diseases in addition to its usage as a probiotic and therapeutic agent. A good instance is the biosurfactant produced using marine B [27–29].
- *Anticancer activity*: Some microbial extracellular glycolipids persuade cell difference with cell proliferation in the human promyelocytic leukemia cell; moreover, presentations of PC12 cells to mouse erythroleukemia cell line increased the acetylcholine esterase activity and cut off the cell cycle at G1 phase by subsequent neurites overgrowth and partial cellular difference, this proposes which MEL persuades neuronal difference in PC12 cells and prepares the work for the usage of microbial extracellular glycolipids as unique reagents for the cancer cells treatment [27,30–32].
- *Immunological adjuvants*: Bacterial lipopeptides organize strong nonpyrogenic, nontoxic immunological adjuvants when mixed by antigens. Development of the humoral kind reply was confirmed when low molecular mass antigens herbicolin A and Iturin AL [29,33].
- Antiviral activity: Antibiotic effects and growth inhibition of human immunodeficiency virus in leukocyte using biosurfactants were given in the literature. Also, research presented due to the enhanced HIV incidence in women, there rises the necessity for a female safe, efficacious, and controlled vaginal current microbicide. Sophorolipids surfactants from *C. bombicola* and sophorolipids structural similarities like the sophorolipid diacetate ethyl ester are the strongest virucidal agent and spermicidal, it was moreover described this substance has a virucidal activity like nonoxynol-9 in contradiction of the human semen [34–36].

- *Other*: Other applications and advantages of biosurfactant in the pharmaceutical industry are the use of surfactants as agents for an immunomodulatory act, exciting stem fibroblast metabolism, but referring to published research results the lack of pulmonary surfactant, a phospholipids protein complex is acceptable for the respiration failure in prematurely born children but genes isolation for protein molecules and bacteria cloning have made promising its fermentation medical application production [27,37].

If the concentration of used surfactants is high enough, especially hydrophobic surfactants, a definite toxicity level occurs in the water and animal food chain and it is dangerous for human and animal health. Subsequently, industries are looking the use biobased surfactants instead of surfactants [38–40]. Due to the biosurfactants composition, biosurfactants are considered more biodegradable and biocompatible than chemically resultant surfactants [41,42]. The biosurfactant biodegradability was calculated as a percentage and measured referring to the surface tension increase of samples of the crude extract, with amounts reaching 81% biodegradability under specific circumstances [38].

Chemically synthesized surfactants are usually categorized using surfactants polarity, although biosurfactants desire to be categorized using biosurfactants microbial source, molecular weight, and action mode [39]. The surfactant hydrophilic head typically includes a polysaccharide, disaccharide, monosaccharide, amino acid, or peptide, but the hydrophobic tail is typically an unsaturated, saturated, branched, linear, or hydroxylated fatty acid [38–41]. The biosurfactants with low molecular weight are more effective than other ones in surface tension reduction of water-air interface and the water-oil interfacial tension, but the higher molecular weight biosurfactants, furthermore are known as bioemulsifiers, are more effective in stabilizing water-in-oil emulsions [42,43]. The most common kinds of biosurfactants from microbial origins and formulations are given in Table 1.1.

Biosurfactant ability to lower interfacial tension and surface tension makes them appropriate for commercial applications [44]. Biosurfactants are more effective because the decrease in the surface tension is more efficient. Since the concentration value of surfactants critical micelle is several times lower for that of chemical surfactants [45–48]. Consequently, for a maximum surface tension decrease, less amount of biosurfactant is required. The pH and temperature tolerance is critical for most systems and biosurfactants biodegradability is another reason for selecting to formulate by biosurfactants instead of chemical surfactants [41–45]. Understanding the structure feature efficiency of biosurfactants is necessary and there has been significant recent progress completed on elucidating the biosurfactants microstructure and their linkages to efficiency criteria. Individual

Table 1.1 Most common kinds of biosurfactants in main origin and application.

Biosurfactant	Main origin	Application
Alkyl polyglucoside	Amylose	
Surfactin	Bacillus subtilis	Petroleum, bioremediation detergents, agriculture, food, pharmaceuticals
Sophorolipids	Candida	
Rhamnolipids	Pseudomonas	

Modified after T Ivanković, J. Hrenović. Surfactants in the environment. Arch. Ind. Hyg. Toxicol. 2010;61(1):95–110.

features of biosurfactants, like bulk rheology, micro rheology, and surface activity have a great effect on their efficiency [49−54].

The main purpose of this chapter is to describe the attention of many researchers in the role of low and high molecular weight biosurfactants in the medicine, biomedical, and pharmaceutical industries. Subsequently, we discussed the application of various biosurfactants applying in the modern pharmaceutical industry. In the first step, the biosurfactants are divided into two low and high molecular weight branches and after that, in any branch, the role of biosurfactants is discussed in detail.

1.2 High molecular weight biosurfactant

1.2.1 Protein

Characteristic protein surfactants, like fungoid hydrophobic, a protein of *Trichoderma reesei*, can be separated into two classes counting Class 1 and 2 referring to differences in hydrophobic patterns and amino acid sequences [55−57]. Interactions of surfactants with proteins have been investigated widely for a few years ago because of their numerous usages in industrial and biological systems. Proteins are the most significant chemical components in living organisms and play a role in all biological systems [1,57]. Surfactants are able of increasing drug transport though causing just minor cell injury [55,56]. Surfactants most recently marketed are synthetic. But interest in biosurfactants has progressively increased through 15 years ago as a driving force for many commercial usages in the food processing, biomedical, pharmaceuticals, and petroleum industries due to biosurfactants having greater diversity, lower toxicity, and better biodegradability than available chemical surfactants [58]. Biosurfactants are structurally different groups of amphipathic molecules with surface-active that are synthesized extracellularly or maybe are a part of a cell membrane using fungi, yeast, and bacteria. Rhamnolipids from *Pseudomonas aeruginosa* are between biosurfactants that have been in the focus of many researchers [55−59].

Sotirova et al. [55] investigated the potential of the biosurfactant PS (alginate + hamnolipid) to permeabilize bacterial cells by two methods: the surfactant effect on resting and growing cells. The cell-permeabilizing influence of surfactants was assessed using calculating protein cell leak. The influence of permeabilization on the cell surface was investigated using scanning-electronic microscopy. Soluble protein was determined using the method presented by Lowry et al. [60] in growing supernatant fluids or permeabilized cells. The influence of concentration of biosurfactant on the protein release and growth of one Gram-positive and two Gram-negative strains was investigated and the results are indicated in Figs. 1.1 and 1.2.

Growth of both Gram-negative strains, *E. coli* and *P. aeruginosa* in the presence of biosurfactant PS (alginate + hamnolipid), as evaluated using the absorption values at 560 nm, was indistinguishable from the controls at all tested concentrations (Fig. 1.1). But the extracellular protein levels are affected in presence of biosurfactant in the growth media. An increase in the value of extracellular protein was registered at all tested biosurfactant concentrations that were more particulate at concentrations greater than concentrations for micelle formation [55].

The effect of the concentration of biosurfactant on the Gram-positive strain *Bacillus subtilis* 168 growth was almost different (Fig. 1.2). The increase of concentration of biosurfactant to 0.06%

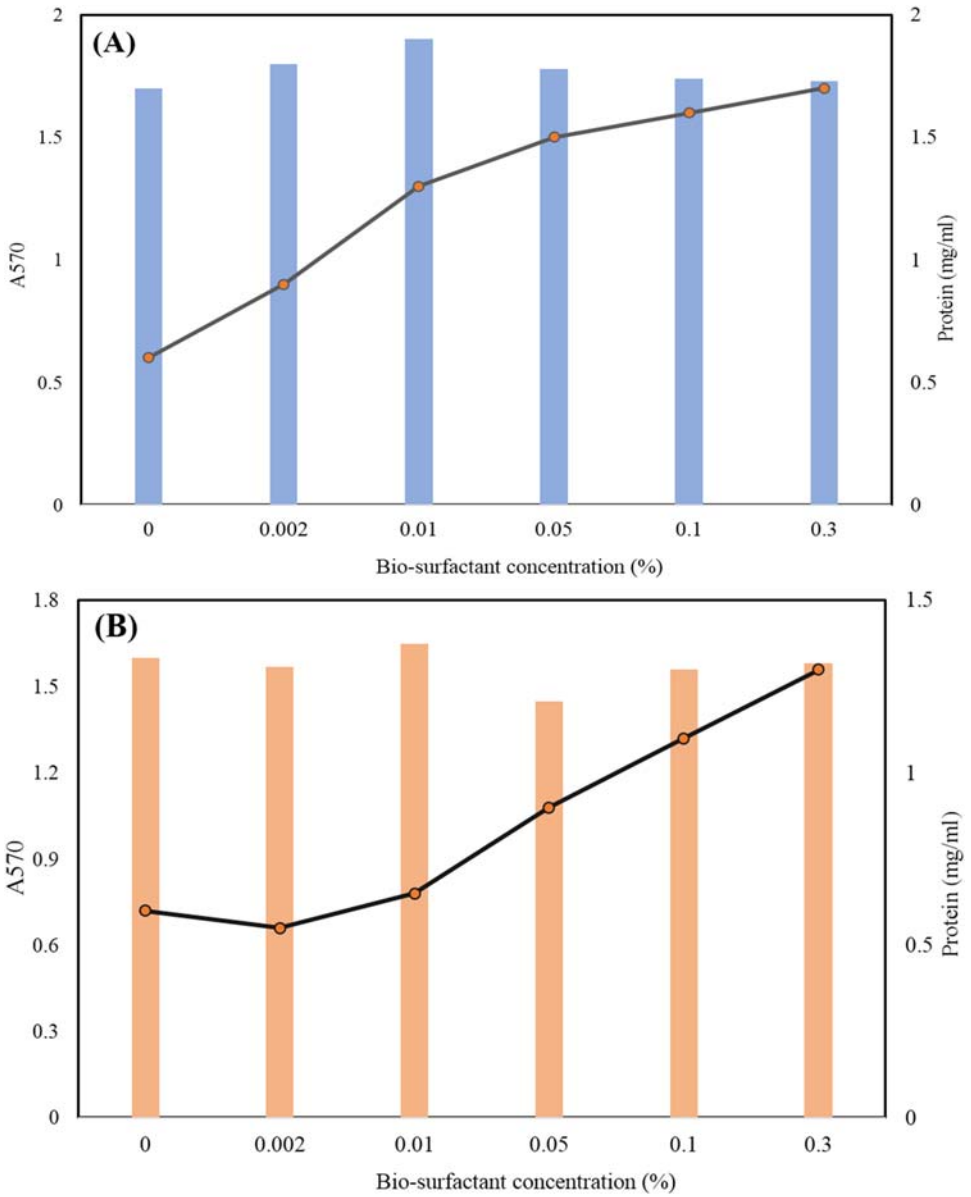

FIGURE 1.1

Influence of various concentrations of biosurfactant PS on the growth (bars) and protein release (dots) of (A): *Ps. Aeruginosa* and (B): *E. coli*.

Modified after AV Sotirova, DI Spasova, DN Galabova, E Karpenko, A Shulga. Rhamnolipid-biosurfactant permeabilizing effects on gram-positive and gram-negative bacterial strains. Curr. Microbiol. 2008;56(6):639–644.

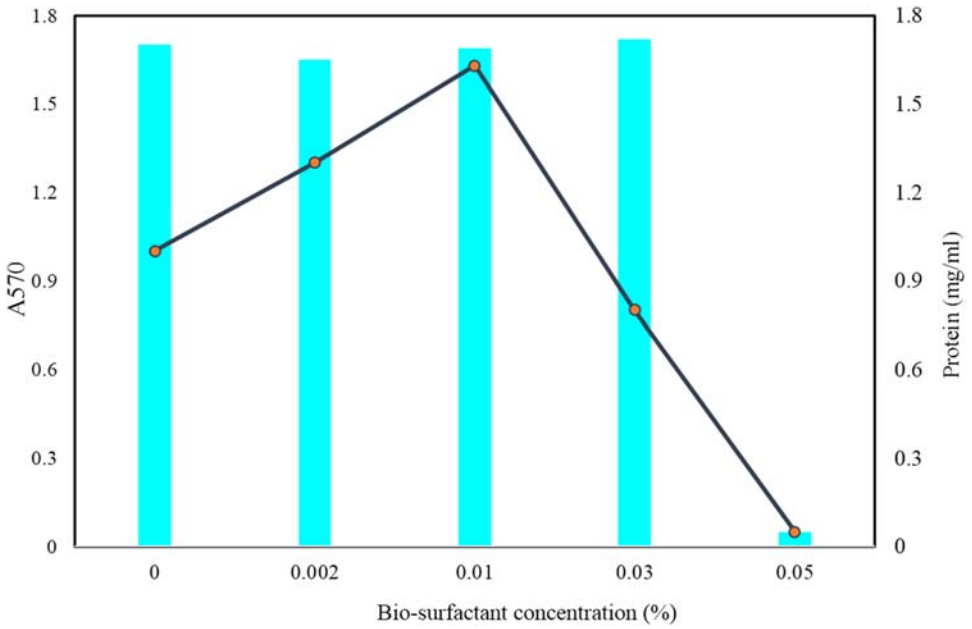

FIGURE 1.2

Influence of various concentrations of biosurfactant PS on the growth (bars) and protein release (dots) of *B. subtilis* 168.

Modified after AV Sotirova, DI Spasova, DN Galabova, E Karpenko, A Shulga. Rhamnolipid-biosurfactant permeabilizing effects on gram-positive and gram-negative bacterial strains. Curr. Microbiol. 2008;56(6):639–644.

leads to complete growth inhibition. But levels of extracellular protein were increased in the presence of biosurfactant PS at concentrations less than and close to concentrations for micelle formation. A maximum increase in protein release $\leq 0.01\%$ was registered [55]. Referring to Fig. 1.2, the extracellular protein levels decreased at biosurfactant concentrations greater than concentrations for micelle formation. A reasonable description for the detected influences could be that biosurfactant PS at concentrations greater than concentrations for micelle formation provokes variations in membrane phospholipid substance, lead an increase in membrane inflexibility. Bacteria change their membrane variability using way of phospholipid head groups to provide the harmful influences of surfactants and solvents. The enhanced level of diphosphatidylglycerol decreases membrane fluidity, again stabilizing cellular membranes in the presence of solvent or biosurfactant [61,62]. The reverse observed results from the biosurfactant PS presence in the medium on the Gram-negative and positive strains growth are related to the bacterial cell surfaces composition. It is known that Gram-negative bacteria have a peptidoglycan thinner layer, significant outer membrane and a periplasmic space via the membrane and cell wall [55]. The outer membrane function as a well-organized permeability barrier is cable to deprive molecules of biosurfactant. The property of the permeability barrier is mainly caused using the lipoprotein and lipopolysaccharide (LPS) layer presence [63].

The influence of various biosurfactant PS concentrations on the permeability of *P. aeruginosa* and *B. subtilis* resting cells was investigated using observing the protein release. According to Fig. 1.3, a separate difference in the extracellular protein levels of both strains after permeabilization is found out. But for *P. aeruginosa* the increase in levels of protein at various concentrations of biosurfactant is not considerable, enhancement in levels of protein for *B. subtilis* resting cells is twofold, at that enhancement in protein levels is $>$ sevenfold at 1.1% [55]. Clearly, the biosurfactant PS indicated well expressed in vivo also in vitro influences on *B. subtilis* 168 culture. A possible description can be related to the higher protein leak in two cases as a permeabilization result by rhamnolipid PS. The biosurfactant possibly forms molecular collections on the surface of bacterial membranes, leading to the formation of the transmembrane pores, which serve as channels to the periplasm [64]. Clearly, this process is very pronounced by Bacillus compared to *Pseudomonas* cells. However, the biosurfactant PS interaction with surface proteins maybe cause the protein direct removal using solubilization; therefore, contributing to the higher protein leakage. It was indicated in a recent study on the rhamnolipid capability to increase the solubility of hydrocarbon that rhamnolipid surfactants have detergency features [65,66].

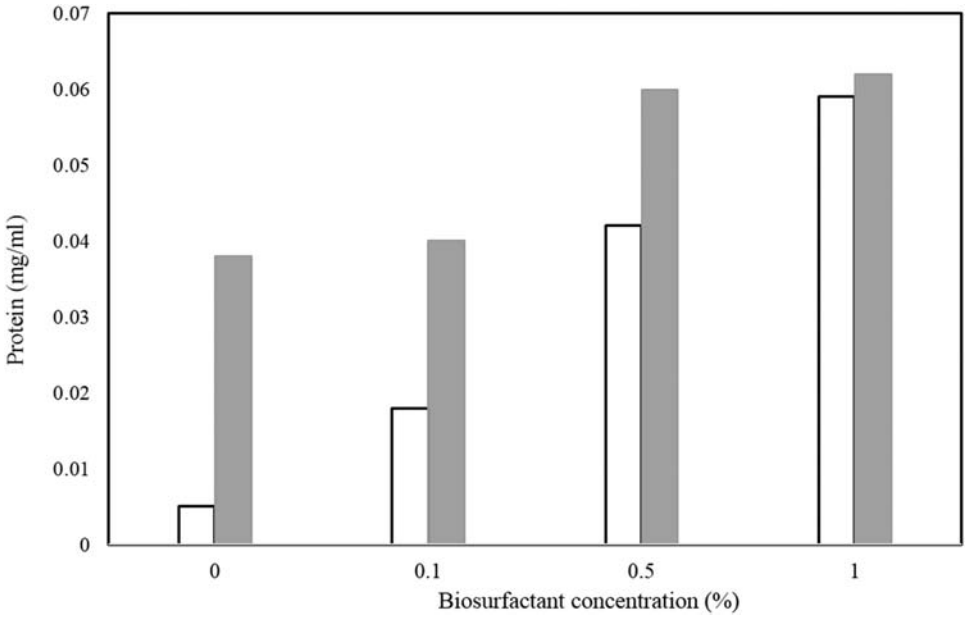

FIGURE 1.3

Extracellular protein of *B. subtilis* 168 resting cells permeabilized using biosurfactant-rhamnolipid (*white* bars) and of *P. aeruginosa* cells (*gray* bars).

Modified after C Hazra, D Kundu, A Chatterjee, A Chaudhari, S Mishra. Poly (methyl methacrylate) (core)-biosurfactant (shell) nanoparticles: size controlled sub100 nm synthesis, characterization, antibacterial activity, cytotoxicity and sustained drug release behavior. Colloids Surf. A: Physicochem. Eng. Asp.0 2014; 449:96–113.

PhaP is a small bacterial protein placed on the surface of polyhydroxyalkanoates (PHA) granules, which are an energy storage material and bacterial carbon, has medical usages [57,66]. Natural PhaP has a high dependency on polyhydroxyalkanoates substances, it can moreover bind unspecifically to other hydrophobic polymers like polystyrene and poly (L-lactide) [67]. Like other amphiphilic proteins, PhaP is a protein molecule preparing the boundary between the hydrophilic cytoplasm and the hydrophobic polyhydroxyalkanoates polymer. Recently, PhaP1 from the strain *Ralstonia eutropha*, is found out to be capable to bind to the lipid inclusions surface [57]. The PhaP capability to bind to a surface of hydrophobic is applied to develop methods for tissue engineering application, drug delivery, and protein purification [67,68]. The protein purification process is according to a self-cleaving intein molten by a target protein and PhaP; the whole fusion protein is bound and expressed to in vivo poly (3-hydroxybutyrate) granules; the target protein is released through intein cleavage collected and induction of the supernatant of the poly (3-hydroxybutyrate) precipitates [57,67].

Wei et al. [57] used PhaP from *Aeromonas hydrophila* containing of amino acid remains, which is investigated for possible application as a biosurfactant. PhaP protein was over-expressed in *Escherichia coli* and purified by 6 × his-tag. The purified PhaP was investigated in several concentrations in H_2O for stable emulsions formations by soybean oil, diesel oil, and lubricating oil, respectively. The obtained results are compared to chemical surfactants containing a liquefied detergent, Tween 20, and sodium dodecyl sulfate. The PhaP-based emulsion formation is shown in Fig. 1.4 and the details of experimental tests are given in Table 1.2.

The results of the influence of PhaP concentration on emulsion stability are shown in Fig. 1.5. Soybean oil, diesel oil, and lubricating oil emulsions including different concentrations of PhaP

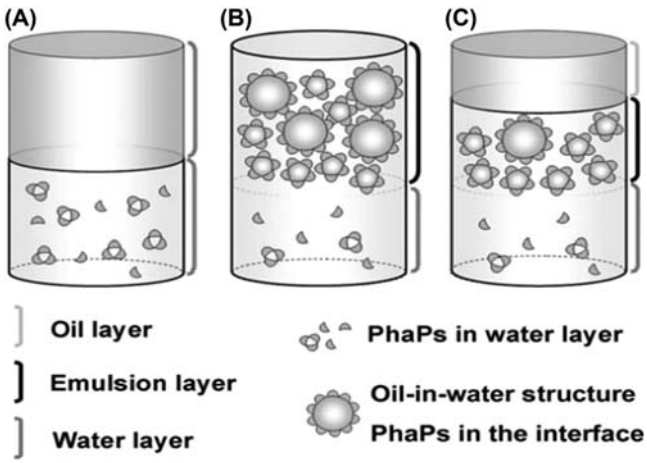

FIGURE 1.4

PhaP as a surfactant. (A) Before sonication or vortex and PhaP is dissolved in water layer. (B) After sonication or vortex. (C) Over time some of the water-in-oil structures became unstable.

Reprinted with permission from DX Wei, CB Chen, G Fang, SY Li, GQ Chen. Application of polyhydroxyalkanoate binding protein PhaP as a biosurfactant. Appl. Microbiol. Biotechnol. 2011;91(4):1037–1047.

Table 1.2 Alteration of emulsion index of lubricating oil and diesel with sodium oleate (SO), SDS, Tween 20, PhaP, BSA and a liquefied detergent (LD) on 2nd and 30th day after vortex treatments, respectively.

Water phase[a]	Lubricating oil	Diesel	Time of storage
SO	6.99 ± 0.14	15.36 ± 1.52	Two days later
SO	6.65 ± 0.63	8.21 ± 1.52	30 days later
SDS	10.0 ± 1.71	50.0 ± 2.89	Two days later
SDS	6.70 ± 1.84	28.6 ± 1.54	30 days later
Tween 20	8.36 ± 2.13	15.33 ± 1.90	Two days later
Tween 20	6.71 ± 0.19	13.66 ± 0.46	30 days later
PhaP	53.8 ± 2.22	69.20 ± 1.72	Two days later
PhaP	51.7 ± 2.20	68.20 ± 2.82	30 days later
BSA	14.58 ± 0.84	12.14 ± 2.34	Two days later
BSA	14.73 ± 0.63	10.42 ± 0.10	30 days later
LD	7.93 ± 1.46	7.78 ± 1.25	Two days later
LD	6.90 ± 0.00	6.78 ± 0.16	30 days later

[a]*All surfactants in water phase were 500 μg/mL.*
Modified after DX Wei, CB Chen, G Fang, SY Li, GQ Chen. Application of polyhydroxyalkanoate binding protein PhaP as a biosurfactant. Appl. Microbiol. Biotechnol. 2011;91(4):1037–1047.

were provided under the same vortex power and several emulsion stabilities were observed. In all cases and under the same vortex condition, the layers of emulsion started to be stable when the concentration of PhaP was higher than 0.2 g/L. Stable emulsions can be observed in concentration of less than 0.05 g/L of PhaP in soybean oil under sonication conditions. Clearly, the more intense mixing presented using sonication permitted the formation of more stable emulsion by the reduced concentration of PhaP substance [57]. PhaP substance stabilized soybean oil-in-water droplets (microemulsion), which indicated sizes ranging from 1.4 to 6.1 μm (Fig. 1.5).

The oil-in-water structures with spherical shape were observed to be larger in 0.1 g/L PhaP (Fig. 1.5A2, B2) compared to those in 1 μg/L PhaP (Fig. 1.5A1, B1). The higher concentration of PhaP leads to the smaller size of the droplet. Consequently, ultra-sonication can provide better mixing for water and oil than the vortexing process (Fig. 1.5A, B). The ultra-sonication generates homogeneous and smaller droplets. Furthermore, droplets including PhaP substance produced the smallest contact angles of 56 degrees (0.5 g/L PhaP) and 57 degrees (1 g/L PhaP) on poly (3-hydroxybutyrate) film compared to 80 degrees for deionized H_2O, 63 degrees for sodium dodecyl sulfate, and 71 degrees for bovine serum albumin. Similarly, the smallest contact angles of 74 degrees (0.5 g/L PhaP) and 71 degrees (1 g/L PhaP) were observed for PhaP substance containing droplets on biaxially oriented polypropylene films [57]. The results indicated that the biosurfactant PS does not use a disruptive action on resting cells opposite to the detrimental influence on growing cells of *B. subtilis*.

Various shampoos to treat dandruff are produced, which contain insoluble ingredients like Zn pyrithione. This ingredient is soluble in both water and oil media. In addition, these antidandruff compounds should contain a great amount of synthesized stabilizing ingredients for not precipitating. By a

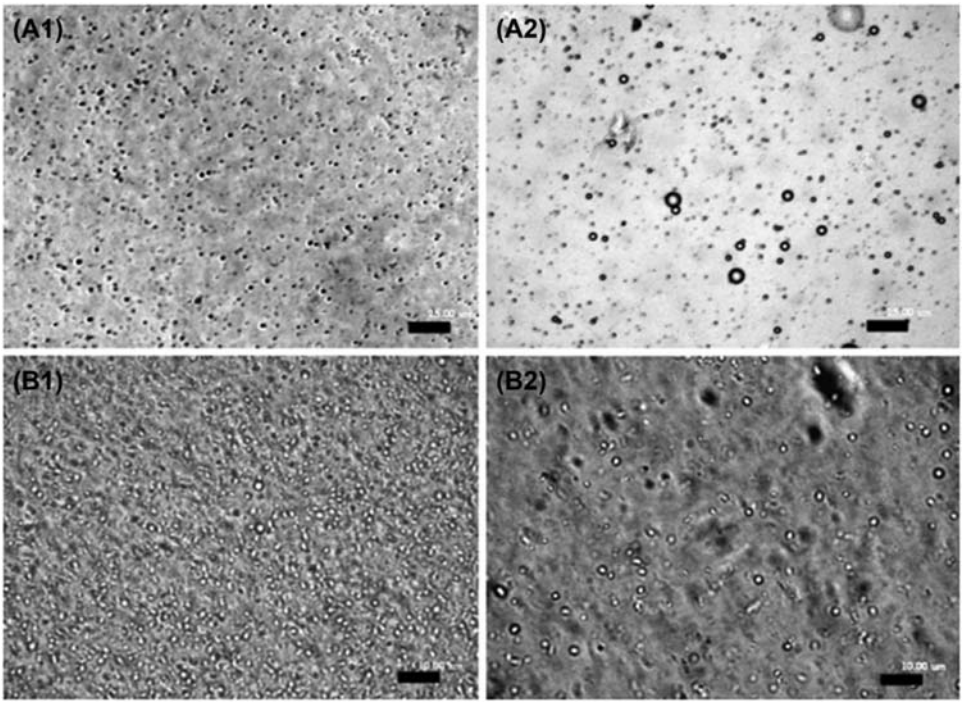

FIGURE 1.5

PhaP stabilized oil-in-water structure under light microscope.1 g/L (A1) and 0.1 g/L (A2) PhaP are applied to disperse soybean oil in aqueous system, respectively; 1 g/L (B1) and 0.1 μ/L (B2) PhaP are applied to disperse soybean oil in aqueous system, respectively.

Reprinted with permission from DX Wei, CB Chen, G Fang, SY Li, GQ Chen. Application of polyhydroxyalkanoate binding protein PhaP as a biosurfactant. Appl. Microbiol. Biotechnol. 2011;91(4):1037–1047.

biosurfactant extract and Tween 80 as surfactant, the stabilization of Zn pyrithione in O/W emulsions is studied. The tea tree oil/water ratio and both biosurfactant and surfactant concentrations are included in an incomplete factorial design [4]. Using a biosurfactant to be a stabilizing agent in antidandruff formulations is a new work to be studied about. For a better result, it is appropriate to use such natural surfactants to have natural and biocompatible formulations. For improving the solubilization and stability of Zn pyrithione in the formulations, which include tea tree oil and Tween 80 the use of biosurfactant under suitable conditions is necessary [19]. The biosurfactant studied here is extracted from corn stream and with an FTIR spectrum, its molecular components are elucidated. This analysis determines that the biosurfactant includes protein-related weak bonds, amide, and amine bonds and also proves the presence of fatty acids. Therefore, from all these observations it can be concluded that this biosurfactant extract contains lipopeptide [8,21]. By looking through the solubility of Zn pyrithione in these formulations, it is found out that the values of each formulation are different from others. In total, the formulations without biosurfactant extract had the worse solubility. Even the presence of Tween 80 did not help as much and still, the solubility was low, whereas the ability of biosurfactant extract to increase the

solubility of Zn pyrithione was better than Tween 80 alone. Moreover, the formulations, which had the same ingredients but different oil/water ratios, their solubility were also different. The ones with a higher ratio had the higher solubility of Zn pyrithione. However, the presence of both surfactant and biosurfactant improves the solubility of Zn pyrithione [4]. Some recent studies [4,8,21] before had shown that a biosurfactant extract could solubilize a material by being adsorbed on the surface so that in these formulations the biosurfactant extract would be adsorbed onto the surface of Zn pyrithione. Because of the biosurfactant extract acting as amphoteric, the stabilization would improve [4,19]. By macroscopic and light microscopy analysis the formulations were characterized. As mentioned before the emulsions, which only contain biosurfactant extract are less stable than the ones that contain both biosurfactant and Tween 80. Some of the formulations with different oil/water ratios remained stable even after 30 days about 93%. In fact, using biosurfactant under specific conditions made an advance in terms of biocompatibility and achieved higher solubility values of Zn pyrithione than the ones that were carried out with organic surfactants. Consequently, applying biosurfactant extract with Tween 80 along with tea tree oil would have been a new option in presenting antidandruff shampoos [19,21]. The biosurfactant extract was able to enhance the solubility of Zn pyrithione in aqueous formulations, up to 148 times in comparison with its solubility in water.

1.2.2 Polysaccharide

Core−shell nanostructured polymers are including two distinguished domains in the shell and core phase, respectively. They have involved important research interests in impact modifiers, textile, and paper manufacturing, conducting materials, drug delivery, and film fabrication [56]. Of these polymeric colloidal nano- and microparticles, poly (methyl methacrylate); moreover, as poly (methyl 2-methylpropenoate), is one of the very broadly explored biomedical substances due to its nonimmunogenicity, nontoxicity, and biocompatibility [69]. Although poly (methyl methacrylate) has a long part as a transporter vehicle for bioactive materials (vaccines, enzymes, genes, proteins, therapeutic drugs), there is a massive challenge to develop economical and robust methods able of producing morphology controlled and size-tunable nanoparticles by a complex architecture [69,70]. Conventional emulsion polymerization and preformed polymer-based methods are still the techniques of selection for making core−shell poly (methyl methacrylate) nanoparticles (n-poly (methyl methacrylate)). But toxicological issues arising from the applying of free radicals, surfactants, and organic solvents through these polymerizations processes can never be removed [69−71].

Hazra et al. [56] synthesized particles (spherical shape) and amorphous polystyrene using the modified microemulsion process in addition to crystalline n-amorphous polystyrene particle (hexagonal shape) using a unique atomized microemulsion process [72]. They used nontoxic, biocompatible, and biodegradable biosurfactants like trehalose lipids of microbial origin, surfactin, and rhamnolipids with very low critical micelle concentrations (CMCs). Also, amorphous polystyrene (core) and biosurfactant (shell) biodegradable bionanocomposites and biocompatible are synthesized for their possibility as drug delivery vehicles [56].

The methyl methacrylate amount in the reaction system affects the resultant polymer particle size. The results are presented in Fig. 1.6A, which indicates an increase in the methyl methacrylate consumption increases in the methyl methacrylate particle size. When the monomer amount was 10^{-2} L, the methyl methacrylate particle size was in the range of 15−20 nm. By increasing the

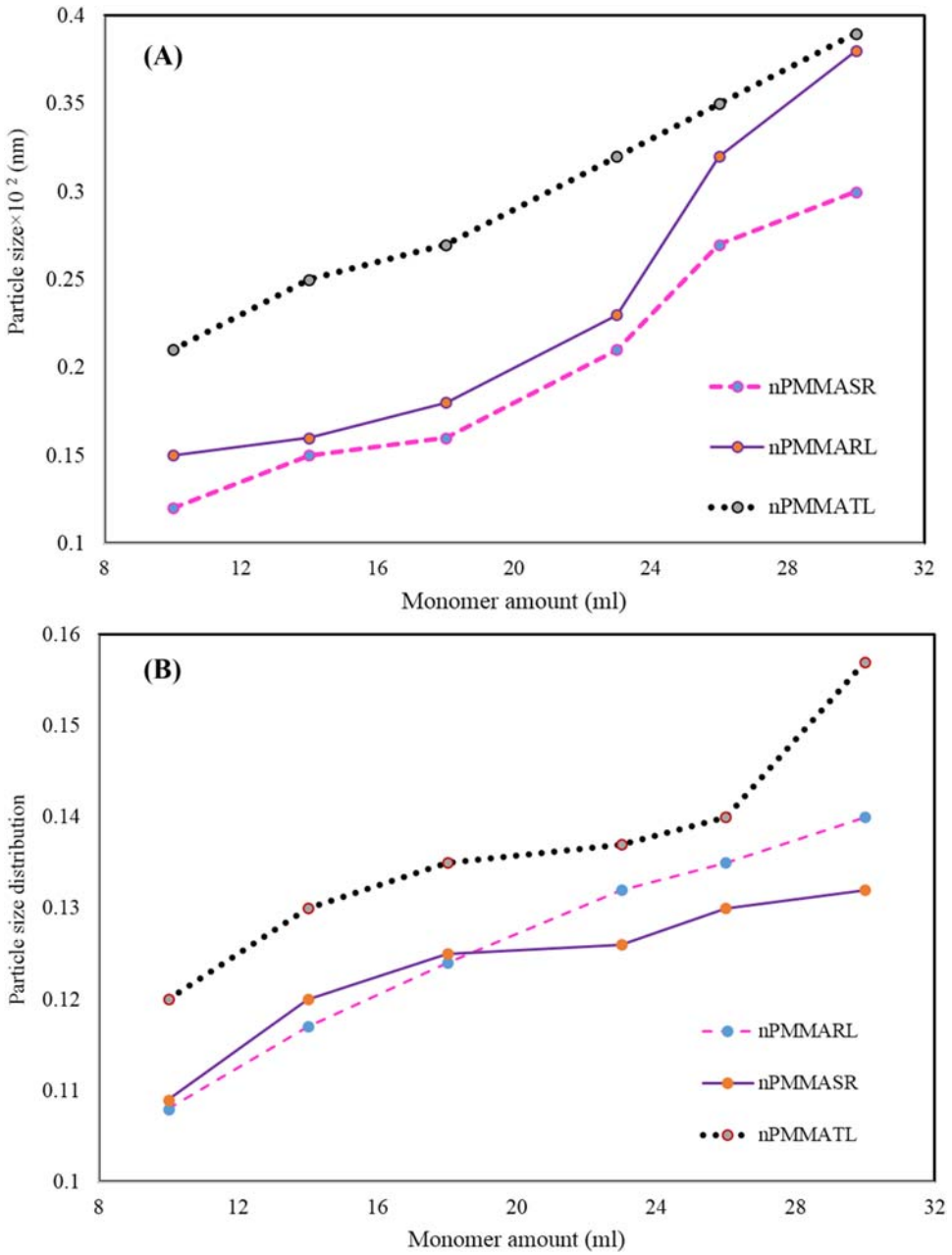

FIGURE 1.6

(A) The monomer amount is 10 ml, (B) The monomer amount is more than 10 ml.

Modified after C Hazra, D Kundu, A Chatterjee, A Chaudhari, S Mishra. Poly (methyl methacrylate) (core)-biosurfactant (shell) nanoparticles: size controlled sub100 nm synthesis, characterization, antibacterial activity, cytotoxicity and sustained drug release behavior. Colloids Surf. A: Physicochem. Eng. Asp. 2014; 449:96–113.

monomer amount, the methyl methacrylate particle size increases. Furthermore, the methyl methacrylate particle size growth rate becomes faster as the additional loading of monomer increases [56].

For instance, when 3×10^{-2} L of methyl methacrylate was added, the methyl methacrylate particle size increases to 29−38 nm and the methyl methacrylate particle size was near double in the size when 10^{-2} L of methyl methacrylate was applied. The methyl methacrylate particle size distribution, moreover, becomes extended by increasing the monomer amount (Fig. 1.6B). Although from Fig. 1.6A, it can be concluded that surfactin is a better emulsifier than trehalose lipids and rhamnolipid in cases of the particle size, Fig. 1.6B indicates that rhamnolipid and surfactin have a rather similar influence on the methyl methacrylate particle size distribution [56].

The influences of the ammonium persulfate amount on methyl methacrylate particle size are studied and the obtained results are presented in Fig. 1.7. This figure indicates that as the initiator concentration increases, the methyl methacrylate particle size in the subsequent emulsion decreases at initial and after that increases slowly.

This phenomenon can be described using the two processes coexistence: (1) oligomeric radicals can move among the polymer particles and aqueous phase and (2) oligomeric radicals can remain to undergo the nucleation process and produce new particles and these processes can control the reaction at various stages. At a low concentration of initiator, the active sites that could grow in particles would be less [56]. Then by the same amount of monomer, the methyl methacrylate particle size would be larger; by the initiator content increasing, more active sites can be created. Consequently, the amount of divided monomer by each active site becomes less and the resulting particles would be small. But when the concentration of the initiator is very high, the number of free radicals divided by one particle increases and some dead polymer particles can be initiated again result in a larger methyl methacrylate particle size [70]. In addition, Fig. 1.7 shows by an increase in the initiator amount, the methyl methacrylate particle size slowly tend to a narrow size distribution.

The obtained results showed using the three kinds of surfactant, the final produced particle size was changed and it was 24.4, 23.5, and 30.4 nm for rhamnolipid, surfactin, and trehalose lipid, respectively, which indicated that the surfactin was very well in reducing the size of n-poly (methyl methacrylate) particles. This is reasoned from the following evidences: (1) the CMC of surfactin is very smaller than those of trehalose lipids and rhamnolipid and by adding the same amount of the biosurfactant, surfactin maybe generate more micelles than the other surfactants and (2), surfactin can transport electrostatic charge on the surface of emulsion particle, thus avoiding ion aggregation results in a good emulsion mechanical stability [56,70]. An increase in the biosurfactants amount, generates a narrower particle size distribution (Fig. 1.8A).

But, outside a certain level, the biosurfactant amount had little influence on particle size distribution. The obtained result shows the cosurfactant does not have any advantage and it can be eliminated from the reaction system. Moreover, the microemulsion particle size did not vary even after six months and it was very stable. Hazra et al. [56] applied a surfactant with a very lower value than the common levels used in the literature [73−75]. The size of the particle and its particle size distribution dependency on the mixed biosurfactant (surfactin + rhamnolipid) are presented in Fig. 1.8B. According to Fig. 1.8B. the particle size decreases as the molar ratio of rhamnolipid/surfactin increases and also the particle size increases as the molar ratio of rhamnolipid/surfactin increases. The same results were observed in the literature [70]. This phenomenon can be described using both used biosurfactants properties. It seems that surfactin and rhamnolipid produced a

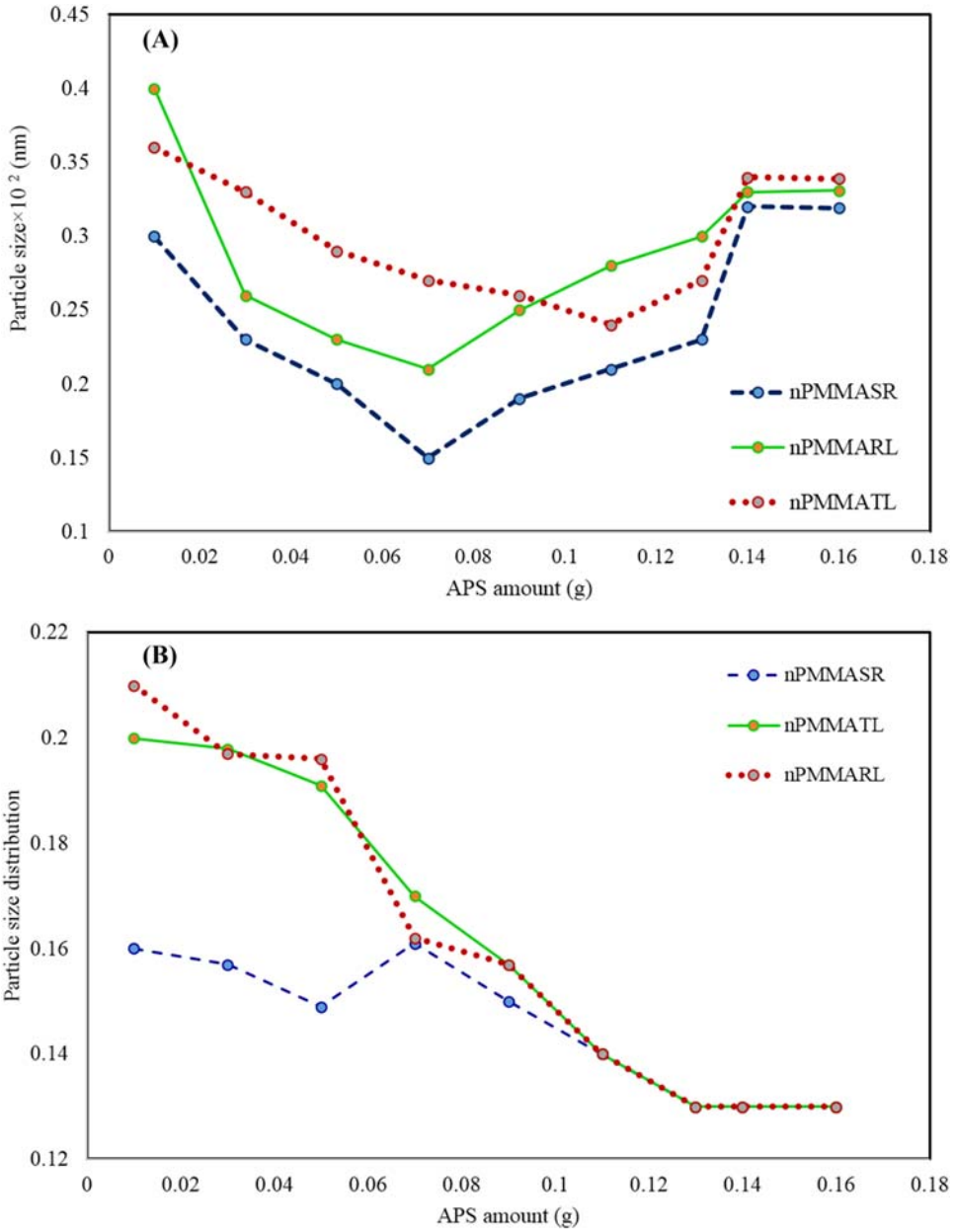

FIGURE 1.7

(A) particle size versus APS amount, (B) particle size distribution versus APS amount.

Modified after C Hazra, D Kundu, A Chatterjee, A Chaudhari, S Mishra. Poly (methyl methacrylate) (core)-biosurfactant (shell) nanoparticles: size controlled sub100 nm synthesis, characterization, antibacterial activity, cytotoxicity and sustained drug release behavior. Colloids Surf. A: Physicochem. Eng. Asp. 2014; 449:96–113.

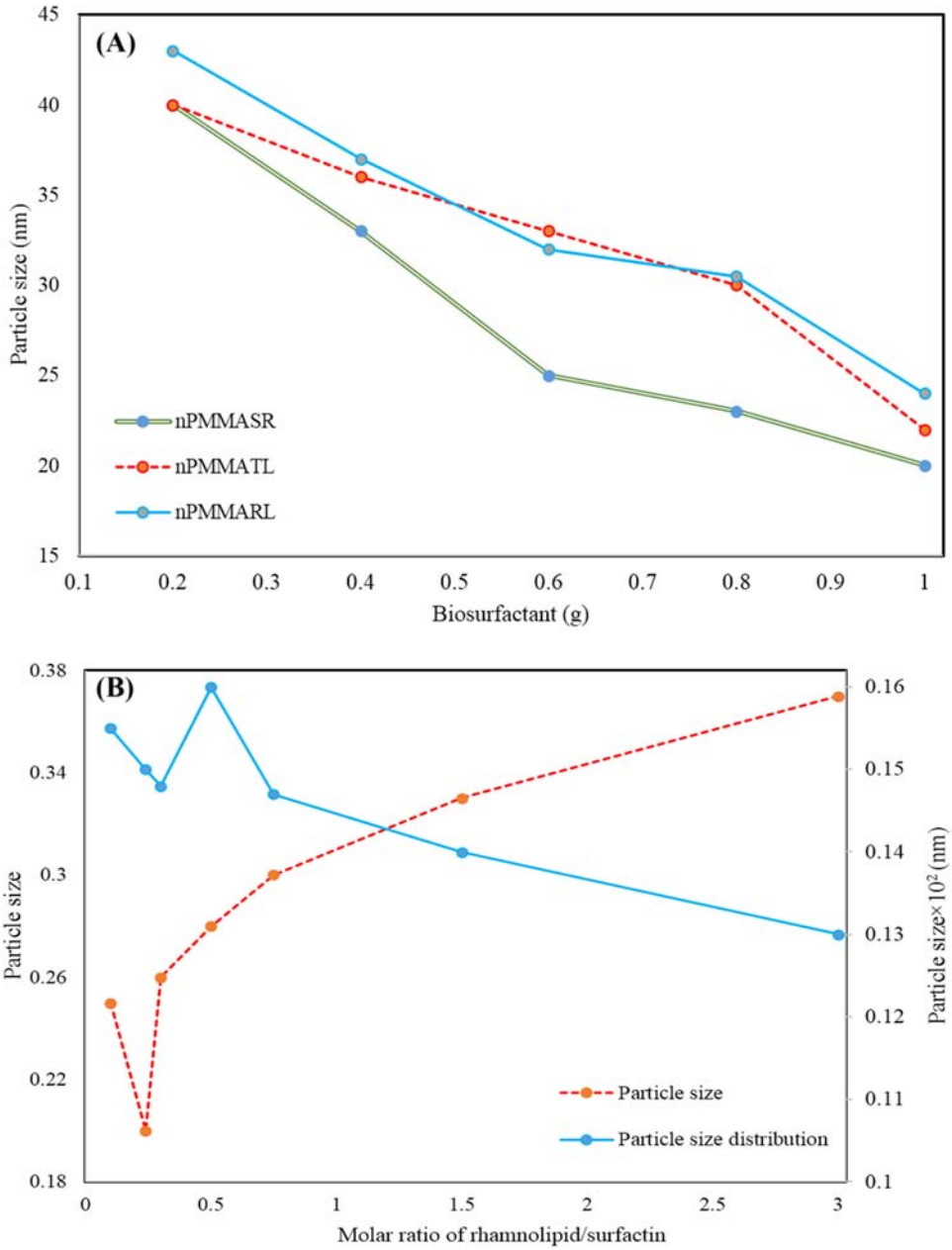

FIGURE 1.8

Influence of (A) biosurfactant amount and (B) mixed biosurfactant (rhamnolipid/surfactin) ratio on the nPMMA particle size.

Modified after C Hazra, D Kundu, A Chatterjee, A Chaudhari, S Mishra. Poly (methyl methacrylate) (core)-biosurfactant (shell) nanoparticles: size controlled sub100 nm synthesis, characterization, antibacterial activity, cytotoxicity and sustained drug release behavior. Colloids Surf. A: Physicochem. Eng. Asp. 2014; 449:96–113.

synergistic influence, which results in the particle size decreasing at a molar ratio of 0.24. Since the CMC and emulsifying influence of rhamnolipid are less than those of surfactin surfactant, the particle size increases by increasing the rhamnolipid amount [56]. Furthermore, Fig. 1.8B shows by increasing the rhamnolipid amount, the particle size distribution slowly becomes narrow. This core (nPMMA)−shell (polysaccharide) system is nontoxic and leads to a decrease in the time of drug delivery.

1.2.3 Lipoprotein

The lipoproteins have been one of the most active features of metabolic health intervention, diagnostics, and research over the past 60 years. Through the mentioned time, the importance of varying concentrations of lipoprotein, composition, and size has been well documented [76]. But, the difficulty of understanding these colloidal particles has been an intimidating research attempt. The most challenging of lipoprotein research aspects have been in understanding the behavior of the particles at the molecular scale [77]. Lipoproteins are natural molecules representing the proteins and lipids' self-assemblies. Lipoproteins, the colloidal components, which transport insoluble lipids in cerebral spinal fluid, lymph, and blood are among the most investigations structures in biology [76−80]. Lipoprotein components involve biomolecules a variety depending on three factors: (1) synthesis of lipoprotein components in the cell that produces them, (2) active remodeling in the lymph or blood substances through their circulation, and (3) artifactual modifications happening through isolation. Discouragingly for the lipoproteins potential as health evaluation indicators, their separation using the methods in both scientific investigation and clinical practice is poorly quantitative [81]. The structure of lipoproteins involves an apolar core, which is mostly composed of triglycerides and cholesteryl esters. This hydrophobic core is bounded using an outer shell containing phospholipids that render the lipoproteins soluble in surface and water apoprotein molecules, which permit cells to take up and recognize the lipoprotein particles [80,82]. Lipoproteins are key transporter of lipids and cholesterol present in the human circulatory system [83]. According to lipoproteins density, they are classified into five main groups: chylomicrons, very low-density lipoproteins (LDLs), intermediate-density lipoproteins, and high-density lipoproteins (HDLs). The most significant ones for the drug delivery process are low-density and HDLs [84,85]. The association of drugs by these two kinds of lipoproteins can control their metabolism and transport in a biological organism. It has been indicated that mixing drugs by LDLs or HDLs before administration leads to an increase of cytotoxic influence of the drugs compared to the situation when the drugs are administered alone [83,86]. There are various benefits of applying LDLs and HDLs as drug carriers for cancer targeting over synthetic drug delivery systems [87−89]. LDLs and HDLs are biodegradable and biocompatible. Both kinds of lipoproteins possess high drug loading ability. Due to its size (< 25 nm), LDLs and HDLs can easily penetrate tumor tissues using increased retention and permeability influence [89−92]. Furthermore, in several types of cancers such as adrenal, lung, and colon cancer, the diseased cells manifest overexpression of LDLs and HDLs receptors [83,88]. The benefits of HDLs for the prediction of heart disease are increasingly well understood. But HDLs are protective against other health problems, mainly infection [90,91].

Lipoprotein is identical to LDL except for the addition of apo-lipoprotein A that is highly glycosylated. Lipoprotein is a mixture of an LDL-like particle and apo-lipoprotein covalently bound to apo-lipoprotein [77]. LDLs, which vary in their density and size, are more or less associated with

heart disease. But it is not known why smaller size is more atherogenic, or what variations to the particles reverse their enhanced risk [78,91]. There is a striking homology between the amino acid sequences of plasminogen and apo-lipoprotein A that is recognized to be a cardiovascular risk factor. Thus, lipoprotein maybe act a significant role in the transition from atherosclerosis to thrombosis, due to it activating monocyte adhesion and migration of macrophage foam cells in the arterial wall [83,92]. Lipoprotein is sometimes considered as a marker of thrombosis. High lipoprotein is positively associated with coronary artery calcification, coronary artery disease, and peripheral arterial disease. It moreover promotes thrombosis using binding to fibrin, consequently blocking the fibrinolytic action of plasmin. Lipoprotein may be a predictor of peripheral and central cardiovascular disease in younger men and women with dyslipidemia [90]. The physiological function of lipoprotein and its influences on the vasculature remains indefinite to date. It was found out that lipoprotein is capable to enter the intima of blood vessels in animals and humans [92]. There it maybe contributes to inflammation of the foam cell, thrombosis, and intima formation-processes, which are identified to be contained in the arteriosclerosis development. Serum levels of lipoprotein indicate a widespread change among race and individuals, whereas they are stable inside an individual over time. Physical exercise, diet, and other environmental effects seem not to alter serum levels of lipoprotein significantly [77,83,93]. Indeed, they are predominantly characterized using the size of the apo-lipoprotein isoforms that are encoded using different changes of the LPA-gene [89,90,92]. Erqou [93] found out low molecular weight of apo-lipoprotein and high lipoprotein A concentrations isoforms associated with a high risk for cardiovascular disease.

HDL are dynamic natural nanoparticles, which are involving diverse biological substances. Many nanoparticles aspects like chemical composition of surface, shape, and size act the main role in their multiple biological functions. HDLs are heterogeneous in their composition aspect (cholesterol esters, cholesterol, phospholipids, and apolipoproteins), they are in every biological and physical sense dynamic and they are very small and unstable. Compared by other lipoproteins, HDLs have a smaller size ranging (8−12 nm diameter) and higher density [90,93]. Due to the novel chemical and physical HDLs properties, combined with HDLs numerous biological roles, HDLs research holds significant potential for improving health. The main protein constituent of HDLs is apo-lipoprotein A-I that comprises nearly 71% of the protein mass in HDLs and is occasionally accompanied using apo-lipoprotein A-II, IV, V, and I. Furthermore, apo-lipoprotein E is a pivotal component of HDLs that occurs in much lower abundance than apo-lipoprotein A [77]. The interaction between lipids and apo-lipoprotein A-I characterizes the final size and shape of HDLs by the hydrophobic face of apo-lipoprotein A-I mediating lipid interactions and the polar face interacting by water. Natural HDLs particles contain discoidal subclass pre-β-HDLs that are generated used an exposure of lipid-free apo-lipoprotein A-I to ATP-binding cassette transporters A1 by the phospholipid/cholesterol transfer activity and two spherical subclasses HDLs 2 and HDLs 3 that include a neutral lipid core containing cholesteryl ester and triglyceride [92]. HDLs 2 and HDLs 3 represent the dominant HDL forms in human plasma. HDLs are referred to as *good cholesterol* using removing extra cholesterol of peripheral tissues and transporting extra cholesterol to the liver for excretion or catabolism through a process well-known as reverse cholesterol transport. Furthermore, HDLs particles possess antiinfective, antiapoptotic, antioxidative, and antiinflammatory features. Consequently, HDLs have been regarded as the main substance that reduces coronary artery disease risk and protects the cardiovascular system [92]. Lately, the potential of applying HDLs particles as drug delivery vehicles has been extensively explored. Many biological properties of HDLs can be

controlled for opening an attractive avenue towards optimum drug delivery process. Firstly, these particles are totally biodegradable and have excellent biocompatibility. After that, as internal materials, they escape removal using the mononuclear phagocyte system and do not trigger immunological responses. Moreover, innate receptor-ligands present in HDLs and the corresponding cellular receptors of these ligands have been indicated to assist HDLs to apply their biological functions [91−93]. HDLs particles that include apo-lipoprotein A-I are well-known to bind several cellular receptors like the scavenger receptor class B type 1 and ATP-binding cassette transporter G1 (ABCG1). The ABCG1 and scavenger class B type 1 receptors have indicated to intercede cholesterol transferring to HDLs particles from the peripheral cells involving foam cells. From the aiming delivery viewpoint, the scavenger receptor class B type 1 receptor is rich in hepatic cells, macrophages, and cancer cells. It has been reported that endogenous HDLs can transport micro RNA, proteins, and lipids from donor cells to recipient cells, suggesting that the intrinsic and functional targeting ability of HDLss makes them a perfect candidate for drug delivery process and intracellular communication [89−93]. But there are moreover various limitations for HDL-mediated drug delivery process. Initially, the source of endogenous HDLs is limited to the purification from human plasma, isolation laborious process and costly, which is very difficult on a large scale. Moreover, the safety concerns of blood-borne contamination can be another main challenge for HDL as a general drug delivery system. To address these topics, a great number of investigated have concentrated on the reconstituted HDLs particles development, which are no-naturally synthesized by phospholipids and apo-lipoprotein/apolipoproteins mimetic peptides as drug delivery platforms or alternative therapeutics. As the specific substances of reconstituted HDLs can be flexibly adjusted, the physiochemical features like zeta potential, uniform size, surface, and core loading of reconstituted HDLs can be simply controlled [92]. In terms of drug loading process, hydrophobic drugs could be combined in the core of reconstituted HDLs while amphiphilic drugs could be introduced in the hydrophilic molecules. Lipid membrane could be carried out either using concentrated by a hydrophobic group to insert in the reconstituted HDLs surface or using encapsulated in the reconstituted HDLs core by the assistance of certain hydrophobic substances. Furthermore, reconstituted HDLs could not just control the biology of HDLs; however furthermore, they dominate the several biological barriers to the drug delivery process. [92].

The lipoprotein lipase existence was first observed in 1943 [94]. Paul et al. [94] observed that intravenous heparin injection can reason the rapid clearance of alimentary lipemia associated with fatty meal absorption. Consequently, a series of investigations revealed, *clearing factor lipase* activated using heparin is a lipolytic enzyme and possesses *antichylomicronemic* features [95]. Subsequently, many investigations have concentrated on lipoprotein lipase roles in the transport and metabolism of lipids and slowly identified the gene function, regulation, synthesis, and structure of the enzyme. To increase the best understanding of lipoprotein lipase function, likely associated pathological events, and lipoprotein lipase as a therapeutic target, it is significant to explore its biosynthesis, its gene involving factors regulating the lipoprotein lipase protein, and the role of lipoprotein lipase in several diseases. The lipoprotein lipase protein monomer is organized in a larger amino-terminal domain, which involves catalytic remains, and a smaller carboxylterminal domain by a flexible peptide involving the two areas [94−97]. The carboxylterminal area involves the prevailing heparin-binding area that is significant for binding lipoproteins. Active lipoprotein lipaseis is a dimeric molecule of two like subunits by a head-to-toe structure [98,99]. Lipoprotein lipaseis is active only when it forms a homodimer. Dissociation of homodimers to monomers

causes loss of the catalytic function [99,100]. Lipoprotein lipase is the rate-limiting enzyme, which facilitates the triglycerides hydrolysis in chylomicrons and very LDLs in circulation and creates chylomicron remnants and intermediate-density lipoproteins and releases fatty acids for tissue energy storage or utilization [101]. Lipoprotein lipaseis is mainly stated in the lungs and spleen, pancreatic islet cells, adrenals, kidneys, mammary gland, liver, nervous system, adipose tissues, skeletal muscle, and heart [99–105]. Lipoprotein lipase in these tissues applies different physiological functions. Lipoprotein lipase plays key role in some pathophysiological and physiological conditions. Abnormal lipoprotein lipaseis explanation and function are indirectly or directly associated with several diseases, like chronic lymphocytic leukemia and stroke, Alzheimer's, diabetes, obesity, atherosclerosis, and hypertriglyceridemia [95].

1.2.3.1 Lipoprotein lipase in diseases pathology

- *Atherosclerosis*: Atherosclerosis is the main reason for cardiovascular disease pathophysiology and universal mortality. Atherosclerotic wounds are considered using vascular smooth muscle cells proliferation, endothelial cells injury, and mononuclear cell infiltration [95]. Whether lipoprotein lipase is anti or proatherogenic, depends on various conditions. Lipoprotein lipase produced using cells in the wall of the artery can mediate the lipoproteins binding to cell surface heparan sulfate proteoglycans, subsequent in endocytosis. In this situation, foam cell formation happens through a receptor-mediated uptake of apoE valueless very low-density lipoproteins and is motivated in the presence of lipoprotein lipase [96–98]. Lipoprotein lipase exists in cells included in atherosclerosis development, like smooth muscle cells, endothelial cells and macrophages. There have been some cross-talks between three types of cells in terms of the regulation of lipoprotein lipase. To additional disclose, their interrelationships assist to understand the lipoprotein lipase functions in atherogenesis.
- *Lipoprotein lipase and macrophage*: Lipoprotein lipase could be produced using monocyte-derived macrophages. Macrophages are noticed in the arteries expressing two forms of lipoprotein lipase [104,105]. Moreover, there is an increase in lipoprotein lipase secretion of inflammatory macrophages created after intraperitoneal inflammatory agent injection [100,106]. Lipoprotein lipase can be produced using macrophage-derived foam cells in human coronary atherosclerotic plaques. The macrophages transformation in lipid-loaded foam cells is a serious initial event in atherosclerosis pathogenesis. Macrophage lipoprotein lipase acts a significant role in atherosclerosis development [95,99]. The lipoprotein hydrolysis products using lipoprotein lipase encourage cholesteryl ester accumulation in macrophages using inhibiting cholesterol flow [107]. Investigations have indicated that macrophage-derived lipoprotein lipase acts as a key role in the inflammatory response in macrophages. Lipoprotein lipase persuades mRNA expression and posttranscriptional modifications of tumor necrosis factor-alpha that enhances interferon gamma dependent NO product and is associated by the response of inflammatory [95,100]. Macrophages treatment by lipoprotein lipase and hydrogen peroxide or macrophages pretreatment by hydrogen peroxide before lipoprotein lipase stimulation decreases the lipoprotein lipase persuaded tumor necrosis factor-alpha release using macrophages, suggesting which, reactive O_2 intermediates influence enhancers of macrophage lipoprotein lipase to produce, modulate macrophage response to lipoprotein lipase and probable encourage the atherosclerosis development. In contrast, lipoprotein lipase suppression can reduce the expression and composition of the inflammatory cytokines of lipid in macrophages [107].

- *Lipoprotein lipase and endothelial cell*: Endothelial cells themselves do not produce lipoprotein lipase. After synthesis using parenchymal cells, lipoprotein lipase is transferred to the luminal surface of vascular endothelial cells using its transporter glycosylphosphatidylinositol to apply its main function of triglyceride hydrolysis. Coronary lipoprotein lipase that originates from cardiomyocytes needs liberation of myocyte heparan sulfate proteoglycans before translocation to vascular lumen [108]. The vascular endothelial growth factor can encourage glycosylphosphatidylinositol mediated translocation of lipoprotein lipase across endothelial cells using activating Notch signaling. For lipoprotein lipase mediated lipolysis to happen, triglyceride-rich lipoprotein should bind to the capillaries lumen. This process is called triglyceride-rich lipoproteins margination. Indication proposes that glycosylphosphatidylinositol bound lipoprotein lipase is the key determinant of triglyceride-rich lipoproteins margination [95]. Lipoprotein lipase that is present on endothelial cells and in subendothelial matrix can importantly increase the lipoprotein retention to the endothelial cell-matrix, representing an initial event in atherosclerosis [106,109]. This enzyme can furthermore increase monocyte adherence that endorses macrophage accumulation in arteries. Lipoprotein lipase derived from endothelial cells mediates cholesterol ester invasion in the wall of the vessel and subsequently endorses proatherogenic modifications. Lipoprotein lipase mediates the acceptance of glycated LDLs in endothelial cells [95,103]. Glycated LDL is a favored oxidative modifications aim, probably contributing to the enhanced atherosclerotic risk of patients by familial hypercholesterolemia and diabetes. Endothelial cells associated lipoprotein lipase in the wall of arterial is a powerful proatherosclerotic factor to persuade vascular cell adhesion molecule and lipid deposition upregulation as showed in lipoprotein lipase [100]. Consideration of triglyceride-rich lipoproteins contributes to the atherosclerotic cardiovascular disease risk. One of the presented methods is that the products of triglyceride-rich lipoprotein lipolysis activate ATF3-JNK transcription factor networks and persuade inflammatory answers in endothelial cells. Triglyceride-rich lipoprotein lipolysis products prepare a proinflammatory incentive to change endothelial barrier function [110].
- *Lipoprotein lipase and vascular smooth muscle cell*: Lipoprotein lipase is moreover produced using vascular smooth muscle cells. Initially, the lipoprotein lipase association by vascular smooth muscle cells is established in neointimal lesions of the usual carotid artery, as striking lipoprotein lipase immunostaining is typically colocalized by neointimal smooth muscle cells in the wall of vascular, proposing that lipoprotein lipase is produced in vascular smooth muscle cells [95]. After that, emerging investigations have demonstrated that vascular smooth muscle cells secrete lipoprotein lipase in atherosclerotic lesions. Finally, lipoprotein lipase was indicated to interact by the extracellular matrix, where lipoprotein lipase increases the atherogenic lipoproteins retention. Vascular smooth muscle cells are converting capable in foam cells and contributing in lesion formation. Lipoprotein lipase may affect vascular smooth muscle cell receptor-mediated lipoproteins uptake through binding by high affinity to various receptors expressed on the vascular smooth muscle cell surface [105–111].
- *Alzheimer disease*: Evidences propose that lipoprotein lipase and its cofactor apo C-II are moreover represented in the brain and central nervous system of various mammalian species, involving its roles in the brain and adaptable energy balance and fatness and cognition, respectively [112]. Brain lipid detecting is essential for adaptable energy balance, in which lipoprotein lipase may act a role. Neuron-specific deletions of lipoprotein lipase in mice propel

to maladaptive replies to unique environment presentation and acute sleep privation and causes in weight gain [99,111]. Furthermore, it has been established that reduced activity of lipoprotein lipase in rodents is associated with enhanced synthesis of novo ceramide and neurogenesis in the hippocampus, suggesting that neuronal lipoprotein lipase, particularly hippocampal lipoprotein lipase, is a significant lipid homeostasis regulator in neurons [95]. Conversely, lipoprotein lipase deficiency is likely related to memory and learning impairment. The hippocampus is the region influenced by associated degradation. It was found out that the levels of hippocampus lipoprotein lipase mRNA, activity, and enzyme mass are all higher than cerebral cerebellum, cortex, and other brain areas [54,83]. It is moreover described that the P + allele in the presence of lipoprotein lipase influences senile plaque densities, neurofibrillary tangles, and brain tissue levels of cholesterol, suggesting that a usual polymorphism in lipoprotein lipase modulates the associated degradation risk level [91]. Lipoprotein lipase is powerfully associated with neurite pathology and levels of lipoprotein lipase are decreased in the associated degradation brain dentate gyrus [95,98,109]. In the neural system, the neuronal synapses networks are the information transmission bridge among fundamental basis and neural for retaining and creating a memory. Consequently, lipoprotein lipase cannot help in lipids recycling; however, furthermore plays as a trophic factor for differentiation and neural survival [95].

- *Cancer*: Cancer is the second death cause in the world and therefore presents a main communal health issue [113]. Also, cancer therapeutics are quickly developing, radiotherapy and chemotherapy continue the mainstay for behavior options. But some methods are often limited with side influences and in other cases [113,114]. Consequently, treatment plans and extra healing aims for cancer are immediately necessary. A growing body of indication proposes that tumors cells need an enhanced cholesterol source and are capable of cholesterol accumulator moreover, complex changes in cholesterol and lipid metabolism are met in tumor cells, and levels of plasma lipid can vary in patients by cancer. Consequently, hypercholesterolemia or hypocholesterolemia, hypotriglyceridemia or hypertriglyceridemia, and reduced HDLs have been presented in human cancers [95,113,114]. Significantly, levels of blood lipid may associate with cancer progression and oncogenesis. Therefore, total cholesterol levels are contrariwise associated with carcinoma in situ risk or nonmelanoma skin cancer, but higher serum levels of total cholesterol have been described in patients with colorectal cancer [113,115]. As an extra relationship between HDLs and cancer, chemotherapy-induced reductions in high-density lipoprotein cholesterol levels have been observed, additional complicating the relationship between cancer and HDLs. Furthermore, several variables (cancer type, tobacco exposure, obesity, and age) can apply a confounding effect on the association among high-density lipoprotein cholesterol levels and cancer. Among these confounders, high-density lipoprotein composition and functionality can act as an important role [113].

HDL and proteins and their related lipids have comprehensive activities. The key atheroprotective function of HDLs is their role in reverse cholesterol transport [113,115]. In addition, there are some nonreverse cholesterol transport atheroprotective activities of apo-lipoprotein AI/high-density lipoprotein, involving antiapoptotic, antiinflammatory, and antioxidant. In the recognized association light among tumorigenesis, immunity, inflammation, and oxidative stress it is suggested that HDLs can be protective against cancer. Dyslipidemic, inflammation, and oxidation states in cancer

can change the anticarcinogenic features of high-density lipoprotein via remodeling and interconversion through removal and addition of apo-lipoprotein, cholesterol, and phospholipids and neutral lipids components [113,116]. Consequently, compositional variations in high-density lipoprotein have been related to the *dysfunctional high-density lipoprotein* concept and specific biomarkers documentation in various presented experimental models will prepare significant information for the patient's evaluation by cancer and the novel antitumor therapies development. The relationship among HDLs, high-density lipoprotein cholesterol, and cancer mortality and incidence is still controversial and can be tumor-kind-dependent, with some investigations reporting negative and other investigations a positive association [117]. This double influence of HDLs in cancer appears in in-vitro investigations. For example, the high-density lipoprotein antioxidant activity is found out to limit prostate cancer cells, but HDLs can stimulate cell migration in breast cancer cell lines, because of high-density lipoprotein oxidative modification in the breast cancer oxidative condition. Moreover, HDLs carry out metabolites and vitamins, hormones, microRNA, enzymes, involving proteins and nonlipid cargo that can act a significant functional role in tumor cell survival [113–117].

1.3 Low molecular weight biosurfactant

1.3.1 Glycolipid

The chemical structure of biosurfactants is in a way that it is composed of one hydrophilic part (amino acids or peptides, di-or polysaccharides; anions or cations) and one hydrophobic part (saturated or unsaturated fatty acids), which can act distinctly as the interface between liquids phases with different polarity degree and hydrogen bonds. Biosurfactants are produced by different microorganisms. Among the main properties of biosurfactants, which is the reason for the increasing tendency to use them, are nontoxicity, functional nature in severe pH and temperature changes, salinity, stable activity, and humidity. However, these compounds are not compatible to synthetic surfactants due to their high cost of production [118–127]. Biosurfactants are classified based on chemical composition, molecular weight (high and low molecular weight biosurfactants), physicochemical properties, function type, ionic charges (anionic, cationic, nonionic, and neutral biosurfactants), and secretory type (intracellular, extracellular, and attached to microbial cells) and microbial origin [117,119]. In terms of molecular weight: glycolipids, lipopeptides, lipoproteins, phospholipids, and fatty acids. Low molecular weight biosurfactants include glycolipids, fatty acids, cyclic and noncyclic lipopeptides, while high molecular weight biosurfactants include polysaccharides, proteins, lipoproteins, and lipopolysaccharides [119]. Glycolipids are composed of a carbohydrate portion attached to fatty acids, which are produced by some microorganisms and have many different structural variations (Fig. 1.9). The most famous glycolipids include Rhamnolipids, trehalolipids, mannosylerythritol-lipids, cellobiose lipids, and sophorolipids. Glycolipids have many applications in the cosmetics, food, and pharmaceutical industries due to their specific structure. Moreover, glycolipid biosurfactants can be used as modulators of enzymatic activities for biotechnological applications. Among biosurfactants, Rhamnolipid has been studied and investigated the most. Production of this substance by *P. aeruginosa* was first reported in 1949 [117–119].

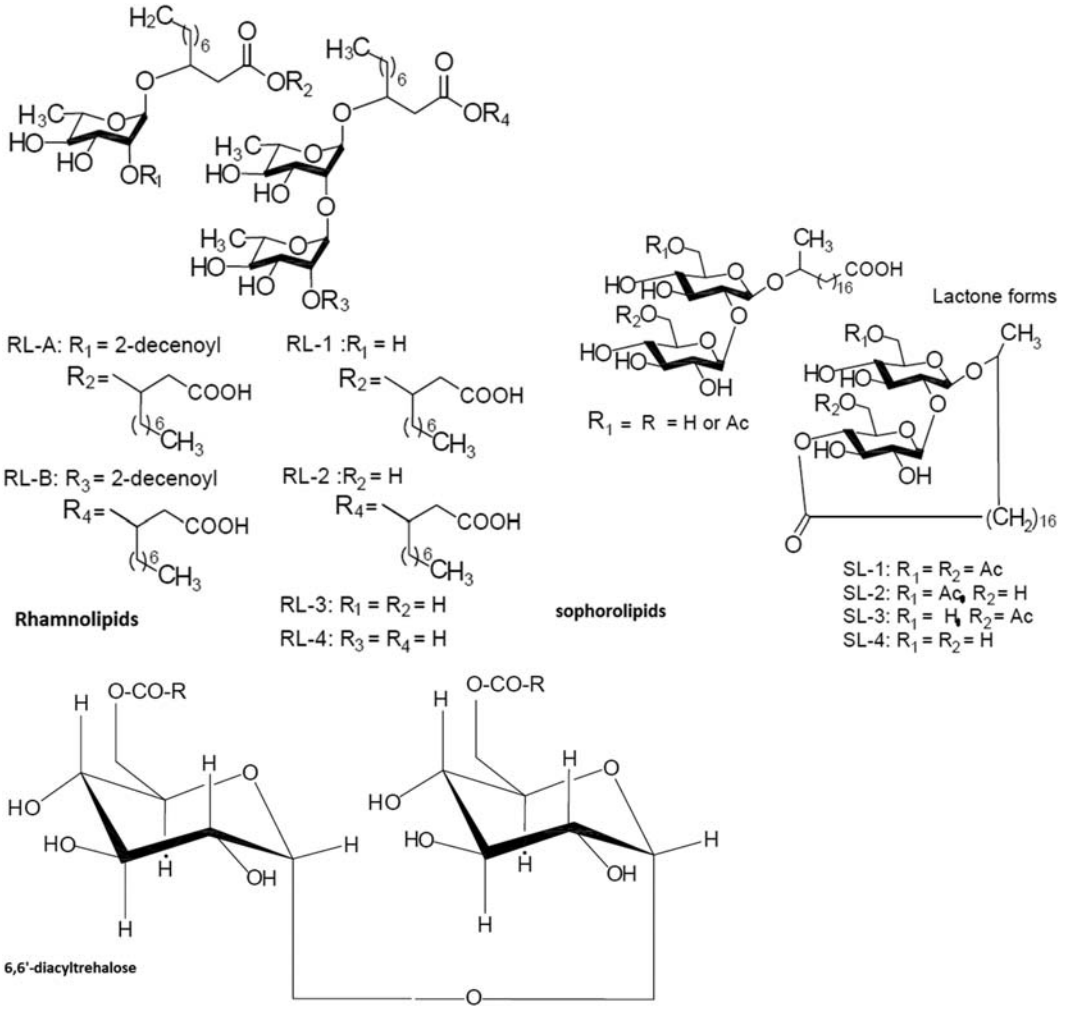

FIGURE 1.9

Chemical structure of the most recognized glycolipids.

Modified after M Inès, G Dhouha. Glycolipid biosurfactants: potential related biomedical and biotechnological applications. Carbohydr. Res. 2015; 416:59–69.

Trehalose lipids are representatives of a large group of glycolipids consisting of a trehalose disaccharide attached to mycolic acids. This group of glycolipids is mainly produced by gram-positive bacteria like *Mycobacterium*, *Nocardia*, and *Corynebacterium* and vary in size and structure. Sophorolipids are mainly produced by yeasts strains like *Candida bombicola*, *C. magnoliae*, *C. apicola*, and *C. bogoriensis* that grow on carbohydrates and lipophilic substrates. In total, they are present in the form of disaccharide sophoroses (2-*O*-β-D-glucopyranosyl-dglucopyranose),

which are β glycosidically bonded to the hydroxyl group at the penultimate carbon of fatty acids. Moreover, we can enumerate the lipids of cellobiose or ustalagic acid that correlates to cellobiose with an *O*-glycosidic bond with the ω-hydroxyl group of the 15, 16-dihydroxyhexadecanoic acid or 2, 15, 16-trihydroxyhexadecanoic acid [116,118]. These types of substances are often produced by *Ustilago maydis*, *Pseudozyma* sp., *Sympodiomycopsis paphiopedili*, and *Cryptococcus humicola*. Microbial glycolipids have come into attention due to properties such as stability at different pH conditions, salinity and temperature tolerance, and functional characteristics such as the ability to reduce surface and interfacial tension, emulsion, and nonemulsion capacities, foaming power, solubilization, and mobilization ability. Table 1.3 lists the main subclasses of glycolipids, their biological activity, and their microbial sources [118].

Considering the physical and chemical properties of biosurfactants and the required degree of purity, there are several ways to extract, purify and identify glycolipids. Among the more common

Table 1.3 Major glycolipids subclasses, biological activities and microbial sources.

Subclasses	Producing strain	Biological activities
Rhamnolipids	*P. aeruginosa* JBR 215 *P. alcaligenes* PCL *P. aeruginosa* J4 *P. desmolyticum* NCIM-2112 *P. aeruginosa* BN10 *P. aeruginosa* #112	Biocontrol agent Mobilization of hydrocarbons Solubilization and mobilization of hydrocarbons Dyes solubilization Antitumor activity Emulsifying activity and washing of hydrocarbons
Glucolipid Trehalolipids	*R. erythropolis* 3C-9	Hydrocarbon solubilization
Trehalose lipid	*R. erythropolis* 51T7 Rhodococcus sp.	Inhibition of phospholipase A2 Hemolytic activity Membrane permeabilizing activity
Trehalose mycolates	*R. erythropolis*, *Arthrobacter paraffineu*, *Mycobacterium phlei*, *Nocardia erythropolis*	–
Trehalose dimycolate	*Mycobacterium tuberculosis*	Immunostimulatory activity
Trehalose mono-, di- and tricorynomycolates	*Rhodococcus erythropolis* strain EK-1 *R. ruber* IEGM 231	Emulsifying and surface-active properties Immunomodulating activity Immunomodulating and antitumor agent
Trehalose dinocardiomycolates	*R. opacus* 1CP	Surface-active compound
Trehalose tetraesters	*R. wratislaviensis* BN38 Micrococcus luteus BN56	Surface-active property
Succinoyl trehalose	Rhodococcus sp. TB-42	Anticancer activity
Lipids	Rhodococcus sp. SD-74	Surface activity
Octaacyl-trehalose	Rhodococcus species H13-A	Surface activity

Table 1.3 Major glycolipids subclasses, biological activities and microbial sources. *Continued*

Subclasses	Producing strain	Biological activities
Sophorolipids	*Candida bombicola* *Wickerhamiella domercqiae* *Starmerella bombicola* MTCC 1910 *Trichosporon asahii* *Starmerella bombicola* NRRL Y-17069 *Wickerhamiella domercqiae*	Surface-active property Anticancer activity Surface-active property Emulsifying activity Reducing and stabilizing agent
Cellobiose lipids	*Sympodiomycopsis paphiopedili* *Cryptococcus humicola* and Pseudozyma Fusiformata *Pseudozyma flocculosa* *Cryptococcus humicola* JCM 1461 *Pseudozyma aphidis* and P. hubeiensis	Antimicrobial activity Fungicidal activity Biocontrol agent Surface-active property Fungicidal activity
Xylolipids	*Lactococcus lactis* *Pichia caribbica*	Antibacterial activity Surface and antibacterial properties
Oligosaccharide lipids	Tsukamurella sp.	Surface and antimicrobial activities
Monoglucosyl diglycerides	*Staphylococcus aureus* *Rhizobium trifolii*	
Mannosylerythritol-lipids	*Candida Antarctica* *Ustilago maydis* *Pseudozyma aphidis* *Pseudozyma tsukubaensis* *Ustilago scitaminea* NBRC 32730 *Pseudozyma churashimaensis*	Hydrocarbon solubilization Hemolytic activity Surface-active property Ceramide-like skin-care property Surface-active and self-assembling properties
Diglucosyl diglycerides	*Streptococcus faecalis*	–
Monoacylglycerols	*Candida ishiwada*	Surface activity
Monoglycosylglycerol and diglycosylglycerol	*Arthrobacter globiformis* and *Arthrobacter scleromae*	–
Polyol-lipids	*Rhodotorula glutinis* and *R. graminis* Aureobasidium sp.	Antiproliferative activity
Lipomannan	*Mycobacterium smegmatis* *Mycobacterium tuberculosis*	Immunomodulatory activities
Lipomannan and lipoarabinomannan	*Mycobacterium chelonae*, *Mycobacterium smegmatis* and *Mycobacterium kansasii*	Proinflammatory and antiinflammatory Activities
Mannosyl-mannitols	*Pseudozyma parantarctica* JCM 11752	Surface-active property
Mannosylribitol lipid and mannosylarabitol lipid	*Pseudozyma parantarctica* JCM 11752	Surface-active property
Mannolipid	*Micrococcus lysodeikticus* *Corynebacterium aquaticum*	–
Mannophosphoinositides	*Corynebacterium aquaticum*	–

Modified after M Inès, G Dhouha. Glycolipid biosurfactants: potential related biomedical and biotechnological applications. Carbohydr. Res. 2015; 416:59-69.

techniques in the extraction of glycolipids are foam fractionation, acid deposition, and solvent extraction. Common purification methods include membrane ultrafiltration techniques, ion-exchange chromatography and adsorption-surface adsorption on resins, and adsorption−adsorption on activated carbon wood such as charcoal [115,118].

Previous studies showed that the bacteria of the *Burkholderia* species are able to produce rhamnolipids. Radhakrishnan and Jyothis [119] reported the production of biosurfactants from endophytic bacteria (bacteria that live inside plant tissues without causing apparent damage to the plant itself) *Burkholderia* sp. *WYAT7* obtained from *Artemisia nilagirica* (Clarke) Pamp plant. They cultivated biosurfactants produced in kerosene (BSB1) and olive oil (BSB2). The production of biosurfactants from bacteria, yeasts, and fungi requires a substrate as a carbon source. Substrates that have been used in researchers' experiments so far include substrates of sugars, oils (like olive oil), alkanes such as kerosene, and organic sludge. The nature of the carbon source available in the environment plays a major part in the production of biosurfactants and also affects one quality and quantity of the produced biosurfactants. But it is still a major problem as to what substrate should be used as a growth medium so that it enables the cells' growth and accumulation of product by the right balance of nutrients [120−124].

The properties of the produced biosurfactants and their chemical nature have been compared through FTIR analysis. The existence of H−O bond and $-CH_2$ and $-CH_3$-acyl chain groups has been reported in both biosurfactants. The peak at 1743.65 cm^{-1} from BSB1 and 1791.87 and 1662.64 cm^{-1} from BSB2 is related to C = O stretching in lipids and fatty acids ester group. C = O stretching bands at 1456.26 cm^{-1}, 1377.17 cm^{-1} from BSB1, 1487.12 cm^{-1}, 1402.25 and 1404.03 cm^{-1} from BSB2 indicate the presence of bonds between the carbon atom and hydroxyl groups in the chemical structure of the glycoside part. The weak absorption peak at 1238.30 cm^{-1} from BSB1 and 1209.37 cm^{-1} from BSB2 indicates stretching vibration bands of ether. Bands at 1024.20 and 603.72 cm^{-1} from BSB2 and 1163.08 cm^{-1}, 997.20, 723.31, and 696.30 cm^{-1} from BSB1 were related to glycosidic linkage stretching vibrations that confirmed glycolipids [119].

BSB1 and BSB2 biosurfactants were also examined for antibacterial effect. The antimicrobial properties of biosurfactants depend on the specific mechanism for killing the organism in comparison with conventional antibiotics. Biosurfactants mainly kill bacterial cells by directly disrupting cell walls or plasma membrane integrity. One way bacteria defend themselves against antibiotics is to form biofilms. A biofilm is a complex structure that causes bacteria to adhere to surfaces and contains colonies of bacteria enclosed in a protective mucosal covering secreted by the bacteria themselves [122]. Biofilms can be created on solid or liquid surfaces as well as on the soft tissues of living organisms. They are usually resistant to common disinfection methods. Biofilm plays an important role in the pathogenicity of several bacteria including *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Serratia marcescens*. The first stage of biofilm formation is adhesion and is a very good time to treat with antiadhesive and antibiofilm compounds. According to reports of Radhakrishnan and Jyothis [119] the antibacterial effect of biosurfactants produced in kerosene was greater than the biosurfactants produced in olive oil so that this effect was reported against bacteria such as *P. aeruginosa*, *E. coli*, *S. paratyphi*, and *B. subtilis* about BSB1 and bacteria *Escherichia coli* and *Salmonella typhi* about BSB2. BSB1 produced for biofilm inhibition was tested and successful inhibition of *Staphylococcus aureus* biofilm has been reported. Kaur et al. [120] reported the production of a biosurfactant from lactic acid bacteria (LAB), which prevents the formation of biofilms in *Escherichia coli*, *Staphylococcus aureus*. Sałek and Euston et al. [121]

examined the disorders in *Bordetella bronchiseptica* biofilms by Rhamnolipids secreted from *P. aeruginosa*. Results indicated the loss of *Bordetella* and *C. tropicalis* biofilm. Dusane et al. [122] also indicated that the Rhamnolipid biosurfactant cover decreased 50% of *Y. lipolytica* biofilm. Vecino et al. [123] reported the beneficial effect of rhamnolipid in preventing deposition and bacterial biofilm formation on the membrane and water purification equipment. Sen et al. [124] reported the antifungal activity of Sophorolipids biosurfactants against pathogenic fungi of *Glutosporium gluosporioids, Fusarium verticilliodes, Fusarium oxysporum f. sp pisi, Corynespora cassiicola* and *Trichophyton rubrum*. They prepared this biosurfactant from a yeast, *Rhodotorula babjevae YS3*. By performing thin-layer chromatography (as a primary method for biosurfactant composition analysis), the biosurfactant prepared reacted positively to sugars using anthrone reagent and to lipids using iodine vapor reagent, which confirms that biosurfactant obtained is glycolipid. Their studies on yeast growth indicated that its growth began without delay and the exponential growth phase lasted up to 168 hours, with a reported bioavailability of 11 g/L during this period. According to reports of Sen et al. [124] room pH was about 7 and there was no significant difference during the experiment. Fig. 1.10 shows the surface stretching changes during the test from the beginning up 240 hours after the starting time of the test. Table 1.4 provides biosurfactant emulsion index (EI) after 24 and 168 hours. Emulsion index, which is also known with the symbol (% E24) determines the effectiveness of bioemulsifier. Antifungal and antimicrobial activity of biosurfactant produced from *Rhodotorula babjevae YS3* (*SL-YS3*) in vitro compared to standard Sophorolipid has been reported against some plant and human fungal pathogens based on MIC values in Table 1.5.

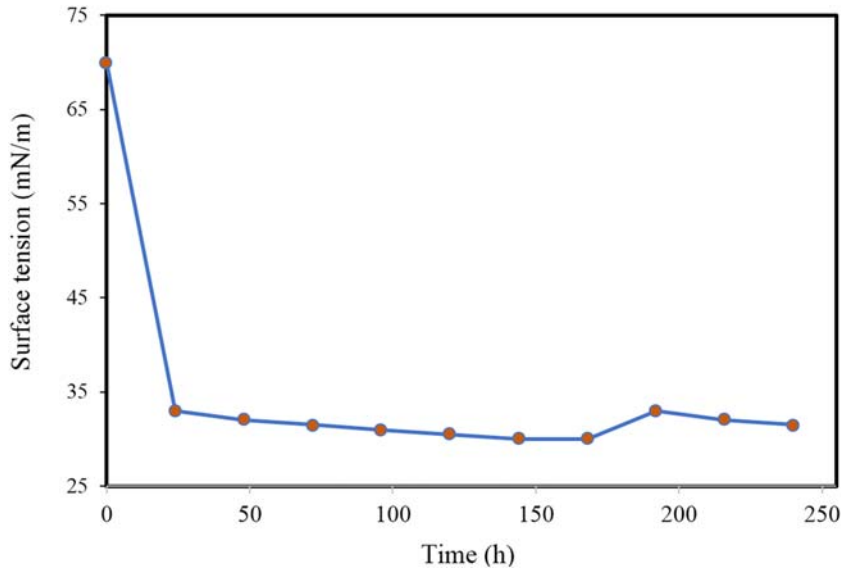

FIGURE 1.10

Surface stretching of *Rhodotorula babjevae* YS3 grown at 19°C, 200 rpm, 5% inoculum (v/v), 10% glucose (w/v) plotted as a function of time.

Modified after S Sen, SN Borah, A Bora, S. Deka. Production, characterization, and antifungal activity of a biosurfactant produced by Rhodotorula babjevae YS3. Microb. Cell Factories. 2017;16(1):1–4.

Table 1.4 Emulsification index (EI) evaluated using the biosurfactant containing culture broth of *Rhodotorula babjevae* YS3 grown at 19°C, 200 rpm, 5% inoculum (v/v), 10% glucose (w/v) after 24 and 168 h.

		Emulsification index (%)	
	Hydrophobic substrates	**24 h**	**168 h**
1	*n*-Hexadecane	25.19 ± 0.12	22.58 ± 0.11
2	Sunflower oil	33.33 ± 0.87	5.26 ± 0.53
3	Motor oil	62.26 ± 0.32	60.00 ± 0.12
4	Diesel	25.00 ± 0.25	5.00 ± 0.43
5	Crude oil	100.00 ± 0.32	83.33 ± 0.23

Values are mean ± SEM of triplicates with three independent experiments.
Modified after S Sen, SN Borah, A Bora, S Deka. Production, characterization, and antifungal activity of a biosurfactant produced by Rhodotorula babjevae YS3. Microb. Cell Factories. 2017;16(1):1–4.

Table 1.5 Antimicrobial activity of the sophorolipid produced by *Rhodotorula babjevae* YS3 (SL-YS3) in omparison to sophorolipid standard, 1,4″-sophorolactone 6′,6″-diacetate (SL-S) against pathogenic fungal strains.

		Minimum inhibitory concentration (MIC, μg/mL)	
	Fungal strains	**SL-YS3**	**SL-S**
1	Colletotrichum gloeosporioides	62	50
2	Fusarium verticilliodes	125	125
3	Fusarium oxysporum f. sp. Pisi	125	125
4	Corynespora cassiicola	>2000	1000
5	Trichophyton rubrum	1000	1000

Values are mean ± SEM of triplicates with three independent experiments.
Modified after S Sen, SN Borah, A Bora, S Deka. Production, characterization, and antifungal activity of a biosurfactant produced by Rhodotorula babjevae YS3. Microb. Cell Factories. 2017;16(1):1–4.

The results showed no inhibitory activity against *C. cassiicola* within the assessed concentration in this study. However, the results for *C. gloeosporioides* and *F. oxysporum f* are satisfactory and promising [124]. Reported cases of biosurfactants from terrestrial bacteria are more than the number of studies on marine bacteria, which given that the marine environment constitutes 70% of the Earth's biosphere and includes diverse groups of microorganisms with unique metabolic, structural, and functional properties, highlights the need for further research in this area. The first preparation of antimicrobial biosurfactant from marine bacteria was isolated from a bacterium called *B. circulans* in India, which has shown significant antimicrobial effects against several drug-resistant human pathogens. Mani et al. [125] investigated the antimicrobial activity of biosurfactants prepared from marine microbe *Staphylococcus saprophyticus SBPS 15* which was obtained from sea water in Jiangsu in China. *Staphylococcus* is a genus known for human and animal infections. Reports indicated that some of the secondary metabolites produced by *Staphylococcus species*

isolated from natural environments have biotechnological and biomedical importance. *Staphylococcus saprophyticus* isolated using multiple screening methods was isolated and its biochemical characteristics and temperature stability were assessed and studied, and the potential antimicrobial effect of biosurfactant against different human pathogens was studied and reported [123]. Results of the FT-IR spectrum and the presence of specific absorption bands for this biosurfactant indicated the presence of long aliphatic fatty acid chains. Alkene, carbonyl, and carboxyl groups were important functional groups of this biosurfactant, and the presence of the carboxyl group indicates the relationship between the sugar group and fatty acids. These results indicate that the prepared biosurfactant belonged to the glycolipid family [128].

Among reagents used in this study, ninhydrin reagent was used to determine peptides, anthron reagent was used to determine sugars, and rhodamine reagent was used to determine lipids. The amount of protein, carbohydrates, and lipids existence was calculated and reported, respectively, using the Lowry method, phenol sulfuric acid method, and totally free fatty acids. Carbohydrate content formed about 74% and lipids, about 26% of biosurfactant. No protein existence was reported. In terms of temperature stability, biosurfactant activities were not diminished up to 80°C and the emulsion activity was reduced above this temperature and was deactivated at a temperature of 100°C [123]. The human bacterial pathogens used in this study include *Escherichia coli, Salmonella typhi, S. paratyphi, Klebsiella pneumoniae, K. oxytoca, Vibrio parahemolyticus, V. cholerae, Proteus mirabilis, S. pneumoniae, B. subtilis, B. cereus stusal*, and *Pathus aucus pathus Aspergillus niger, A. flavus, Candida albicans, Cryptococcus neoformans*, and *C. gattii*. Among the twelve human bacterial pathogens tested, the purified biosurfactant from SBPS 15 showed antimicrobial activity against seven types [125]. In explaining the antibiotic function of biosurfactants, it can be said that they cause the formation of pores and ion channels in the membrane of bilayer lipids. Therefore, they are able to disrupt the integrity as well as the permeability of membrane in microorganisms [118]. Maximum inhibition zone and minimum inhibitory concentration (MIC) of the mentioned biosurfactant related to the following bacteria and fungi, *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae, Bacillus subtilis, Salmonella paratyphi*, and *Staphylococcus aureus* and *Cryptococcus neoformans, Candida albicans*, and *Aspergillus niger* are provided in Table 1.6. According to this table, it can be concluded that there is inhibitory activity on both gram-positive and gram-negative strains with minimum MIC values, which indicates a promising and diverse antimicrobial potential of SBPS 15 biosurfactants. According to this study, the isolated biosurfactant has shown potential antimicrobial activity range against strains of pathogenic bacteria and fungi [125]. Khopade et al. [126] isolated a biosurfactant of marine species *Streptomyces B3*, which is from the family of the glycolipid and has shown antimicrobial activity against different human pathogens.

Among other cases of biosurfactants isolation from marine bacteria are biosurfactants produced from *Brachybacterium paraconglomeratum* (*MSA21*), which lives inside a kind of sea sponge. Estimations have been reported for protein levels using the Lowry method, for Carbohydrates by Chaplin and Kennedy method, lipids by Sadasivam and Manickam methods, and glycolipid concentrations by Chandrasekaran and Bennett methods. Active compounds extracted were tested against human pathogens *Candida albicans, Escherichia coli, Proteus mirabilis, hemolytic Streptococcus* sp., *Pseudomonas aeruginosa, Micrococcus luteus, Staphylococcus epidermidis, Enterobacter faecalis, Klebsiella pneumoniae, Bacillus* sp., and *Staphylococcus aureus*. The results of spectroscopy showed the glycolipidic being of isolated biosurfactant. The results of liquid chromatography

Table 1.6 Antimicrobial activity of a purified glycolipid biosurfactant from a marine Staphylococcus saprophyticus SBPS 15 against different human pathogenic clinical isolates.

S. No.	Bacterial pathogens	Zone of inhibition (mm)	Minimum inhibitory concentration (MIC, l g/mL)
1	*Escherichia coli*	20	12
2	*Salmonella typhi*	No activity	No activity
3	*Salmonella paratyphi*	13	32
4	*Klebsiella pneumoniae*	23	4
5	*Klebsiella oxytoca*	No activity	No activity
6	*Vibrio cholerae*	18	64
7	*Vibrio parahemolyticus*	No activity	No activity
8	*Proteus mirabilis*	No activity	No activity
9	*Pseudomonas aeruginosa*	20	32
10	*Bacillus subtilis*	15	48
11	*Bacillus cereus*	No activity	No activity
12	*Staphylococcus aureus Fungal pathogens*	11	12
13	*Aspergillus niger*	15	16
14	*Aspergillus flavus*	No activity	No activity
15	*Candida albicans*	21	32
16	*Cryptococcus neoformans*	22	32
17	*Cryptococcus gattii*	No activity	No activity

Modified after P Mani, G Dineshkumar, T Jayaseelan, K Deepalakshmi, CG Kumar, SS Balan. Antimicrobial activities of a promising glycolipid biosurfactant from a novel marine Staphylococcus saprophyticus *SBPS 15. 3 Biotech. 2016;6(2):163.*

identified the type of glycolipid prepared as rhamonolipid. Rhamnolipids, which is one of the most well-known glycolipids is produced from bacterial strains *P. aeruginosa, B. megaterium*, and *Pseudozyma parantarctica*. According to the report of Inès and Dhouha [118], rhamnolipids derived from *P. aeruginosa*, have shown antimicrobial activity against *Neurospora crassa, Staphylococcus aureus* and *Micrococcus luteus* with 256 micrograms per milliliter and *B. cereus* and *Mucor miehei* with 64 μg/mL. According to the report of Kiran et al. [127] biosurfactant produced by *B. paraconglomeratum MSA21* has continued its emulsion activity without showing any significant reduction in terms of temperature stability up to 121°C. The mentioned biosurfactant has always been stable in the pH interval of 5−9. Although this stability has been reported lower in Alkaline pH compared to other pH intervals. It has also been stable in the interval of 1−4, relative to NaCl concentration. The emulsification index for produced biosurfactant from *B. paraconglomeratum MSA21* has been invariably high over the synthetic surfactants like 1% of SDS, Tween 20 and Tween 80. Research results have indicated that the formed emulsion had been stable for more than 2 months at a temperature of about 24°C. Another known glycolipid is sophorolipid. According to the report of Inès and Dhouha [118] sophorolipids derived from *C. bombicola* has inhibited the growth of Gram-negative bacteria *Escherichia coli; P. aeruginosa* with MIC about 30 and 1 μg/mL at a contact time of 2 and 4 hours and of Gram-positive bacteria *S. aureus, B. subtilis* about 6 and

1 μg/mL at a contact time of 4 hours. It is also reported that sophorolipids strengthen the antibacterial activity of some antibiotics against *E. coli* and *S. aureus*. According to Kim et al. [128]. The antibacterial effect of sophorolipid biosurfactant produced from *C. bombicola* has been proven against bacteria *B. subtilis, Staphylococcus xylosus*, *Streptococcus mutans*, and *Propionibacterium acne*. According to researchers, the antifungal activities of glycolipidic biosurfactants such as *P. aeruginosa rhamnolipid*, sophoroselipid and mannosylerythriol lipids are promising. Sha et al. [129] isolated rhamnolipid derived from *P. aeruginosa* and assessed its antifungal activity against plant's pathogenic fungi. Sajna et al. [130] reported the antifungal activity of sophorolipids biosurfactants against several different fungi including *Saccharomyces, Cladosporium, Aspergillus, Fusarium, Penicillium, Gloeophyllum*, and *Schizophyllum*. Haferburg et al. [131] used rhamnolipids for the treatment of viral infections in Nicotiana glutinosa leaves infected with the Tobacco mosaic (TMV) virus. According to them, the treatment has been successful. Hoq et al. [132] tested trehalose Lipids isolated from Mycobacterium tuberculosis and sophorolipids prepared from *C. bombicola* as antiviral agents. Chen et al. [133] and Shao et al. [134] studied Glycolipid biosurfactants as an antitumor. They tested the effects of cytotoxic sophorolipids on cancer cells of liver, lungs, blood and gullet. The results showed the anticancer activity of this substance. However, the results were different for each different type of a cancer cell. The glycolipid biosurfactant produced has significance due to its antibacterial and antibiofilm potential against clinical bacterial pathogens.

1.3.2 Cyclic and acyclic lipopeptides

Most researches conducted on biosurfactants is related to glycolipids and lipopeptides groups. Lipopeptide biosurfactants (LPBs) are related molecules that exhibit antibacterial, antifungal, antiviral, and antiadhesive activity and are of interest to researchers due to their antibiotic activity. The results of antimicrobial tests of LPBs are often very promising. It is hoped that with more and more complete research in this area, lipopeptides could replace traditional antibiotics [135,136]. The mechanism of action of lipopeptides to kill bacteria is different compared to conventional antibiotics. Lipopeptides impact the bacterial cell membrane due to their detergent properties and form ion transport channels in the bacterial cell membrane. LPBs also tend to bind to bacterial cell membranes due to their hydrophobic interactions. In addition to antimicrobial properties, lipopeptides have shown strong antibiophilic properties, which is very important for disinfecting various surfaces, especially surgical instruments. Extracellular polymer matrix in biofilms increases bacterial retention on surfaces and reduces sensitivity to host defense systems, antibiotics, and other drugs [136]. On the other hand, the involvement of biosurfactants in the adhesion and repulsion of microbes has been widely reported. Adsorption of biosurfactants to solid surfaces is a strategy to reduce the adhesion of pathogenic microorganisms in both biomedical and food industries [137].

Hajfarajollah et al. [137] reported, for the first time, biosurfactant production from the probiotic bacteria *P. freudenreichii* subsp. They reported CMC, emulsion activity (E_{24}), surface stress (ST), and oil-spreading test (OST) to evaluate the biosurfactant active surface properties. In investigating the chemical nature of biosurfactant by thin-layer chromatography using iodine vapor and ninhydrin reagent, the yellow spots indicated the presence of polar lipids while the red spots indicated the presence of peptides. The results indicated the presence of lipopeptide biosurfactant. FTIR and NMR spectroscopy are used to identify the types of chemical bonds and functional groups existing in the chemical structures of biosurfactants, which the presence of amine and hydroxyl groups of

protein, presence of protein amides, carboxyl groups, and CH_3 and CH_2 groups in alkyl chains, $C-CH_2$-and $C-CH_3$-groups in aliphatic chains, α amino acids of peptide fragment with fatty acid functional group and ester and carboxylic groups have been reported. IR spectra of biosurfactant produced by Hajfarajollah et al. [137] has been compared with LPBs produced by different species and it has been reported that it is similar to *Bacillus species* and surfactin biosurfactant [135−137].

The CMC amount of pure biosurfactant was reported to be 1.59 mg/mL. According to Busscher et al. [138], the minimum reduction in surface tension for any microorganism as a biosurfactant producer should be more than 8 mN/m. The produced biosurfactants were reported to be capable of reducing water surface tension from 72 to about 38 mN/m, while the ST of efficient biosurfactants such as surfactin and rhamnolipids in their CMCs were reported to be in the range of 27−36 mN/m. Table 1.7 compares the CMC and ST of produced biosurfactants with those of several other biosurfactants. *Propionib* species Bacteria cannot grow below pH 5.0 and also, extreme sensitivity to pH changes can alter the structure and activity of surfactants and reduce their solubility in water [137].

According to the reports, biosurfactant produced is active at pH between 6 and 8 and is resistant to salinity to NaCl concentrations of about 0−30 g/L. Microorganisms that are more tolerant of salinity are suitable for cleaning oil contaminants from seawater. Thermal stability is another reportedly important feature of this biosurfactant, which make it possible to use it in the food industry since it is produced by Gram-positive propionic acid bacteria (PAB), which has been developed by the US Food and Drug Administration and is known to be in generally recognized as Safe (GRAS) status. Non-coagulant and nonpathogenic bacteria *P. freudenreichii* are currently used in the food and pharmaceutical industries to produce vitamin B12, which is produced by intracellular and propionic acid and acetic acid, which are extracellularly excreted by bacteria [137,138]. However, the highest production of biosurfactants was reported to be from *Pseudomonas* sp., *Acinetobacter* sp., *Bacillus* sp., and *Arthrobacter* sp., that due to the pathogenic nature of the producing microorganisms, the use of these compounds is not suitable in the food industry [139].

P. aeruginosa, B. subtilis, R. erythropolis, S. aureus, E. leucillus monos, Alternaria alternantherae, S. typhimurium, and *Klebsi microorganisms* were used to evaluate the antimicrobial and antiadhesive properties [137]. Fig. 1.11 shows the LPBs growth inhibition ability against bacteria and fungi at concentrations of 50−3.2 mg/mL.

Table 1.7 Comparison of the surface stress and critical micelle concentration of biosurfactants from some probiotic microorganisms.

Strain	ST mg/mL	CMC mN/m
Propionibacterium freudenreichii in this work	38.2	1.6
Lactobacillus fermentum RC-14	39	1
Lactobacillus fermentum B54	39	2.5
Lactobacillus paracasei	41.8	2.5
Streptococcus thermophilusA	36	20
Lactococcus lactis	40	3.5
Lactobacillus delbrueckii	–	2

Modified after H Hajfarajollah, B Mokhtarani, KA Noghabi. Newly antibacterial and antiadhesive lipopeptide biosurfactant secreted by a probiotic strain, Propionibacterium freudenreichii. *Appl. Biochem. Biotechnol. 2014;174(8):2725−2740.*

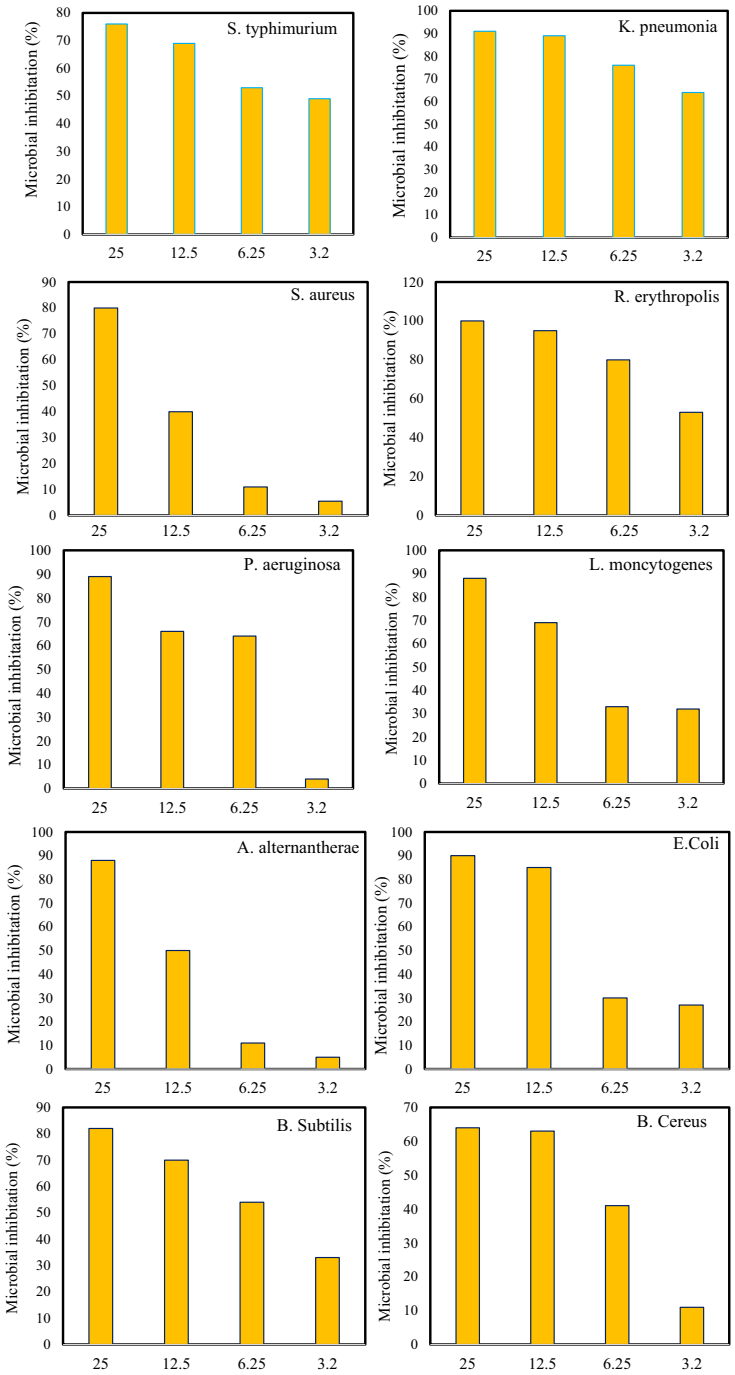

FIGURE 1.11

Microbial inhibition percentages obtained from the antimicrobial assays with the crude biosurfactant isolated from *P. freudenreichii* at different concentrations.

Modified after H Hajfarajollah, B Mokhtarani, KA Noghabi. Newly antibacterial and antiadhesive lipopeptide biosurfactant secreted by a probiotic strain, Propionibacterium freudenreichii. *Appl. Biochem. Biotechnol. 2014;174(8):2725–2740.*

The results of Hajfarajollah et al. [137] indicated the ability of full inhibition of the *R. erythropolis* growth at a concentration of 25 mg/mL and a low inhibitory effect against *B. cereus* at a lipopeptide concentration of 25 mg/mL by the produced biosurfactant. Considering the concentration of biosurfactant, its antiadhesion activity against four bacterial strains, *S. aureus, P. aeruginosa, B. cereus*, and *E. coli* was reported differently. The results in Table 1.8 show that the highest percentage of antiadhesion was reported for *P. aeruginosa* (67.1%) at a concentration of 40 g/L and the lowest percentage for *S. aureus* was reported at the same concentration.

Hajfarajollah et al. [137] produced and tested biosurfactants once in the presence of various carbon sources such as glucose, sucrose, maltose, starch, kerosene, glycerol, sunflower oil, and frying oil. In total, three categories of carbon sources, namely hydrocarbons, carbohydrates, and vegetable oils, are used to grow bacteria. Changing the carbon source from glucose to vegetable oils (i.e., sunflower oil or frying oil) was reported to cause bacteria to use a different metabolic pathway, eventually producing more biosurfactants. The maximum emulsifying activity (E_{24}-72%) was reported in frying sunflower oil whereas the lowest activity was in kerosene and starch. The values of E_{24} are given in Table 1.9.

Large variations in E_{24} values indicate that emulsion activity is strongly influenced by the type of carbon source [137]. Cyclic lipopeptides are produced by different gram-positive and gram-negative groups of bacteria. Gandhimathi et al. [140] reported the production of lipopeptide biosurfactant from *Nocardiopsis alba MSA10* present in a type of sea sponge called Porifera. Bacteria comprise up to 60% of the sponge biomass. The produced biosurfactant showed the most optimal activity at pH 7, temperature 30°C, and salinity of 1%. Biosurfactants are produced in glucose supplementation as a carbon source and python as a nitrogen source. Carbohydrates, proteins, and lipids were reported as the produced biosurfactant compounds so that the percentage of carbohydrate combinations was higher than that of lipids and proteins. Gandhimathi et al. [140] obtained the emulsion index (E_{24}) based on the height of the emulsified layer (H_{EL}) and the total height of the liquid column (H_S).

$$E_{24} = H_{EL}/H_S \times 100 \tag{1.1}$$

Produced pure biosurfactant was tested on human pathogens such as *C. albicans, E. coli, P. mirabilis, Hemolytic streptococcus, P. aeruginosa, Micrococcus luteus, S. epidermidis, E. faecalis, K. pneumoniaee, B. subtilis*, which the highest activity was reported against *B. subtilis* and *E. faecalis*

Table 1.8 Antiadhesive properties of the produced biosurfactant.

		Inhibition of microbial adhesion (%)				
		Biosurfactant (g/L)				
Microorganism PBS	**Control**	**2.5**	**5**	**10**	**25**	**40**
B. cereus	0	2 ± 0.1	2.1 ± 0.1	14.5 ± 0.2	21 ± 0.4	39.1 ± 0.6
P. aeruginosa	0	4.5 ± 0.4	12.5 ± 0.2	37.2 ± 0.6	45.5 ± 0.4	67.1 ± 0.2
E. coli	0	1 ± 0.4	7.7 ± 0.3	13.1 ± 0.4	25.9 ± 0.5	47.7 ± 0.6
S. aureus	0	0	2 ± 0.1	9.1 ± 0.1	16.4 ± 0.3	32.3 ± 0.4

Modified after H Hajfarajollah, B Mokhtarani, KA Noghabi. Newly antibacterial and antiadhesive lipopeptide biosurfactant secreted by a probiotic strain, Propionibacterium freudenreichii. *Appl. Biochem. Biotechnol. 2014;174(8):2725–2740.*

Table 1.9 Physical characteristics of produced biosurfactant by *Propionibacterium freudenreichii* using different carbon sources in 30°C and 130 rpm.

Carbon source	Min ST	Max OST (cm)	Max E24%
Glucose	38 ± 0.5	2.8 ± 0.2	57 ± 2
Sucrose	39 ± 0.9	2.1 ± 0.3	49 ± 3
Maltose	42.1 ± 0.8	1.8 ± 0.2	51 ± 1
Kerosene	44 ± 0.7	1.1 ± 0.1	14 ± 1
Glycerol	41.8 ± 0.9	1.5 ± 0.3	23 ± 1
Starch	42.6 ± 0.7	0.5 ± 0.1	5 ± 2
Sun flower oil	29.2 ± 0.2	14 ± 0.9	71 ± 3
Waste frying oil	29 ± 0.3	15 ± 0.8	72 ± 2

Modified after H Hajfarajollah, B Mokhtarani, KA Noghabi. Newly antibacterial and antiadhesive lipopeptide biosurfactant secreted by a probiotic strain, Propionibacterium freudenreichii. *Appl. Biochem. Biotechnol. 2014;174(8):2725–2740.*

and the pathogenic yeast *C. albicans*, and no activity was observed against *E. coli*, *Hemolytic streptococcus* and *P. aeruginosa* [140].

The bacterium *L. chungkukjangi* belongs to the *Bacillus genus* and many species of this genus are mostly known for producing LPBs. The chemical structure of a biosurfactant also varies from one microorganism to another. Bhardwaj et al. [135] prepared a biosurfactant from *Lysinibacillus chungkukjangi* in bran oil sludge as a carbon source, which reduces the surface tension of the medium from 72 to 27.9 mN/m. FTIR and 1H-NMR spectroscopy were used to identify functional groups in crude biosurfactants. The results of FTIR spectroscopy indicated the presence of N-H groups of the peptide bond, CH−, $-CH_2$ and CH_3- groups, carbonyl group as well as the presence of aliphatic chains and −C−N bond in biosurfactant structure. Results of 1H-NMR spectra indicated the presence of −NH, $-CH_2O-$, $-OCH_2-$ and $-OCH_3$ groups and aromatic rings in biosurfactant structure, the presence of $-CH=CH-$ parts connected to electronegative groups, the presence of $CH_2-C=O-$ group and the oxygen (O−) and nitrogen (N−) atoms attached to the carbon produced atom as well as carboxylic acid derivatives and the C = O aldehydes or ketones group. In terms of emulsion activity, the data also show that the produced biosurfactants emulsify various hydrocarbons of olive oil (100%), rice bran oil (85.71%), kerosene (34.48%), n-dodecane (14.28%), and n-hexane (8.33%). As it appears from the research results [135], the produced biosurfactants have the potential of providing a solvent for the huge amount of fat and oil present in the wastewater of the cosmetics industries.

Marine bacteria produce several essential enzymes, such as lipase, to convert and use organic substances available in marine ecosystems, especially in oil-contaminated areas. Lipases are a subclass of glycerol ester hydrolyzes catalyzing the hydrolysis of triacylglycerols to dicylglycerols, monosylglycerols, free fatty acids, and glycerol [141]. There are many reports about the lipase activity of marine microorganisms even from deep-sea sediment samples [142]. Several marine actinomycetes [143] and fungi [144] can produce lipase proteins. So far, there have been many reports of the production of lipases by microorganisms, such as *Candida* sp., *Pseudomonas* sp., *Bacillus* sp., and *Rhizopus* sp. [145–147]. Microbial lipases are key enzymes in various industries including food, paper, textile, leather and detergents, wastewater treatment, pharmaceuticals,

cosmetics, synthesis of surfactants, polymers as well as in the fermentation of vegetables and the processing of meat products [8,141,148–152]. Kayanadath et al. [141] produced biosurfactants from the bacteria *Halomonas* sp., a type of lipolytic marine bacterium from the Bay of Bengal drilling sites, and reported its characteristics along with antimicrobial activities. Since marine sediments are rich in bacterial diversity, the results of 40 isolates were reported positive for lipase production from a total of 99 selected bacterial colonies. The optimum temperature and pH for maximum enzyme activity were 30°C and 6 at a salt concentration of 15 g/L, respectively.

Isolated lipase activity varies with different carbon sources such as rice bran oil, coconut oil, gingelly oil, and olive oil. Among the various organic substrates used (rice bran oil, gingelly oil, olive oil, and coconut oil), gingelly oil presented the maximum lipase production at a concentration of 5%. Different functional groups in the biosurfactant were identified by FT-IR analysis. Some of these groups include C–H, O–H, C = O, and C–O, C = C bonds for alkenes, O–H bond, C = O bond due to ester or ether group, and N–H bond, and C–N, N = O, and NO_2 bonds were also present in some biosurfactants. All these bonds confirm the lipid nature of biosurfactants [141]. The obtained biosurfactants were effective in color decomposition and also inhibited the formation of biofilm by *Vibrio cholerae* and *Salmonella typhi*. The highest percentage of biofilm formation inhibition (99.5% for *V. cholerae* and 99.8% for *S. typhi*) was observed at a biosurfactant concentration of 125 μg/mL (Fig. 1.12).

Ohadi et al. [136] studied the production of a biosurfactant from *Acinetobacter junii (AjL)* and its potential inhibitory activity against bacterial pathogens. They reported the MIC of *AjL* against three gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus*) and four gram-negative bacteria (*Pseudomonas aeruginosa, Klebsiella neumonia, Escherichia coli, Salmonella typhi*) as well as two fungi species (*Candida albicans, C. utilis*). The results indicated that AjL has effective antibacterial activity at concentrations approximately below the CMC. Regarding the antifungal properties of *AjL*, it was found that the obtained MIC values were lower

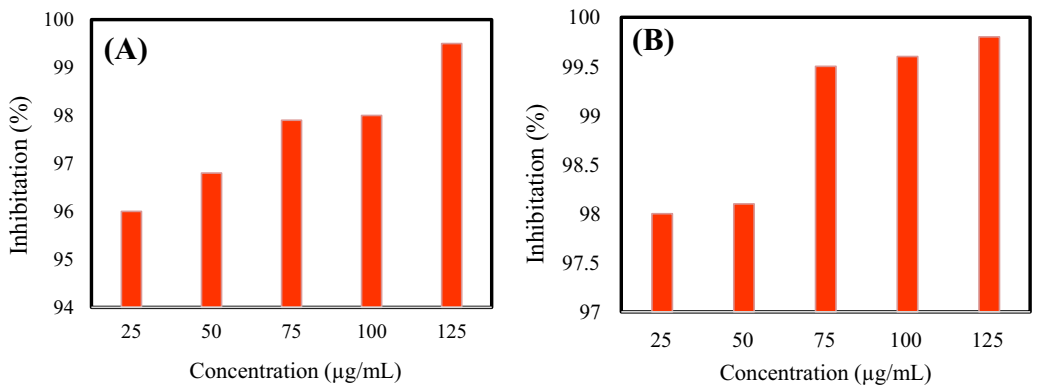

FIGURE 1.12

Antibiofilm activity against opportunistic pathogenic bacteria (A) *Vibrio cholera* and (B) *Salmonella typhi*. Values are of triplicate experiemnt and expressed as mean ± S.D.

Modified after S Kayanadath, VK Nathan, P Ammini. Anti-biofilm activity of biosurfactant derived from Halomonas sp., a lipolytic marine bacterium from the bay of bengal. Microbiology. 2019;88(5):585–599.

than those of standard antifungals and showed almost 100% inhibition against both species. The results obtained from biofilm formation showed that AjL disrupted the protein biofilm of *Mirabilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* at 1250 μg/mL and the concentration of 2500 μg/mL The results of *AjL* antimicrobial activity are given in Table 1.10. Researches indicated that the percentage of mortality in both bacteria and fungi depends on the dose of biosurfactant, which can be observed in Figs. 1.13 and 1.14.

Ohadi et al. [136] discussed that the reason for this type of response may be due to the interaction of *AjL* with membrane structures and stated that the amphiphilic properties of biosurfactants alter the interaction with phospholipids, which in turn alters the permeability of the cytoplasmic membrane. As a result, due to the formation of pores in the cell wall, its contents leak out and cause the death of the bacterial cell. Fig. 1.15 shows the antibiofilm activity of three species. The results obtained from antibiofilm activity indicated that, with the *AjL* at 1250 μg/mL, the formed biofilm of *S. aureus, P. mirabilis*, and *P. aeruginosa* were disrupted up to 35%, 10%, and 32%, respectively, and at concentration of 2500 μg/mL, they reached to 52%, 31%, and 70%, respectively. Promising results have been reported, but the exact mechanism involved in antibiofilm activity is not yet known. The high diversity of lipopeptide producing microorganisms and difference in chemical structure suggest that the lipopeptide compounds may serve different, and possibly multiple, purposes.

1.3.3 Trehalose lipid biosurfactant with phospholipid

There are some interactions between *succinoyl* bacterial trehalose lipid biosurfactant and phospholipid vesicles, which are studied with some techniques such as calorimetric, fluorescence and absorption spectroscopical. Measuring the surface tension of trehalose lipid led to the amount of CMC, which was 300 μm. Here membrane incorporation is preferred over micellization, because

Table 1.10 Antifungal and antibacterial activities of the *Aj*L compared to the standard antimicrobial drugs.

	*Aj*L	Ciprofloxacin	Fluconazole
Compound Microorganisms	**MIC (μg/mL)**		
Candida utilis	5.0	–	8.0
Candida albicans	5.0	–	8.0
Salmonella typhi	5.0	2.0	–
Escherichia coli	5.0	2.0	–
Klebsiella pneumoniae	5.0	2.0	–
Pseudomonas aeruginosa	5.0	2.7	–
Micrococcus luteus	5.0	1.3	–
Bacillus subtilis	5.0	4.0	–
Staphylococcus aureus	5.0	2.0	–

Modified after M Ohadi, H Forootanfar, G Dehghannoudeh, T Eslaminejad, A Ameri, M Shakibaie, et al. Antimicrobial, antibiofilm, and antiproliferative activities of lipopeptide biosurfactant produced by Acinetobacter junii *B6. Microb. pathogenesis. 2020; 138:103806.*

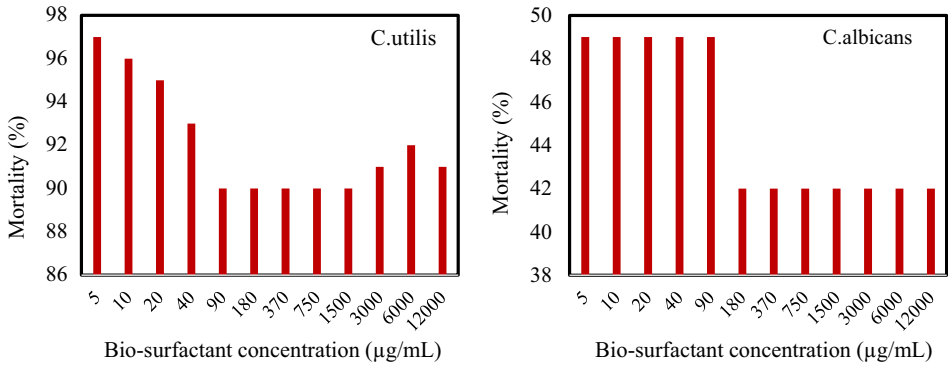

FIGURE 1.13

Antifungal activity of the *Aj*L at different concentration.

Modified after KV Sajna, RK Sukumaran, H Jayamurthy, KK Reddy, S Kanjilal, RB Prasad, et al. Studies on biosurfactants from Pseudozyma sp. NII 08165 and their potential application as laundry detergent additives. Biochem. Eng. J. 2013; 78:85–92.

trehalose lipid shows manners like a weak detergent. If trehalose lipid merges into phosphatidylcholine membranes and sets apart, there will be the leakage of small solutes. These consequences generally show the biological actions related to the membrane, of this trehalose lipid biosurfactant [8,146–153]. The trehalose biosurfactants, which consist of glycolipid have interesting physicochemical and biological properties. For inducing the distinctness of leukemia cell lines and to prevent kinase activity *succinoyl* trehalose lipid is used. The perturbing actions on phospholipid bilayer mainly cause all these biological activities of trehalose lipid surfactants. The trehalose lipid would apply its membrane-perturbing actions by a mechanism that is defined by encountering large differences as a function of the lipid structure of the membrane, which is the goal [154]. The trehalose lipid is having a hydrophobic tail consisting of three fatty acids and a polar head group. There is a group of carboxyl in the *succinoyl* chain that might be either neutral or negative, which depends on the pH. For determining the thermodynamics of trehalose lipid and POPC (1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) membranes bond, an ITC (Isothermal Titration Calorimetry) experiment is run in which at a concentration below CMC a lipid dispersion was added to a biosurfactant solution by titration. In these experiments, the biosurfactant would partition into the membrane. A plot of δhi/δnL against the concentration of lipid is shown in Fig. 1.16, where δhi is the heats of reaction that can be determined by integration of the peaks in titration and δnL is the amount of lipid injected. Fluorescent probes entrapped into POPC LUVs monitor the outflow of aqueous vesicle quantity to the external medium. The time during, which CF (5(6)-Carboxyfluorescein) outflows in POPC LUVs (Large unilamellar vesicles) at varied trehalose lipid concentrations is shown in Fig. 1.17.

There is a lag of time in the leakage process, this lag period does not depend on the trehalose lipid concentration. But after this period both the concentration of glycolipid and the rate of leakage increase and this leakage depends on the trehalose concentration. The results showed that there would be a decrease in turbidity when biosurfactant is added. That might be because of membrane solubilization [154]. The leakage reaches its maximum amount at a concentration of about 200 μm

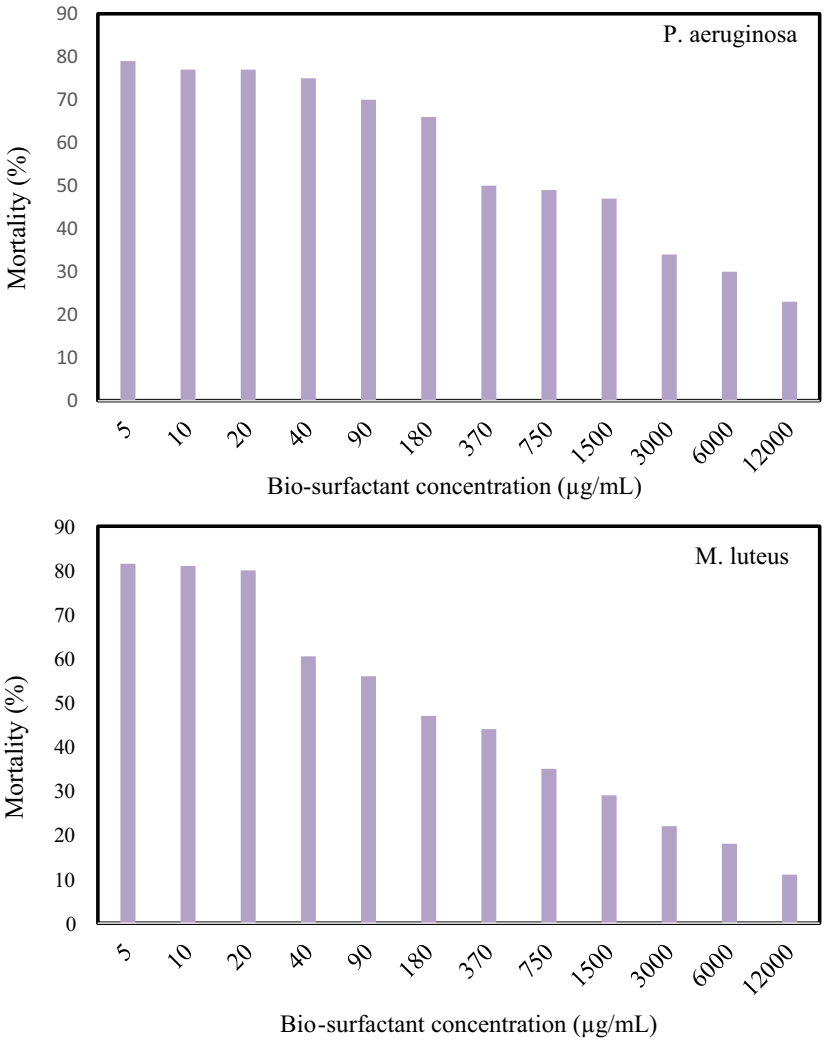

FIGURE 1.14

Antibacterial activities of the *Aj*L at different concentration on the Gram-negative bacteria (*P. aeruginosa*) and Gram-positive bacteria (*M. luteus*).

Modified after KV Sajna, RK Sukumaran, H Jayamurthy, KK Reddy, S Kanjilal, RB Prasad, et al. Studies on biosurfactants from Pseudozyma sp. NII 08165 and their potential application as laundry detergent additives. Biochem. Eng. J. 2013; 78:85–92.

of trehalose lipid, which is near to the value of CMC. Membrane formation affects CF leakage rate. Cholesterol and POPE (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine) in the membrane would cause a reduction in the initial rate of leakage (Fig. 1.18).

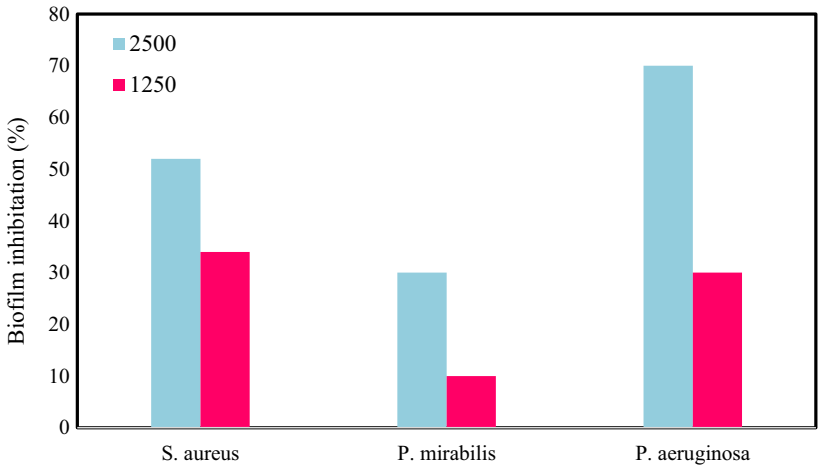

FIGURE 1.15

Antibiofilm activity of the *Aj*L on the three biofilm producers; *S. aureus, P. mirabilis*, and *P. aeruginosa*.

Modified after KV Sajna, RK Sukumaran, H Jayamurthy, KK Reddy, S Kanjilal, RB Prasad, et al. Studies on biosurfactants from Pseudozyma sp. NII 08165 and their potential application as laundry detergent additives. Biochem. Eng. J. 2013; 78:85–92.

As it is found out from the previous section the aim of this work [154] is to figure out the interaction between bacterial trehalose lipid biosurfactant and phospholipid membranes, which causes permeabilization in the membrane. POPC has a role that presents membrane vesicles so that the results showed some new biological membrane actions of the glycolipid. The authors mentioned that trehalose lipid acts like a weak detergent that prefers to be inserted in membranes over micellization. For producing membrane disintegration, the detergent/lipid ratio must be more than 1, which shows trehalose lipid motivated the revealing of low molecular weight solutes. Like CF the existence of an initial lag period, as mentioned before, suggested that some steps were needed for acceptable interpolation of the glycolipid into the aim bilayer. For addressing these flurescentprobes, which are reactive to trehalose lipid, are used namely, FD4 and FPE. There are two stages before leakage, the first one is adsorption of trehalose to the membrane, and the second one is flip flop to the monolayer. The result shows that the first stage is fast and the other is slow. The rate of the flip flop of trehalose lipid in comparison with free fatty acids was slow but when it was compared to phospholipid flip flop it was fast [154]. It can be understood that trehalose lipid merged into POPC membranes within domains that are consist of pure glycolipid. It is shown by differential scanning calorimetry. These domains have a defecting role in the lipid membrane. It was figured out that POPE and cholesterol lowered the leakage; however, IysoPC (1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine) caused an enhance.

Cholesterol has some effects, the inclusion of 40% or 50% cholesterol in phosphatidylcholine membranes scrapped the passive membrane leading to CF. It also has a protecting effect against trehalose lipid motivated membrane permeation, which would be the consequence of the growth in the motional order of phospholipid acyl chains. It is considered that bacterial membranes do not have any cholesterol while plasma membranes of eukaryotic cells maximum containing are 50%;

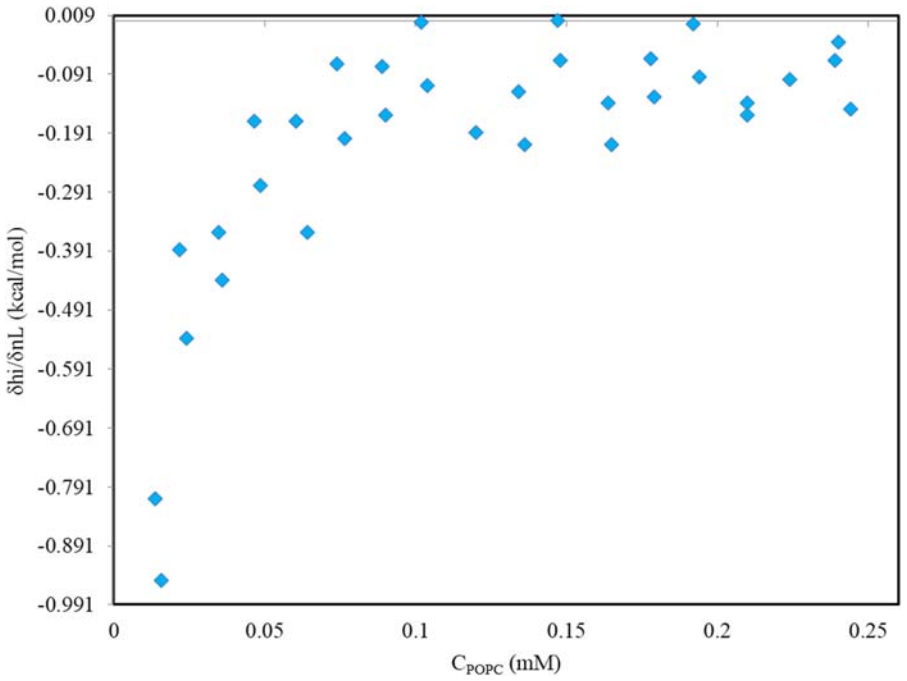

FIGURE 1.16

ITC definition of the division of trehalose lipid into POPC vesicles. δhi/δnL is the heats of injection per mole of the injected lipid, which is plotted against the total concentration of lipid. This test was done at 25ǫC.

Modified after A Zaragoza, FJ Aranda, MJ Espuny, JA Teruel, A Marqués, A Manresa, et al. Mechanism of membrane permeabilization by a bacterial trehalose lipid biosurfactant produced by Rhodococcus sp. Langmuir. 2009;25(14):7892–7898.

therefore, it is said that the effects of cholesterol would be the cause of particular interest. This result helped to find out the application of surfactants in preventing sexual disease. In total, there would be a common impact on the physical characteristics of the POPC bilayer by effects of POPE and cholesterol [154]. Trehalose lipid is a weak surfactant and it has the capacity as lipid membrane and the trehalose lipid CMC was dependent on pH.

1.3.4 Lipopeptide

The production of microbial assisted biosurfactant lipopeptide, which has pharmaceutical applications is discussed in this section. Lipopeptides are an amazing group of biosurfactant, which have great surface properties. The groups of these molecules would merge with other substances which are going to produce significant materials. Here the isolation of an anionic lipopeptide, which produces bacterium is investigated while it is going to have some pharmaceutical applications [146]. There are too many numbers of isolated *Bacillus* utilizing oil expansion technique, blood agar hemolysis, and pH sensitivity, the *B. mojavensis* can produce a large amount of lipopeptide

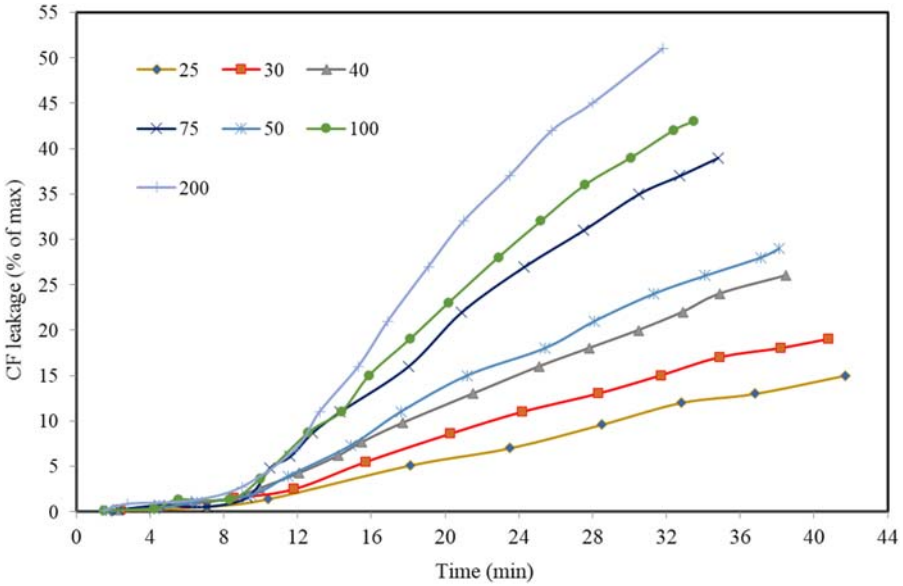

FIGURE 1.17

The CF leakage in POPC LUVs is plotted against time at different concentrations of trehalose.

Modified after A Zaragoza, FJ Aranda, MJ Espuny, JA Teruel, A Marqués, A Manresa, et al. Mechanism of membrane permeabilization by a bacterial trehalose lipid biosurfactant produced by Rhodococcus sp. Langmuir. 2009;25(14):7892–7898.

biosurfactant. By the metal salt precipitation method, the extraction of anionic lipopeptides complex was performed and their properties would be explored by TLC, FTIR. Some analyses for detecting the lipopeptides gained from the most anionic fraction were applied that prove the presence of the surfactin isoform in the released substance. These lipopeptides had the ability to reduce surface tension, the lack of toxicity, and antibacterial activity. In addition, it is considered that it is obvious they can be a natural product with applications in the medicinal industry [147]. Lipopeptides have one or more fatty acid chains, which are connected to a peptidyl group. There are so many kinds of lipid chains that would lead to different types of isoforms. An anionic lipopeptide, produces microorganisms having antibacterial activity and is used in drug delivery, and has an effect on the advance of water-soluble drugs bioavailability. For producing lipopeptides the *Bacillus* strains are so important. In the first step of screening, the spore of bacteria starts to form. When the culture on a medium contains glycerol that has a role of carbon source there would be the maximum production of the anionic lipopeptide. Also, when the media includes formate and sodium citrate the colonies made a concentrical zone on the petri dish. The analysis of this via TLC proved the presence of amino acids in the complex. This sampling took place in winter and the samples preserved in a sealed pocket were brought into contact with seasonal temperature, which changes every 3 years [146]. The connection between cells and revealed lipopeptides was shown by the growth curve and the amount of anionic extracellular metabolite chart (Fig. 1.19). As it is obvious from Fig. 1.19, in the first 20 hours there is a rise in population with a minimum

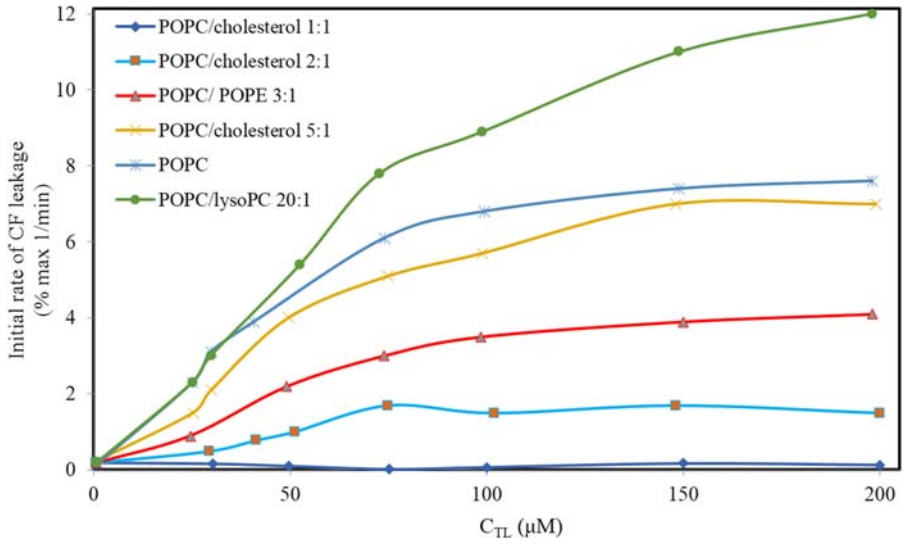

FIGURE 1.18

The initial rate of trehalose lipid-induced CF leakage from LUVs is effected by membrane lipid composition. This rate of CF leakage is plotted against different concentrations of trehalose lipid (CTL).

Modified after A Zaragoza, FJ Aranda, MJ Espuny, JA Teruel, A Marqués, A Manresa, et al. Mechanism of membrane permeabilization by a bacterial trehalose lipid biosurfactant produced by Rhodococcus sp. Langmuir. 2009;25(14):7892–7898.

amount of lipopeptides. The production of lipopeptides increases suddenly in the next 50 hours. For investigating the surface characteristics of the released lipopeptides, FTIR analysis is used. The results of this analysis show peaks of NH and CH at 2600−2950 cm^{-1}. At the surface of the lipopeptide is a carboxylic acid group, which can be proved by four regions that are shown by spectrum (Fig. 1.20).

It can be expressed that the extract did not prevent the growth of *E. coli*. It was shown that the viability of treated PBMC (peripheral blood mononuclear cells) increased but PC12 (a cell line derived from a pheochromocytoma of the rat adrenal medulla) did not change compared to negative control [148]. There were two strong bands on the released sample, which were purified including 0.2 and 0.5 M NaCl, which was shown by monitoring of the peptide [146]. Chromatography displayed the presence of Glycin, Glutamic acid, and Aspartic acid in all extracted samples as amino acids (Table 1.11).

It can be shown with mass spectra that there were three various clusters of peaks at m/z values between 800 and 940, between 1030 and 1153, and between 1290 and 1350. These three cluster peaks are assigned to an isoform of kurstakins, surfactins, and fengycins. For isolating the bacterium possesses significant lipopeptides with the screening method. *B. mojavensis* strain was chosen from so many *Bacillus* species [149]. A cyclic lipopeptide surfactin was found that cultivated on nutrient agar medium. This surfactant has an antifungal activity, which is suggested to be used to control biological agents. Three lipopeptides were produced, which were cultivated on a medium consisting of sucrose as a carbon source [155]. Some of the biosurfactant and anionic lipopeptides

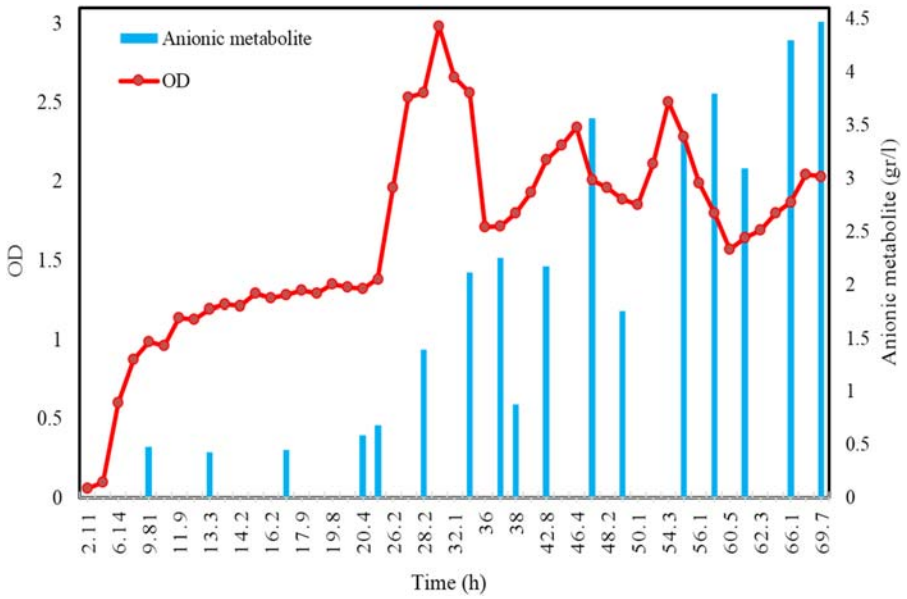

FIGURE 1.19

The curve of growth and the production of the isolated strain which has relations between releasing lipopeptides and cells.

Modified after M Fanaei, G Emtiazi. Microbial assisted (Bacillus mojavensis) production of biosurfactant lipopeptide with potential pharmaceutical applications and its characterization by MALDI-TOF-MS analysis. J. Mol. Liq. 2018; 268:707–714.

can be produced by *B. mojavensis*, which proves that the types of carbon source have an important effect on the production of biosurfactant [156–159]. It was mentioned that the *B. mojavensis*' extracellular strain 32 A was in a medium containing glutamic acid as carbon source, which can motivate the accumulation of particles. In this study [146] the *B. mojavensis* strain was in a medium that its carbon source was glycerol. Three clusters of peaks gained from chromatography were displayed by MOLDI-TOF analysis (Matrix-assisted laser desorption ionization time-of-flight tandem mass spectrometry). The production of surfactin and fengycin isomers was shown by these results but another substance was found that could be related to the isoforms of kurstakin lipopeptides. It was shown from the analysis of amino acids that anionic amino acids caused the rising of the percentage of amino acids. Applicable materials are made when these groups interface with other components. This lipopeptide is used for pharmaceutical and medical applications because of its surface tension-reducing ability, lack of toxicity, and antibacterial activity [146,160–164].

There is a glycolipid biosurfactant produced by marine yeast, which is purified and characterized based on different spectral analyses. This biosurfactant's name is Cybersan. There have been some researches on marine microbes because of their products, which might relate to discovering drugs [160]. After that Cybersan presented a surface tension at critical micellar concentration and had a stable state at constant pH and temperature. Some biosurfactants are produced from bacteria whereas some of them are produced from different terrestrial yeasts. Recently marine yeasts have

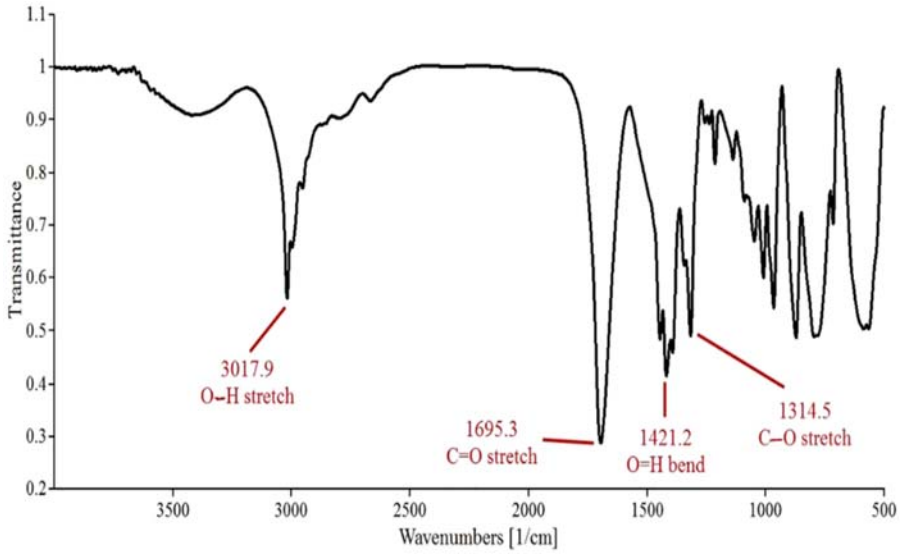

FIGURE 1.20

The FTIR spectrum of the extracted lipopeptide is shown to know much about the structural characteristics and also about the existence of carboxylic group.

*Reprinted with permission from M Fanaei, G Emtiazi. Microbial assisted (*Bacillus mojavensis*) production of biosurfactant lipopeptide with potential pharmaceutical applications and its characterization by MALDI-TOF-MS analysis. J. Mol. Liq. 2018; 268:707–714.*

Table 1.11 The fractions from anion exchange chromatography and percentage of amino acids in the sample.

Amino acid	Fraction 0.2 M (%)	Fraction 0.5 M (%)	Whole extracted sample (%)
Asp	14.30	11.90	10.44
Glu	9.30	9.39	16.05
Ser	6.14	5.20	4.5
Gly	20.11	22.30	14.4
Thr	5.14	3.20	2.5
Arg	10.64	6.77	1.6
Ala	10.48	14.22	7.9
Tyr	1.80	2.32	1.03
Val	2.52	2.45	5.7
Phe	–	–	0.86
Ile	2.71	3.56	2.56
Lys	8.33	6.99	4.9
Leu	6.00	8.93	23.64

*Modified after M Fanaei, G Emtiazi. Microbial assisted (*Bacillus mojavensis*) production of biosurfactant lipopeptide with potential pharmaceutical applications and its characterization by MALDI-TOF-MS analysis. J. Mol. Liq. 2018; 268:707–714.*

been useful to produce a wide range of biomolecules, which have possible applications in the food and pharmaceutical industries [162]. More researches on this marine yeast showed some of its interesting features like the barotolerance, thermos ability, new high osmotic tolerance, and new chemical diversity. For producing marine yeasts from hydrocarbon polluted sea precipitate samples, Balan et al. [160] have paid attention to isolation for potential nonhemolytic biosurfactant which produce marine yeast from hydrocarbon. During the isolation process a SWYM broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1% glucose, prepared using 100 mL natural sea water at 34 ppt and the final medium adjusted to pH 5.5 with 1 M HCl), consists of chloramphenicol for bacterial inhibition, is needed for the collected sea sediment samples to be enriched in. This SWYM broth and its compositions would selectively enhance the growth of marine yeasts. After all these the SWYM broth was enriched and then the marine yeast world be isolated from every sample and purified. The isolated marine yeast culture individually cultured in a broth consists of 1% crude oil as a carbon source [163]. This carbon source helps marine yeast to grow selectively. Marine biosurfactant producers have too many kinds of chemical and functional characteristics, using a one-single method for isolating is hard so that several screening methodologies are needed for isolating. In this study [160] three methods were presented for the necessary properties of a biosurfactant and its application in therapeutics. For knowing the interfacial and surface activity of a biosurfactant an oil displacement test was performed. The significance of biosurfactant in various industrial applications is due to the biosurfactant adsorption at various interfaces, which results in a wide range of surface tension amounts. These two features of biosurfactant namely, emulsification and surface activity, are separate and this may happen that some biosurfactants, which represent a great reduction in surface tension could not exhibit emulsification activities. The molecular identification, which uses ITS (internal transcribed spacer) revealed the most exact procedure for various levels of delineation among fungal diversity [165]. There is a medium consisting of glucose mineral salts, which was constructed in natural sea water, in which the marine yeast *C. saturnus* strain SBPN-27 was cultured. This was prepared for assessing the growth kinetics profile of the biosurfactant as a function of time. As it was mentioned before for observing the production of surfactant in the broth, an emulsification index was used. This released the beginning of secretion from a logarithmic growth phase and the peak was at the late logarithm phase (Fig. 1.21).

As observed in Fig. 1.21 the biosurfactant predominantly was produced during the exponential phase. Some variations were observed between biosurfactant activity and yield, which caused the revealing of cell debris from tested yeast [160]. After 72 hours of fermentation, the biosurfactant was released by dichloromethane and purified with reverse-phase silica gel column chromatography. The characterization of refined biosurfactant by TLC (Thin-Layer Chromatography) proved that it contained sugar and lipid components. The refined biosurfactant was acid hydrolyzed that released only a single peak at the retention time as shown in Fig. 1.22A.

According to the NIST webbook mass spectrum, the hydrophobic part of biosurfactant consists of 3-hydroxyheptadecanoic acid with a molecular mass of 286.4, and the 3-hydroxyheptadecanoic acid methyl ester is with 300.4 molecular mass based on the methylated fatty acid analysis method as shown in Fig. 1.21B. These results confirmed that galactose is the hydrophilic part of the refined biosurfactant. The performance of a biosurfactant depends on its surface activity at various physiochemical states, which affects its applications [163–166]. Cybersan decreased the surface tension of water and this makes the CMC to be about 30 mg/L, but increasing the CMC of Cybersan had no effect on the surface tension. With pH changing from 3 to 9, there was not any reduction in

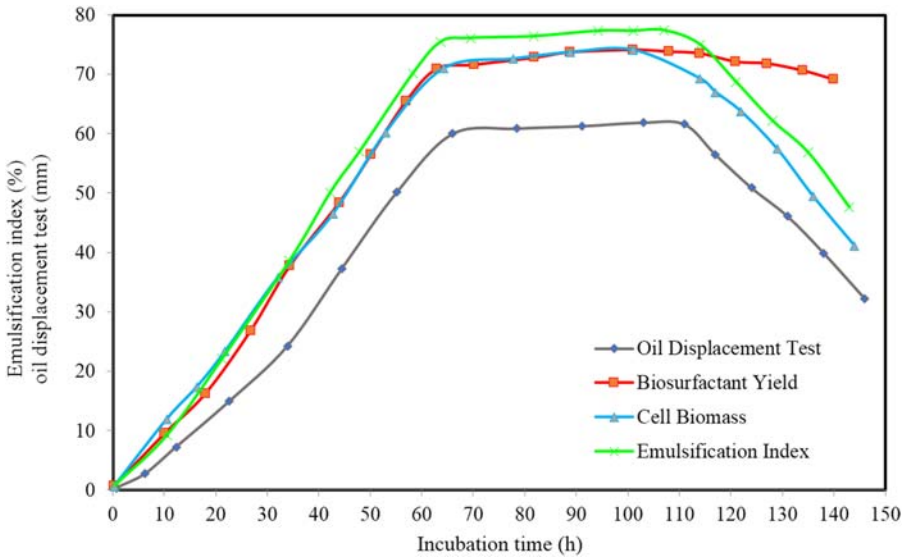

FIGURE 1.21

The growth curve of marine yeast as a function of time. The medium of this experiment was GMS at the 30o̲C. This was monitored up to 6 days.

Modified after SS Balan, CG Kumar, S Jayalakshmi. Physicochemical, structural and biological evaluation of Cybersan (trigalactomargarate), a new glycolipid biosurfactant produced by a marine yeast, Cyberlindnera saturnus *strain SBPN-27. Process. Biochem. 2019; 80:171–180.*

surface tension of water but there were considerable changes in surface activity at pH of 2 to 10 and regained its activity while the conditions back into the stable states. Cybersan would have a loss of surface activity and is denatured above pH 10. These results were the same for glycolipid biosurfactant produced by a marine *S. saprophyticus* strain SBPS-15, which showed the surface activity is steady up to 90°C and would have a loss at 100°C and is denatured completely above 110°C [160].

The results proved that Cybarsan presented physicochemical stability, which caused its application in making stable emulsions at defined conditions and in many products formulations. Cybarsan also showed antimicrobial activity, in which the percentage of growth inhibition depends on the amount of concentration used (Table 1.12).

When the glycolipid biosurfactant is isolated from the marine *S. saprophyticus* strain SBPS-15, it shows antimicrobial activity against many kinds of bacteria. There was a type of biosurfactant that prohibits microbial growth against different corrosive bacterial strains [167]. At the screening level of Cybersan, in which the nonhemolytic activity was observed, using 3T3 fibroblast cell culture the cytotoxicity was evaluated and showed no significant toxicity. 200 μg/mL Cybersan concentration released 96.8% ± 0.7% cell viability and it also displayed complete growth inhibition of various clinical bacteria isolated. As the concentration increased the cell viability decreased. In addition, some other biosurfactants like rhamnolipid and sophorolipidand surfactin released higher

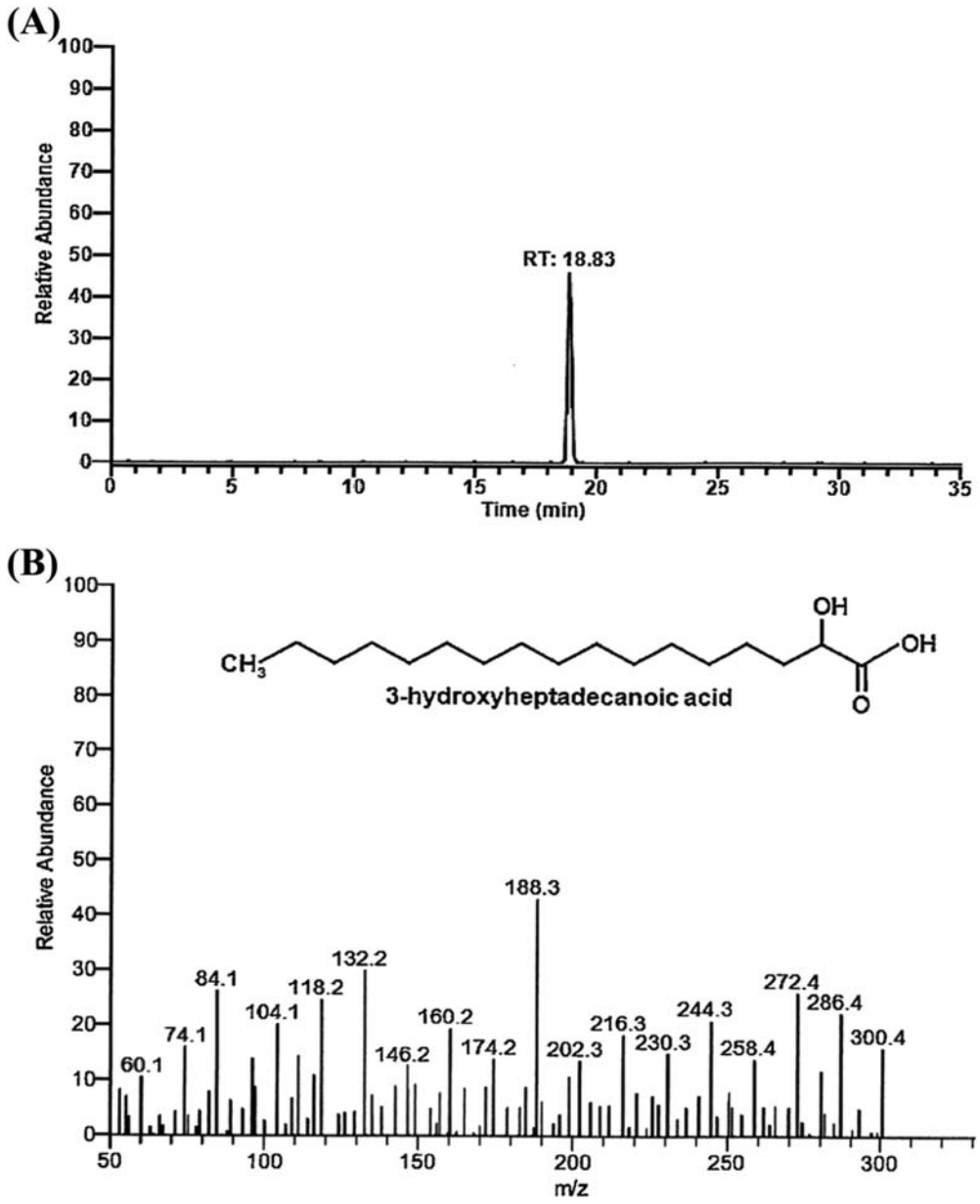

FIGURE 1.22

(A) Gas chromatography of the solvent which release fatty acid peak at the time 18.83. (B) The mass spectral of that peak was analyzed and was known as 3-hydroxyheptadecanoic acid.

Reprinted with permission from SS Balan, CG Kumar, S Jayalakshmi. Physicochemical, structural and biological evaluation of Cybersan (trigalactomargarate), a new glycolipid biosurfactant produced by a marine yeast, Cyberlindnera saturnus *strain SBPN-27. Process. Biochem. 2019; 80:171–180.*

Table 1.12 Microbial growth prevention of Cybersan at different concentrations of biosurfactant.

Test organisms	Biosurfactant concentration (μg/mL)					
	6.25	12.5	25	50	100	200
Staphylococcus aureus	3 ± 0.1	15 ± 0.2	29 ± 0.2	43 ± 0.3	55 ± 0.2	69 ± 0.1
Bacillus cereus	31 ± 0.2	46 ± 0.2	63 ± 0.3	77 ± 0.2	91 ± 0.3	100−0
Bacillus subtilis	27 ± 0.1	42 ± 0.2	57 ± 0.3	73 ± 0.3	87 ± 0.2	100−2
Streptococcus pneumoniae	−	5 ± 0.1	14 ± 0.1	27 ± 0.3	38 ± 0.3	49 ± 0.2
Proteus mirabilis	−	2 ± 0.1	9 ± 0.1	18 ± 0.2	29 ± 0.3	37 ± 0.3
Vibrio cholerae	15 ± 0.1	29 ± 0.2	42 ± 0.1	53 ± 0.3	65 ± 0.2	77 ± 0.2
Vibrio parahemolyticus	27 ± 0.2	46 ± 0.1	68 ± 0.3	80 ± 0.2	91 ± 0.1	100 + 0
Klebsiella oxytoca	14 ± 0.2	29 ± 0.3	41 ± 0.1	55 ± 0.2	69 ± 0.3	86 ± 0.2
Klebsiella pneumoniae	32 ± 0.2	47 ± 0.1	69 ± 0.2	82 ± 0.3	93 ± 0.2	100−0
Salmonella paratyphi	11 ± 0.2	21 ± 0.2	35 ± 0.3	48 ± 0.3	62 ± 0.3	79 ± 0.3
Salmonella typhi	−	−	5 ± 0.2	14 ± 0.2	26 ± 0.2	36 ± 0.3
Escherichia coli	35 ± 0.2	49 ± 0.2	65 ± 0.3	79 ± 0.2	92 ± 0.1	100−0

The values are reported as mean ± standard deviations.
Modified after SS Balan, CG Kumar, S Jayalakshmi. Physicochemical, structural and biological evaluation of Cybersan (trigalactomargarate), a new glycolipid biosurfactant produced by a marine yeast, Cyberlindnera saturnus *strain SBPN-27. Process. Biochem. 2019; 80:171−180.*

cytotoxicity against mammalian cells [166]. An exact biological evaluation happens because of the toxicity of an antimicrobial agent against mammalian cells, this shows that for the production of safe biomedical drugs there would be some adverse effects.

1.3.5 Acetylated acidic sophorolipid

One of the greatest alternatives to classical compounds based on petrol is microbial production of biosurfactant due to their high biodegradability, low toxicity, and also because of having biological processes. The small number of chemical structures available and the low productivities are the main problems, which limit the application of biosurfactants so that microbial biosurfactants offer opportunities to widen the available molecules. They also have direct synthesis, which is a better option [168−170]. For production and purification of acetylated acidic sophorolipids (aSL-COOH) a one-step biological process is performed by utilizing a strain in the lactone esterase gene. The biosynthesis of sophorolipid has the main part, which happens in the cytoplasmic environment consequently that this is an intracellular action. In the plasma mono-, non, diacetylatedsophorolipids are produced [171]. For the production of kg scale of acidic sophorolipids with a high degree of

acetylation, a method was prepared using the *Starmerellabombicola lactone esteraseknockout* strain (Δsble). To characterize this, multireaction monitoring was used to assign the expression levels of the SL cluster proteins under different situations. By studying another strain, *S. bombicolawild* type (WT), and comparing it with Δsble strain, it was found out that this new strain can be used to produce a large scale of acetylated acidic sophorolipids [172,173]. As we know the biosurfactants are biodegradable and have low toxicity and also are used as antimicrobial agents and also anticancer. The acetylated acidic of sophorolipids are assumed poorly toxic. The results proved that aSL-COOH was nontoxic for the aquatic environment. The antibiofilm and antimicrobial properties of aSL-COOH were evaluated on three bacterial strains, one Gram-negative *P. aeruginosa* and, two Gram-positive *E. faecalis* and *S. epidermidis*. With these strains, the growth had an increase in shaking conditions, and biofilm was better in static cultures [172]. Evidence proved that lactonic and acidic forms of sophorolipids have different antagonistic biological activities when they are merged with unpurified preparations [169].

An interesting observation from the tests was that the effect of the one Gram-negative strain was not as obvious as it was for the two Gram-positive ones so that the results showed a delay in biofilm and growth formation rather than an inhibition. The sophorolipids are a kind of biological biosurfactants, which can be used as household detergents and for that might have great applications in the human's future life. Fermentation caused 90% of the impact from the production phase and that is because of the utilization of reproducible resources [171]. In another analysis compared the acidic sophorolipids with industrial products like biobased soaps, fatty alcohol sulfate, esterquat, fatty alcohol, and alkyl polyglucosides. These biosurfactants are produced chemically so that they are called the first generation. They are produced by renewable recourses. By the results, the acidic sophorolipids are similar to the reference products. In terms of damage to ecosystems, the damage caused by sophorolipids is due to the production of glucose, rapeseed oil, and much use of water. In addition, the most of impact on recourses is caused by electricity and glucose production; however, soap and esterquats have less impact on them. The application of glycolipidic biosurfactants, which are made from fermentation, is limited due to their missing purity for dedicated applications and also the lack of structural control. Because of the SL-mediated antibacterial action, this has some advantages in the field of biosurfactants therapeutics as antimicrobial application. Acetylated acidic sophorolipid biosurfactants is advantageous for both gram-positive and negative bacterial, however, it was more prominent for the gram-positive of them.

1.4 Conclusions

Biosurfactants are one of the most useful chemical groups that have significant functional properties and structural diversity, making them an attractive compound to apply in a wide change of biotechnological, environmental, and industrial applications. These features have significantly developed the biosurfactants applications in the food, oil, and especially in pharmaceutical industries for the past ten decades. Biosurfactants are amphipathic surface-active substances that show the range of a physiological function. The market of biosurfactants is inexpensive and manufacturers will have to develop biosurfactant production in cost-effective and eco-friendly manner. Increasing attention in biosurfactants was conducted to an intense study for cost-efficient and environment-friendly biosurfactant production. Biosurfactants are organized according to chemical

structure and microbial basis and they divide into two classes: high molecular weight and low molecular weight. In this chapter, the applications of protein, polysaccharide, and lipoprotein as high molecular weight and glycolipid, cyclic and acyclic lipopeptide, trehalose lipid, lipopeptide, and acetylated acidic sophorolipid as low molecular weight in pharmaceutical industries were investigated. The results of investigations indicated resented of biosurfactant PS as protein in culture media is neutral to the tested bacteria growth and high concentrations of biosurfactant PS (greater than CMC) leads to toxic conditions for bacterial strains. The content of novel poly (methyl methacrylate) is increased when the amount of added polysaccharide biosurfactant is increased. These nontoxic core (nPMMA)−shell (polysaccharide) system decreased the time of drug delivery to 10 hours. Lipoprotein as biosurfactant has a significant effect on the treatment of many diseases like atherosclerosis, muscle cell, Alzheimer and cancer. Dysfunctions and variations in lipoprotein biology are accountable for degenerative and chronic diseases that are afflicting the world today. In the case of low-molecular weight, a glycolipid that has shown good antibacterial, antibiofilm, and antiadhesive properties and is a strong candidate for replacement with common antibiotics due to their natural environmentally friendly nature. Lipopeptides are produced by different groups of bacteria. The mechanism of action of lipopeptides to kill bacteria is the formation of ion transport channels, which is due to the washing properties of lipopeptides. Trehalose lipid is a weak surfactant that prefers membrane insertion over micellization and the capacity as lipid membrane is significant for some usages like the drugs external administration and biologically active substances. Lipopeptide is a fascinating group of biosurfactants that possess significant surface features that it has antibacterial activity and produces from *Bacilli* by oil-spreading method. Lipopeptide can reduce surface tension very well and it is one of a candidate for pharmaceutical and medical applications. Acetylated acidic sophorolipid biosurfactants obtained using fermentation offer a well-meaning alternative to petrochemical surfactants that are dependent on finite fossil resources and it is useful for both gram-negative and positive bacterial strains; however, it was more prominent for the gram-positive of them.

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CHAPTER

Application of biosurfactant as an adjuvant in medicine

2

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2.1 Introduction

The diverse amphiphilic molecules possessing distinct chemical structures and those expressed by microorganisms are referred to as biosurfactants/biomimetic surfactants/natural surfactants. Biosurfactants are the secondary metabolites of their source microorganism and play an important role in their survival by enabling transport of nutrients, meddling with quorum sensing mechanisms or host-guest interactions and, or deterring and controlling the effect of harmful microorganisms by biological means [1]. Biosurfactants are usually water soluble with a low critical micellar concentration 10^{-5} M that remains unchanged even upon varying salt type and concentration. On account of their source, minimal toxicity, biodegradability, mild production conditions, and environmentally friendly nature [1] biosurfactants have attracted applications not only in the medical field (Fig. 2.1)

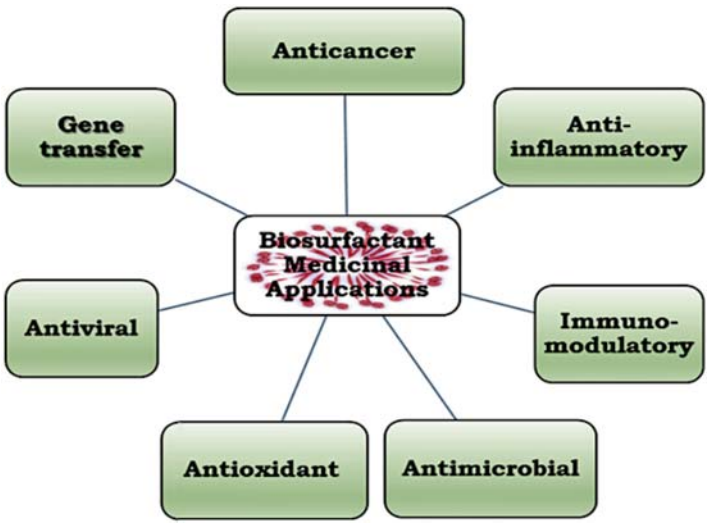

FIGURE 2.1

Applications of biosurfactant in medicine.

Green Sustainable Process for Chemical and Environmental Engineering and Science. DOI: https://doi.org/10.1016/B978-0-323-85146-6.00018-8

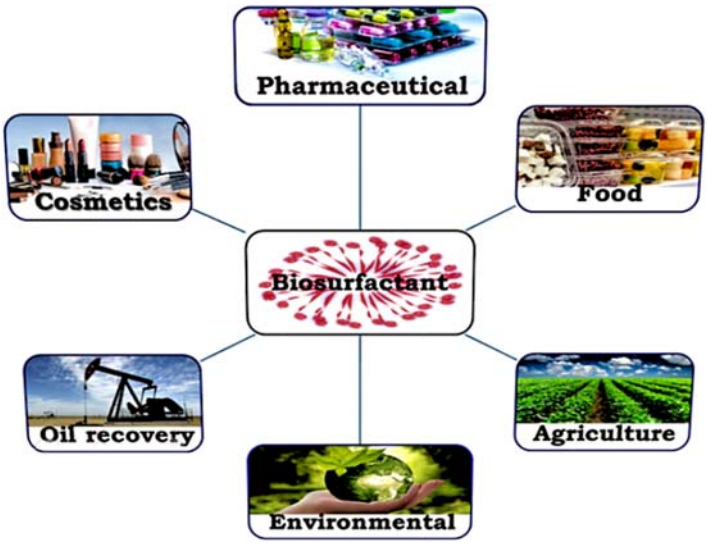

FIGURE 2.2

General applications of biosurfactants.

but also in food, cosmetics, oil-recovery, energy-saving technology, and environmental remediation [2,3] (Fig. 2.2).

Biosurfactants are typically categorized as low (glycolipids and lipopeptides) and high molecular weight (proteins, polysaccharides, lipoproteins) entities. Due to their simple structures, the former class of biosurfactants has impressive surface-active properties. Rhamnolipids (glycolipid) and surfactin (lipopeptide) are a few of the widely studied biosurfactants, with the addition of some newly reported ones like nucleolipids and full peptides. Their hydrophobic component comprises either an alkyl chain or amino acid sequence. Whereas the hydrophilic component varies and can be a lipid head, short peptide, DNA strand, or sugar ring. Thus the chemical nature of the hydrophilic head, type, and size governs the surface activity, physical and biological properties.

Besides their encouraging anticancer and antimicrobial properties, biosurfactants display antiadhesive character against pathogens and also constitute a critical component of microemulsion-based drug delivery systems. Biosurfactants can easily penetrate the cell membranes of cancer cells leading to their lysis and cell death [4]. These natural surfactants can destabilize the adhesion of pathogens on surfaces due to their biphasic partition at the interfaces. Novel therapeutic applications of biosurfactants in the field of nanotechnology, those based on their micellization behavior are reported in the literature [5]. Nonetheless, it seems clear that biosurfactants are versatile and valuable entities for biomedical applications, but some may pose harm and must be wisely examined. For instance, *Pseudomonas aeruginosa* expresses glycolipids for medicinal applications, still, it is also a causative agent for severe nosocomial infections [6,7]. Another concern related to biosurfactants is their high cost of production and low purity which can limit their usage. However, bearing in mind that just minute quantities of biosurfactants are required for medical purposes, hopes are

high for their further exploration in this regard. Under these premises, in this chapter we have shed light on the diverse biosurfactants, further we have discussed their structure−property relationship, their emerging medicinal applications, and their probable mechanism of action.

2.2 Biosurfactant types and structure−activity relationship

The structurally diverse amphipathic natural surface-active agents, possessing a plethora of properties and derived from microbial sources such as yeast, bacteria, and fungi are referred to as biosurfactants. Several rewards are associated with biosurfactants which have resulted in an upsurge in their commercial applications over the chemical counterparts. These primarily include eco-friendly, low toxicity, good biodegradability, and bioactive nature. These natural surfactants can be characterized based on their structural dissimilarity, molecular weight, manner of action, or the microorganisms producing them [8]. Usually, any biosurfactant comprises of the hydrophilic head (fatty acids, DNA nucleotides, nucleosides) and a hydrophobic tail (peptides, cations, anions, amino acids, long alkyl chain) region. Biosurfactants with high and low molecular weights are known to stabilize oil-in-water emulsions and diminish the surface tension at the air-water interface, respectively. The molecular weight of biosurfactants generally falls between 500 and 1500 Da [9]. The classification and the structure−activity -relationship of the biosurfactants are described in the following sections.

2.3 Lipopeptides

Lipopeptides are amphiphilic molecules integrating a linear or cyclic peptide head group tethered to one or more lipid chains comprising the tail, through an amide bond. The head group can be either positively or negatively charged depending upon the charge carried by the amino acid forming the peptide sequence. If this peptide is cyclic in nature, then the in vivo stability of the lipopeptides is significantly enhanced. Usually, the chain length, constituent amino acid residues, their surface charges, stereochemistry of amino-acid at the C-terminal in addition to temperature and pH are known to control the surface activity and micellization behavior of these lipopeptides. Impurities, if present, can pose serious problems in the purification of these lipid biosurfactants. Solid-phase synthesis technologies can be implemented to synthesize judiciously designed lipopeptides containing the different amino acid sequences. However, this route being economically unviable, biosynthetic methods are preferred for the synthesis of lipopeptides [10]. Fabricating genetically engineered strains is yet another promising and feasible approach for the biosynthesis of lipopeptides possessing desired molecular skeletons suitable for particular applications. Some notable examples include Daptomycin, Caspofungin, Micafungin, Aridulafungin, Viscosin, Plymyxin B, etc.

2.4 Surfactin and surfactin derived

Produced by *Bacillus subtilis*, surfactin is a water-soluble cyclic lipopeptide made of saddle-shaped heptapeptide head and 13−15 long hydrocarbon tail (Fig. 2.3). Typically the Asp and Glu appear

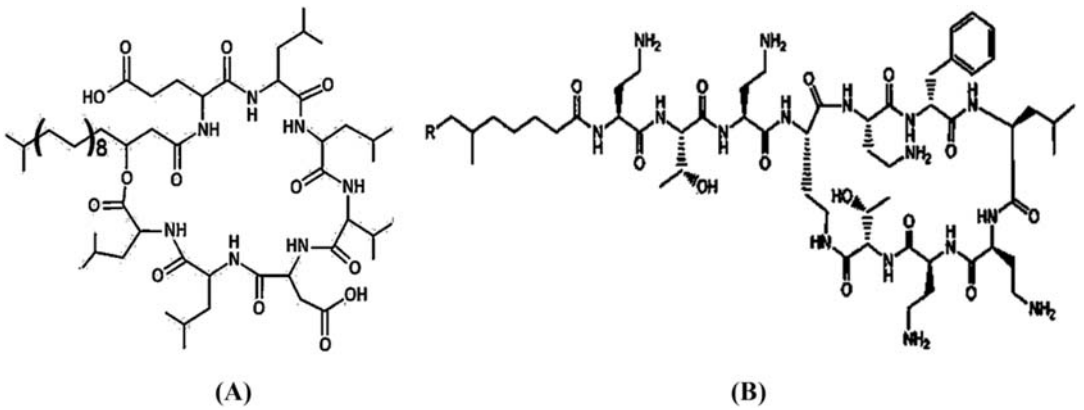

FIGURE 2.3

Chemical structure of surfactin (A) and polymyxin B (B).

in the head, albeit the nature of the peptide and hydrocarbon chain expressed is reliant upon the bacterial strain and culture conditions. The ability to lower surface tension, in conjunction with amphiphilic nature, allows its applications in both hydrophobic and hydrophilic surfaces. By the rational tailoring of the peptide sequence and the number of carbon atoms in the tail region can result in various surfactin derivatives bestowing different functions viz cell-adhesion, reversible cross-linking, and directed mineralization. To increase the yield, Wu et al. [11] have reported the synthesis of surfactin via genetic engineering approach.

2.5 Nucleolipids

The biomimetic surfactant comprising of a nucleobase/ nucleoside/ nucleotide/ short oligonucleotide (head region) attached to a hydrophobic tail (saturated or unsaturated or lipid or cholesterol or polymer) fall under the category of nucleolipids [12]. Bacterial and marine sponges generally are the natural sources of these DNA-like surfactants, while they can be synthesized by judicious design and incorporation of chemical moieties to render them with aqueous solubility. In a DNA surfactant, adsorption and aggregation are elicited by the tail region, but most importantly, provide stability to the entire structural framework. Whereas, the head region endows aqueous solubility, polarity and acts as a source of information. The examples include as illustrated in Fig. 2.4.

2.6 Glycolipids

Glycolipids are lipids combining a carbohydrate moiety (mono, di, or polysaccharides) covalently conjugated to long-chain aliphatic acids or aliphatic acids bearing a hydroxyl group [13]. A range of natural glycolipids originate from many microbial species varying in structure and bioactivity

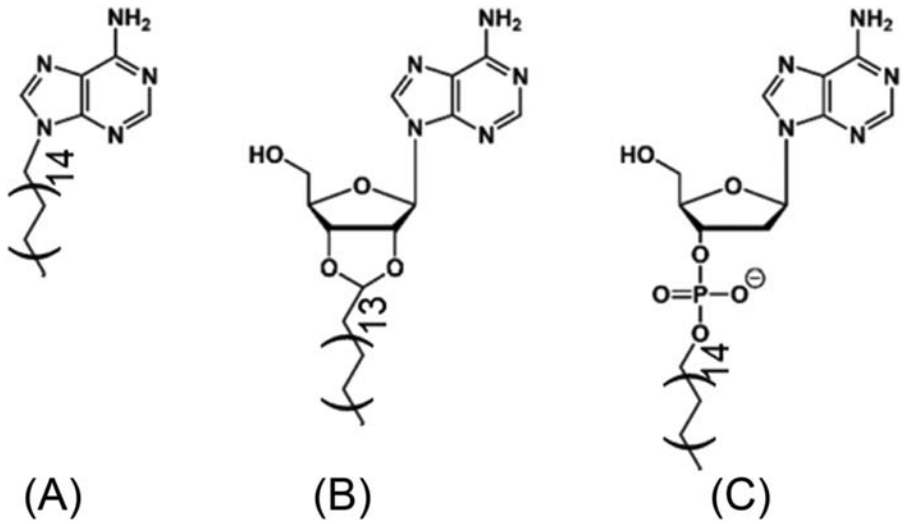

FIGURE 2.4

Chemical structures of (A) lipid nucleobase, (B) lipid nucleoside, and (C) lipid nucleotide.

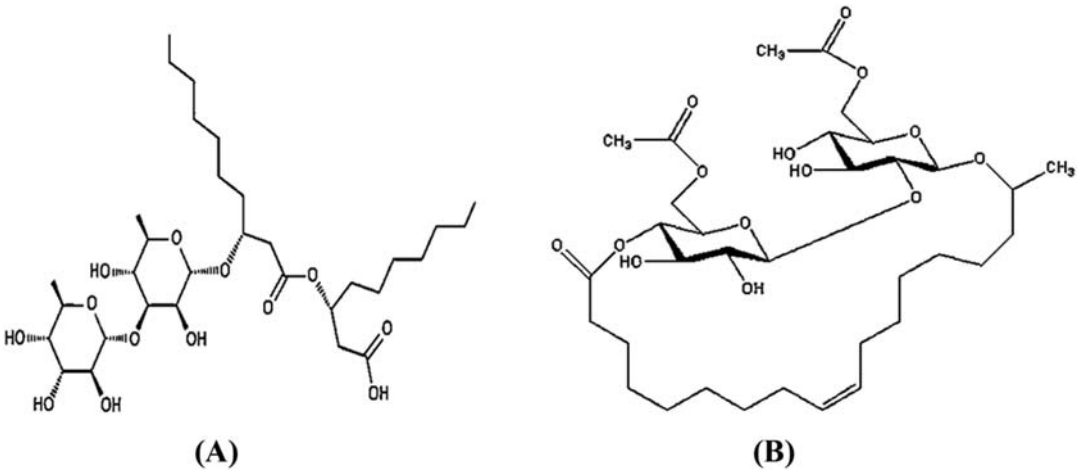

FIGURE 2.5

Chemical structures of (A) Rhamnolipid, (B) lactonic sophorolipid.

namely: Rhamnolipid obtained from *Pseudomonas* species, terahalose lipids of *Mycobacterium* and sophorolipids found from yeasts, and Mannosylerythritol lipid obtained from *Candida* strains [14].

The head and the tail of rhamnolipids (Fig. 2.5) are made up of glycosyl moiety and fatty acids of varying length. The extent of branching and unsaturation in the tail region can be modified by altering the concentration of *Pseudomonas* species, environmental and growth conditions leading to

the production of rhamnolipid congeners [15,16]. For example, longer alkyl chain rhamnolipids are produced by *Burkholderia* species, in contrast to those produced by *Pseudomonas aeruginosa*, by using diverse starting materials, such as alkenes, monosaccharide's, olive oil, etc. Even minute differences in the source compound will govern the physicochemical properties of rhamnolipids differently. *Pseudomonas aeruginosa* owing to its pathogenicity, may cause severe health fears from the biomedical application point of view. Therefore, to tackle the issue of pathogenicity, *Pseudomonas aeruginosa* is either engineered or genes for rhamnolipid production are inserted into a nonpathogenic bacterial strain to ensure maximum safety. Alternatively, in vivo or in vitro enzymatic degradation of the toxins may also reduce the extent of pathogenicity. For another glycolipid, sophorolipids, which exist in the lactonic and acidic form, the major natural source is the yeast *Candida bombicola* [17]. Due to challenges associated with separation and purification with the natural genesis of glycolipids by a microorganism, chemically synthesizing them, by rational and strategic molecular design, delivers a smart option both in terms of cost-effectiveness, and tuning the structure and functions as per the needs of industrial applications. For instance, there is a rise in the synthesis of sophorolipids from waste and low-cost substrates for enhancing the prospects of reduced cost production, thereby increasing their use and sustainability [18–20].

2.7 Full peptides

Another unique class of natural surfactants is the full peptides which are self-sufficient and self-efficient in terms of their chemical nature. The name itself suggests entities comprising of only amino acid sequences combined and arranged in "*n*" number of possibilities, imparting the required amphiphilic character. Even full peptides can be constructed using several copies of a single amino held together via amide linkage with promising self-assembling features and resulting in the formation of nanospheres, nanofibres, nanovesicles, nanotubes, and other well-ordered nanostructures [21]. The difference in the self-ordering capability is attributed to the different amino acid sequences that make up the full peptide. Zhang and coworkers [22,23] have very well described the design of full peptides through the synthesis and characterization of V6D1 and A6D1. In their work, they have shown that the hydrophilic head and the hydrophobic tails bearing charged and hydrophobic amino acids respectively can be tailored using 20 natural or other unnatural amino acids rendering vivid physicochemical properties. Amino acids that mimic phospholipids, such as those based on phosphoserine, can be used as hydrophilic heads. Peptides such as pS1A6 and pS1V6 possess unique critical micelle concentrations, comparable self-assembly performance like phospholipids [21].

2.8 Medicinal properties of biosurfactants

The structural diversity, uniqueness, surface activity, versatility, and multifarious properties have made biosurfactants as one of the most sought after molecular entities favorable for various applications (Table 2.1) including medical or therapeutic applications as described below:

Table 2.1 Different classes, types, their microbial source, and the applications of/ activities exhibited by biosurfactants.

Biosurfactant class	Type	Source organism	Application/activity
Lipopeptides	Surfactin	*Bacillus subtilis, Lactobacillus surlactin*	Antiviral activity against human immunodeficiency virus 1 (HIV-1), antitumor performance against Ehrlich's ascite carcinoma cells, antimicrobial and antifungal activities
	Viscosin	*Pseudomonas fluorescens*	Impede the movement of metastatic prostate cancer cells
	Peptide-lipid	*Bacillus licheniformis*	Possess antibiotic activity
	Daptomycin	*Streptomyces roseosporus*	Antifungal and antibiotics, food safety applications
Nucleolipids	Lipid nucleobase: Agelasines	*Agelas Sponge*	Strong activity against *Mycobacterium tuberculosis*
	Lipid nucleoside: liposidomycin	*Streptomyces griseosporeus*	Antibiotic: inhibits phosphor-*N*-acetylmuramylpentapeptide translocase
	Lipid nucleotide tunicamycins	*Streptomyces lysosuperficus*	Antimicrobial including antifungal, antiviral, and antitumor activities
Glycolipids	Rhamnolipids	*Pseudomonas aeruginosa*	Antimicrobial activity against *Mycobacterium tuberculosis*
	Sophorolipids	*Torulopsis bombicola*	Impedes growth, seizes cell cycle, induces apoptosis in hepatic cancer cells, impedes the growth of esophagl tumor cells
	Trehalolipids	*Rhodococcus erythropolis*	Antiviral activity against Herplex simplex virus and influenza virus
Polymeric surfactants	Emulsan	*Acinetobacter calcoaceticus*	Antimicrobial activity against *Photobacterium phosphoreum*
	Biodispersan	*Acinetobacter calcoaceticus*	Used to disperse 10% limestone in water and also prevents flocculation
	Alasan	*Acinetobacter radioresistens*	Used as bioemulsifier for skincare and food applications

2.9 Biosurfactants as antitumor agents

One of the most prominent discoveries of the bioactivity of biosurfactants is their capacity to act as prospective anticancer agents by directing several cellular functions like inducing apoptosis, cell differentiation, immune response, and cell growth arrest which are instrumental in cancer advancement [24]. To begin with glycolipids, demonstration of apoptosis in mouse malignant melanoma B16 cells is evidenced by their accrual in the subG0/G1 phase, DNA fragmentation, and build-up of chromatin upon the concentration-dependent action of mannosylerythritol lipids. These results provide a testament to the antitumor activity of these lipids hindering protein kinase C activity, involving crucial cellular events viz, cell differentiation, growth, and death. Further,

mannosylerythritol lipids have also shown their potential to initiate HL60 cell differentiation as evidenced by the expression of Fc receptors and phagocytic events in HL60 cells. Thus, mannosylerythritol glycolipids not only elicit cell differentiation but also cause apoptosis in plenty of cancer cell lines [25−27]. In another instance, inhibition of cell growth and cell distinction is demonstrated by the action of Succinoyl trehalose lipids on HL60 and KU812 cancer cell lines [25].

Sophorolipids have been shown to impede the protein kinase C activity in the HL60 human leukemia cell lines by inducing cell differentiation ascribed to an explicit communication with the plasma membrane [25]. In another instance, apoptosis in H7402 human liver cancer cells is observed by the sophorolipids produced by *Wickerhamiella domercqiae*, as a consequence of caspase-3 activation, arresting the cell cycle at G1 phase and enhanced calcium ion production in the cancer cell cytoplasm [28]. From their work, Fu et al. [29] established structure−activity relationship of several sophorolipids derivatives and their action on human pancreatic carcinoma cells. They found that the methyl ester derivative of sophorolipids showed the most effective anticancer activity hinting at the possible involvement of different mechanisms of action. On similar grounds, it is revealed by Shao and coworkers [30] that the cytotoxic effect of sophorolipids on human esophagal cancer cell lines is promising for derivatives possessing higher degrees of acetylation over the lower degree acetylated derivatives. Sophorolipids with unsaturation in the fatty acid chain also demonstrated a potent anticancer effect, while the ones containing acid functionality are found to be totally ineffective. Consequently, structure-activity dependent mechanism of anticancer effect is proposed by them: [29,30] sophorolipids bearing either unsaturation or acetyl groups or presence of lactone rings are capable of causing an enhanced anticancer activity over the other derivatives. In the case of glycolipids, the crude glycolipid derived from *Sphingobacterium detergens* has demonstrated a potential inhibitory effect on the growth and proliferation of Caco2 human colorectal cancer cells [31]. Amongst the lipopeptides, surfactin has shown remarkable anticancer activity following the different mechanisms of action, irrespective of the cancer cell lines under study and the source from where the surfactin is derived [32]. Kim et al. have established the antitumor efficacy of surfactin by arresting the cell cycle and impeding the phosphoinositide 3 kinase and protein kinase B activity [33]. Surfactin isolated from *B. subtilis*, on one hand, caused an inhibitory effect in a dose-dependent fashion [34], while on the other it induced apoptosis following a reactive oxygen species initiated caspase pathway in the human breast cancer cell lines [35]. In another instance, surfactin prompted its anticancer activity by obstructing the cell cycle at the G2/M phase in the breast cancer cell lines [36], and caused cytotoxicity on hepatic carcinoma and human chronic myelogenous leukemia cells in a dose-dependent fashion [37]. Surfactin is also known to prompt apoptosis in HepG2 cells by interfering with reactive oxygen species regulated protein kinase pathways. Monoolein is another such lipopeptide, derived from dematiaceous fungus *Exophiala dermatitidis*, which has shown its antiproliferative activity in a dose-dependent fashion by inducing severe morphological changes on cervical cancer and leukemia cell lines [38].

Although biosurfactants have shown a promising anticancer activity on numerous cell lines following varied mechanisms, however, some drawbacks remain associated with their anticancer potency. Firstly, most of the studies report the cytotoxicity results on the single cancer cell lines without the use of any control or comparing the results with normal cell lines. Secondly, these biosurfactants are highly unspecific in nature which in the long run can weigh down their probability to function as anticancer agents. Thirdly, there is a lack of concrete support on their mechanism of action. Fourthly, in vivo studies are not performed to corroborate the in vitro results.

Biosurfactants are known to interact with certain biomolecules like lipids in the normal and cancer cell membrane. These lipids (e.g., phosphatidylcholine and sphingomyelin) are known to maintain cellular integrity, confer structure, and function of cell membranes. Sphingomyelin and phosphatidylcholine are known to make up the cell membranes of normal and cancer cells respectively [39]. Any alteration in the lipid profile can modulate the surface activity and upon interaction with surface-active natural surfactants can lead to morphological changes in the cell membrane and subsequent cell death [39–42]. Variations in the level of these two lipid constituents can greatly affect the membrane permeability of the cancer cells. Since biosurfactants can change the lipid profiles and hence the membrane permeability, this further can form the basis of rational designing of biosurfactant-based anticancer agents or drug delivery vehicles. Thus, the knack of natural surfactants to disintegrate the cell membranes by enhanced membrane permeability can trigger a cascade of cell events like lysis of cell and metabolic leakage, which taken altogether are viewed as their plausible mechanism of anticancer activity [43].

2.10 Biosurfactants as antiviral agents

Surfactin and its derivatives have been known to exhibit impressive antiviral activity. Pumilacidin, produced by Gram-positive *Bacillus pumilis*, has demonstrated antiviral activity on herpes simplex virus [44], inhibits ATPase dependent hydrogen-potassium pump, and guards against gastric ulcers in vivo. Itokawa and group have revealed the antiviral efficiency of surfactin against human immunodeficiency virus 1. The mode of antiviral activity of surfactin can be endorsed due to its capability to interact with the lipid membrane of the virus, leading to permeability changes and finally causing disruption of the outer membrane by micellization. In some cases, through the formation of ion channels, the viral capsid also undergoes disruption [45]. At higher concentrations, hemolytic effect is more likely, while at concentrations below 20 μM, no harmful effects are reported. Additionally, surfactin has shown its antiviral potency on plethora of viruses like herpes simplex virus, feline calicivirus, suid herpes virus, murine encephalomyocarditis virus, semliki forest virus, vesicular stomatitis virus, etc. [46]. The replication of many viruses, such as bursal disease virus, porcine parvovirus, pseudorabies virus, etc. is shown to be curtailed in vitro by the antiviral activity of surfactin and feengycin produced by *Bacillus subtilis* [47]. Here again, the mechanism of antiviral action is the same as described above. Further, sophorolipids have also been found to be active as an antiviral agent against the human immunodeficiency virus [48]. Rhamnolipid alginate complex is found to be effective against herpes simplex virus types 1 and 2 at concentrations below the critical micelle concentration [49]. Very recently, a breakthrough in the biosurfactant based antiviral agents are seen on the rise as lipopeptides based vaccines have been invented against several viral infections such as human immune deficiency virus, hepatitis B, and especially human papillomavirus as it is associated with different types of cancer including cervical cancer, etc. [50]. A series of HIV-lipopeptides has been invented by the French National Agency for AIDS research (ANRS) based on an induced cytotoxic T lymphocyte response. Simian immunodeficiency virus has also been used as a model for vaccination using lipopeptides, which trigger a cytotoxic T lymphocyte response [51]. Theradigm, a lipopeptide, constituting a peptide sequence from a cytotoxic T lymphocyte linked to a T-helper peptide epitope TetTox and two lipid chains comprising palmitoyl group, has been used to develop a vaccine for chronic hepatitis B virus.

2.11 Biosurfactants as antibacterial agents

Yakimov et al. [52] established the antibacterial activity of lichenysin A, which derives its origin from *Bacillus licheniformis*. The antibacterial effect of lichenysin is attributed to its chelating properties as demonstrated by Grangemard and group [53]. The growth and adhesion of several yeasts and bacterial strains are truncated, as a consequence of the antibacterial activity of the biosurfactant derived from dairy *Streptococcus thermophilus* strains [54]. A similar antiadhesion property is also assessed by *Lactobacillus lactis* and *Streptococcus thermophiles A* derived biosurfactants against two yeast and four bacterial strains [55]. From the obtained results it is concluded that, for all the pathogens tested, after 4 hours, the biosurfactants could significantly diminish the adhesion of bacterial pathogens.

Lipopeptides constitute an important class of biosurfactants with potent antibacterial activity credited to their micellization properties [50]. Surfactin is one such lipopeptide with notable antibacterial activity on several Gram-positive and Gram-negative bacteria [50]. Biosurfactants obtained from several bacterial strains viz; *Mycobacterium smegmatis*, *Bacillus circulans*, *Escherichia coli*, *Serratia marcescens*, *Micrococcus flavus*, *Bacillus pumilus*, and *Proteus vulgaris* also exhibit promising antibacterial activity against various Gram-positive and Gram-negative bacteria [56]. These biosurfactants are structurally analogous to surfactin lipopeptides and lichenysin. Huang and group revealed the antibacterial activity of surfactin and polylysine against *Salmonella enteritidis* in dairy products with a minimum inhibition concentration of 6.25 and 31.25 μg/mL respectively. Apart from surfactin, *Bacillus subtilis* is known to produce several biosurfactants such as fengycin, surfactin, and the iturin congeners, such as Iturins, Mycosubtilins, and Bacillomycins [44] with promising antibacterial properties. Surfactin and fengycin are described to be able to impede endospore formation of *Bacillus cereus* by damaging their surface structure spores. Cationic polymyxin B produced by *Bacillus polymyxa*, binds to the anionic outermost membrane of Gram-negative bacteria resulting in micellization due to the established electrostatic interaction and displays its antibacterial action by subsequently disrupting the membrane integrity [57]. Thus, polymyxins have been assessed to exhibit their antibacterial activity against many pathogenic bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholera, Acinetobacter baumannii, and several other species belonging to Enterobacter*, *Enterococcus*, *Klebsiella*, *Acinetobacter*, *Haemophilus*, *Pasteurella* species, *Salmonella*, and *Shigella* genus [58].

Daptomycin (CubicinR) is an antimicrobial lipopeptide that is produced by *Streptomyces roseosporus*, approved by FDA in 2003 and is under development for future applications as antibacterial agent against skin infections. This lipopeptide-based natural surfactant is also very active against Methicillin-resistant *Staphylococcus aureus* [58]. Another notable cyclic lipopeptide showing antibiotic activity against tubercle bacillus is Viscosin, isolated from pseudomonas libanensis [59]. Viscosin is exceedingly surface-active which is known to hinder the cancer cell movement [60].

Rhamnolipid demonstrates significant antibacterial activity against *B. subtilis* with a minimum inhibitory concentration of 8 μg/mL. As per the literature reports, Mannosylerythritol lipids obtained from *Candida antarctica* strains display their antibacterial activity against Gram-positive bacteria. Nitschke et al. reported the antibacterial and antifungal performance of rhamnolipids isolated from *Psedumonas aeruginosa* on several bacterial species and fungi such as *Mucor miehei*, *Micrococcus luteus*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Neurospora crassa*,

Staphylococcus saprophyticus. The biosurfactant derived from *Tsukamurella* sp. strain DSM 44370, has also shown their promising antibacterial activity on Gram-positive bacteria. Kitatsuji and group, assessed the antibacterial activity of biosurfactants derived from microorganisms capable of disintegrating filamentous bacteria. The antimicrobial lipopeptides Lichenysins, polymyxin B, and pumilacidins are derived from *B. licheniformis*, *B. polymyxa*, and *B. pumilus*, respectively. Polymyxin B and polymyxin E (colistin) illustrate antibiotic activity against several Gram-negative bacteria. Upon examination of urethral catheters, it is observed that the biofilm formation in *Salmonella enterica* is inhibited as a consequence of the antibacterial action of surfactin. Because surfactin can generate selective cationic channels in lipid bilayers, it can easily assist the transport of cations across the membrane. This mechanism forms the basis of the antibiotic activity and directs further for probable applications in drug delivery. Through rational molecular design, Makovitzki and group presented several ultra-short antibacterial and antifungal lipopeptides comprising of four L- and D-amino acids, including lysine and leucine which displayed promising antibacterial activity against both human and plant pathogens.

2.12 Biosurfactants as drug-delivery agents

With new inventions in the field of drug delivery systems, such as polymeric, particulate, macromolecular, cellular carriers, lipid particles, microemulsions, micelles, polymeric, liposomes, niosomes, virosomes, sphingosomes, etc., it has become possible to provide prophylactic measures against various diseases efficiently. Quintessentially, drug delivery systems should possess the following features: (1) ideal drug loading capacity to reach the target site with greater bioavailability (2) precise release of the drug when required. In recent times, microemulsions have gained more attention as favorable drug delivery systems because of the ease in formulations and more importantly several alternative routes (oral, ocular, nasal, intravenous, topical, transdermal, parenteral) are available for drug administration using microemulsions as drug vehicles. Microemulsion-based drug cargoes constitute an aqueous phase, an oil phase, and a surfactant along with a cosolvent or cosurfactant. Of these, the nature of surfactant is or prime importance as it agglomerates or self-associates to form templates possessing different structures. Although impressive in their performance, drug delivery systems tend to fail when the constituent elements are not biocompatible and biodegradable in nature. Thus, biosurfactants carry the immense potential to particularly fill this lacuna and offer a safer, greener, and biocompatible replacement to chemical surfactants in microemulsions.

As far as microemulsion-based drug delivery systems are based, their nature and stability can be controlled by judicious assortment of the most suitable biosurfactant. For rhamnolipid and surfactin to be used as biosurfactants for microemulsion formulation, it has to be borne in mind that their micellization is greatly affected by the environment. Due to the intricate nature of the head functionality (e.g., amino acids in lipopeptide and saccharides in glycolipids) the further evaluation of their structure becomes difficult. Additionally, these biosurfactants tend to lose their carboxylic acid proton under alkaline conditions, become hydrophilic in nature, and undergo a structural transformation from micellar to lamellar. These properties of biosurfactants can be harnessed to formulate novel drug delivery systems for (1) specific routes of administration and (2) which can be triggered by external stimuli like pH, temperature, or salt concentration. A very exhaustive review

on the same has been recently published and is currently out of the scope of this article. Therefore, readers are directed to refer to the article by Gudina et al. [61].

It is known that biosurfactants can be deployed for targeted or triggered drug delivery, however, further research in this area is the need of the hour. Shim and coworkers, employed cationic surfactin liposome for the delivery of small interfering RNA in cervical cells. This formulation greatly enhanced the specificity of the system in terms of improved uptake of the small interfering RNA over the liposomes sans biosurfactant. In another instance, Cheow and Hadinoto, established the efficiency of rhamnolipids, obtained from *Pseudomonas aeruginosa* biofilm, to prompt the release of antibiotics condensed inside lipid-polymer coated hybrid nanoparticles.

2.13 Biosurfactants as antiadhesive agents

The capability of biosurfactants to adhere to solid surfaces of implant materials or infection sites prevents microbial colonization significantly. Thus, they can be effectively deployed as antiadhesive agents in the medical field [62]. Prior coating of the vinyl urethral catheters by using surfactin, caused a diminution in the production of biofilm by several bacteria namely *Salmonella typhimurium*, *Salmonella enterica*, *Escherichia coli*, and *Proteus mirabilis* [63]. These results are very compelling considering the significance of urinary tract infections in AIDS patients caused by the *Salmonella* species. The natural surfactant surfactin derived from *Lactobacillus* functions as an appropriate antiadhesive coating for catheter materials [64]. The natural habitats of the female urogenital tract, *Lactobacillus* species, are believed to control the vaginal microbiota by contesting with other microbes for epithelial cell attachment and biosurfactant production. The biosurfactants derived from *Lactobacillus acidophilus* prevent biofilm production by uropathogens and yeast on silicone rubber [65,66]. Heinemann and group, demonstrated that the biosurfactants produced by *Lactobacillus fermentum* prevented the adhesion of uropathogenic bacteria such as *Enterococcus faecalis* [67]. Busscher and group reported that the biosurfactant produced by diary *Streptococcus thermophiles* prevented the growth and adhesion of numerous yeast and bacterial strains onto silicone rubber [54]. Rodrigues and group have worked on similar grounds to design strategies that can reduce and inhibit colonization of microbes on the surface of prostheses made up of silicone rubber [68,69]. In another report, the deposition of bacterial and yeast strains isolated from explanted voice prostheses is prevented by the biosurfactants derived from *Lactobacillus lactis* and *Streptococcus thermophiles*. Rhamnolipid biosurfactants could be employed as cleaning solutions for prostheses, increasing their shelf-life thereby benefiting the laryngectomized patients. Another striking feature of natural surfactants is in combating infections and inflammation in the human body. For instance, the pulmonary surfactant secreted by the epithelial lung cells curbs the invasion of any infection and inflammation by reducing the surface tension in the lung [70].

2.14 Biosurfactants as antimicrobial agents

Biosurfactants are known to function as antimicrobial agents. For example, the biosurfactants derived from probiotic bacteria *Lactobacillus lactis* and *Streptococcus thermophilus* demonstrate

their antimicrobial activity against a series of yeast and bacteria from explanted voce prostheses. They exhibit high antimicrobial performance against the species accountable for prostheses failure, that is, *Candida tropicalis* GB 9/9. Reid and coworkers [71,72], in their remarkable work have revealed natural biosurfactants produced by *Lactobacilli* strain can aid in the repair and maintenance of urogenital and intestinal microbiota. This impressive strategy is first clinically used to deliver probiotic lactobacilli to the vagina signifying its use as an alternative regimen in place of using antibiotics [71]. Gan and coworkers [73] demonstrated the infections caused by *Streptococcus aureus* and its adherence to surgical implants are curtailed by the biosurfactants isolated from probiotic strain *Lactobacillus fermentum* RC-14. In another study, *Lactobacillus species* by their produced biosurfactants impeded the attachment between *Escherichia coli* and intestinal epithelial cells by triggering the secretion of epithelial mucins [74]. These results suggest the mechanism of action of these biosurfactants, wherein certain signaling factors on their surface interact with the causative pathogens, preventing their adhesion and controlling the spread of infections. Thus, the biosurfactants can possibly be viewed to efficiently function as coatings for implant materials.

There are several evidences on the antimicrobial performances of various glycolipids [75]. The rhamnolipids secreted by *Pseudomonas aeruginosa* AT10 found in wastes of oil refineries, demonstrated excellent antifungal properties. Golubev et al. [76] reported that the glycolipid obtained from *Pseudozyma fusiformata* (Ustilaginales) acted as an antifungal agent inhibiting the activity of $>80\%$ of yeast species at ambient temperatures and low pH conditions [77].

2.15 Biosurfactants: mechanism of interaction

The natural surfactants or biosurfactants are amphiphilic or polyphilic polymers that interact at the interphase of the heterogeneous media. At the interphase, a conditioning film is formed as a consequence of immobilization of the organic molecules or the biosurfactant, which greatly modulates the surface energy and wettability properties [78]. This in turn may play an instrumental role in the inhibition of bacterial adhesion and hence their detachment from the surface. For about one hour of exposure, the composition and the orientation of the biosurfactants are determined by the surface properties. After 4 hours, the composition of the biosurfactant remains constant. Due to the amphiphilic nature of the biosurfactants, other molecular forces of attraction also come into play which governs the adsorption of biosurfactants to the interfaces. Natural interfaces being mostly negatively charged, ionic conditions and pH become critical parameters to evaluate the interaction between biosurfactants and the interface. This property is very well exemplified by Gottenbos and group [79], where an effective antibacterial activity is displayed on Gram-negative bacteria only when the surface is positively charged. The chemical structure of biosurfactants also impacts its performance at interfaces but how their orientation and localization will, is the area that requires more research considering the complexity of in vivo systems.

2.16 Conclusion

Biosurfactants have attracted the attention of researchers on account of their versatility, biocompatibility, biodegradability, and most importantly their tendencies to undergo micellization rendering

different polarities that form the basis of a wide range of applications. These versatile and amphiphilic natural surfactants have found suitable applications in cosmetics, agriculture, different industries, petroleum, and most notably in the biomedical field which is the focus of this article. These biosurfactants have shown encouraging antitumor, antibacterial, antiviral performances, are deployed as antiadhesive coatings for medical insertion parts, are used in the formulation of vaccines, in gene therapy as well as have established themselves as prime components of the drug delivery vehicles. They are also assimilated into probiotic preparations to battle against urogenital tract infections. Encouraging alternatives to produce effective biosurfactants with enhanced antimicrobial performance and minimal toxicity against human cells can be accomplished by the genetic modifications in the microbes producing biosurfactants. The antitumor activity of biosurfactants is evaluated only on the cancer cell lines but no comparison is made with normal cell lines or positive control, which is an area of concern. Another major concern in the practical applications of biosurfactants in the medical field is the expensive production cost and low yield. Current times demand the development of better, affordable, production technologies that manufacture biosurfactants of a higher grade suitable for biomedical applications. Thus, with the advent of newer technologies, it can be envisioned that the biosurfactants will begin to compete with and supersede favorably their synthetic equivalents in the surfactant industry. The probable mechanism of action for the biosurfactants is known but further studies are still required to establish a relationship between the molecular structure, related activity, and physicochemical properties. The biosurfactants have shown promising biological activities but still, there is a lot of scopes to further escalate this knowledge so that biosurfactants can be employed to the fullest of their potential in the medical field.

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CHAPTER

Applications of biosurfactants in dentistry

3

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3.1 Introduction

Biosurfactants are bioactive compounds that have a structurally diverse characterization. They are amphipathic in nature and thus accumulate in between the interfaces of two liquid layers with different polarities. A surfactant molecule comprises the hydrophobic chain which often comprises the long aliphatic hydrocarbon chain, whereas the hydrophilic moiety may be ionic, nonionic, or even amphoteric in nature. A general surfactant moiety is shown in Fig. 3.1.

These moieties make the hydrophilic compounds more soluble hence reducing their interfacial tensions at different surfaces. At a particular concentration called the critical micelle concentration (CMC), the surfactant moieties assemble themselves in the formation of micelles, at a point where the lowest stable surface tension is achieved.

Most biosurfactants are cationic, anionic, or neutral. Those containing the amine group are cationic in nature. The hydrophilic moiety can be a carboxyl acid, alcohol, cyclic peptide, carbohydrate, amino acid, and the hydrophobic moiety is a long fatty acid chain.

Surfactants that are chemically synthesized are generally categorized based on their polarity whereas biosurfactants are classified based on their microbial origin and chemical compositions, namely, glycolipids, lipopeptides and lipoproteins, surfactins, lichenysin, fatty acids, phospholipids, and neutral lipids, polymeric surfactants and particulate biosurfactants. A variety of biosurfactants are naturally produced by numerous microorganisms in the environment. These are the new therapeutic agents to control and eradicate pathogenic microbes due to their ability to disturb the cell membranes, disrupt protein conformations and affect the adhesion properties of microbes. There is an increasing demand for these molecular compounds in medical and dental fields due to their surface activity-related properties. Moreover, these are highly biocompatible and biodegradable in nature.

A general way of classifying biosurfactants is through their origin and chemical composition and their molar mass range from 500 to 1500 Da. They can be further classified based on chemical properties and mode of action. However, in the year 1999, Rosenberg et al. classified biosurfactants based on molecular mass [1]. The high-molecular-weight compounds, also known as bioemulsifiers,

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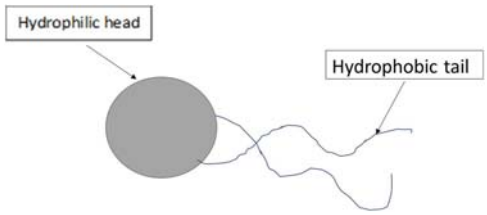

FIGURE 3.1

Surfactant moiety with polar and nonpolar ends.

are better as emulsion stabilizing agents while the low-molecular-weight compounds have significantly lower surface and interfacial tension. Some examples are as follows:

1. Low-molecular-weight compounds, for example, rhamnolipids, lipopeptides, sophorolipids, trehalolipids.
2. High-molecular-weight compounds, for example, lipoprotein and polymeric surfactants.

3.2 Oral biofilm

The oral cavity supports up to 1000 diverse varieties of microorganisms. There is a wide range of microorganisms that actively contribute to the normal physiology and host defense of the mouth. In general, these communities exist together with the host, and any deviation in the balance of the oral microbiota results in a disturbance of this symbiotic relationship ultimately affecting the normal survival of the host. The microbial population inside the oral cavity exists in two states: (1) planktonic state and (2) biofilm state (Fig. 3.2).

Planktonic (free-floating) microbes are loosely adherent to the tooth surface and other tissues and are therefore easy to deal with. However, when these microbes evolve into complex structures with a matrix-like composition, they are known as biofilms. These biofilms are more resistant to hygiene practices, protecting the resident microbes.

Biofilm formation is a dynamic process that mainly occurs in three stages: (1) initial attachment, (2) growth and maturation, and (3) detachment or steady state. The first phase is critical, as in this phase, the bacterial cell initially attaches itself to the substratum or to any other cell with a protective extracellular matrix. In the second phase, the biofilm matures by the formation of a three-dimensional structure along with the bacteria and extracellular polymeric substances (EPSs). In the last phase, cells from this biofilm detach as single entities or clusters and colonize other sites. EPS holds the biofilm complex and provides a suitable matrix for all intercellular interactions making it antibiotic tolerant. Once biofilms are formed, several proteolytic enzymes are produced within it, damaging the soft and hard tissues in the oral cavity and also affecting the defense mechanism of the host. The biofilm is composed of 91% of water, 5% microorganisms, 2% EPS matrix and, the other 2% is made up of proteinaceous and genetic content (DNA and RNA).

Dental plaque is a mature polymicrobial biofilm in the mouth that mainly affects the oral health in form of dental caries and periodontal diseases. It appears as tenacious, thin films, which may

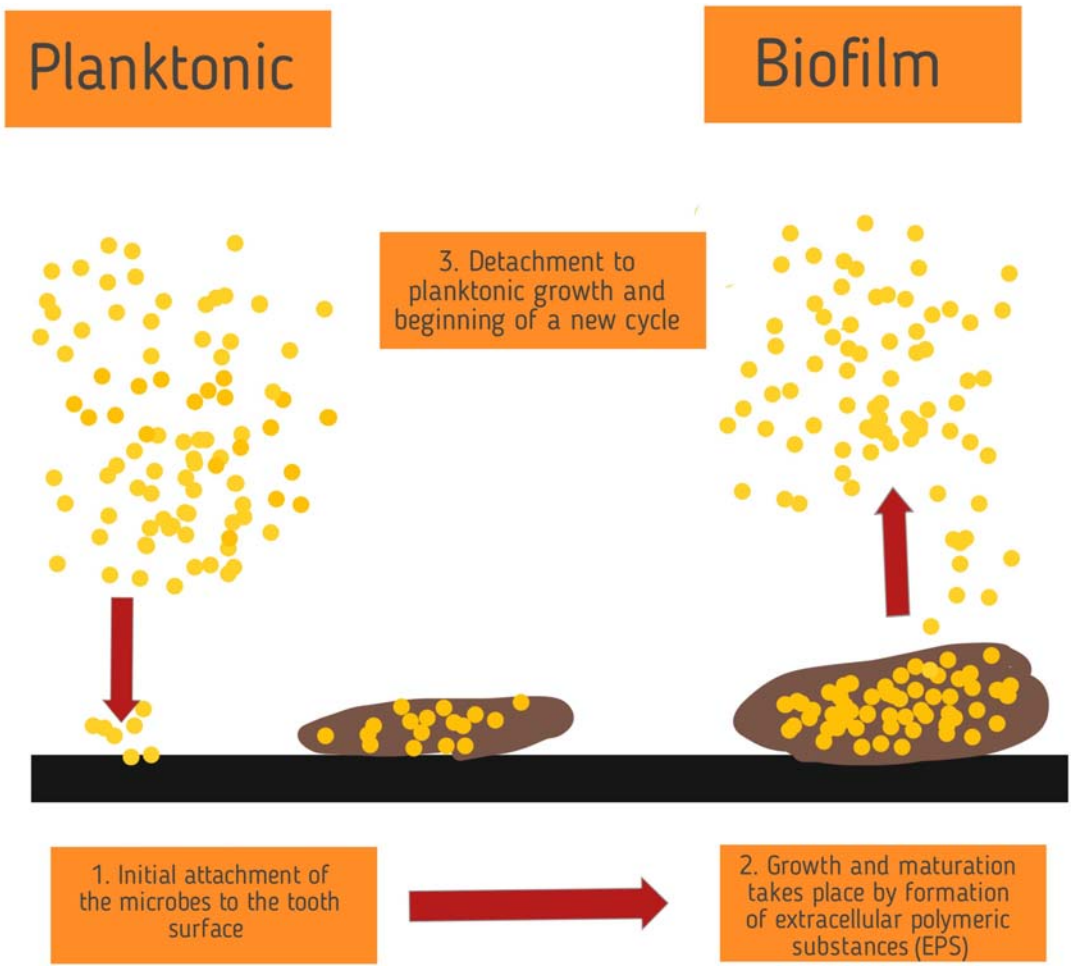

FIGURE 3.2

Schematic diagram of a biofilm formation. The biofilm formation begins with a reversible attachment of planktonic cells (small *circles*) that is followed by adhesion to the tooth surface (*black bar*). (1) The initial attachment of the microorganism takes place on the tooth surface. (2) Growth and maturation of the biofilm take place by formation of extracellular polymeric substance. (3) Detachment of the microbes to planktonic phase which begins a new cycle of biofilm formation.

accumulate on surfaces like teeth and dental prostheses. A characteristic property of dental plaque is that it resists its removal by physiologic or oral cleansing forces such as saliva and tongue movement but is removable by tooth brushing. Before dental plaque, a glycoprotein known as acquired pellicle is formed on the tooth surface that is derived from saliva. This may act as food for the microbial component of plaque and makes the surface receptive to colonization by specific bacteria.

This pellicle formation starts within minutes after professional prophylaxis and microbes get attached to the pellicle within one hour. Dental plaque consists of mainly two components: salivary components and microorganisms. It also contains lipids and carbohydrates which act as a reservoir for the fermentable substrates. The inorganic components such as calcium and phosphate in dental plaque are much higher than saliva in the oral cavity. Gingival crevicular fluid is found in the sulcus between the tooth and marginal gingiva and is the main source of nutrients, proteins, and glycoproteins for the development of dental plaque. Three basic groups of microorganisms are found in abundance in dental plaque: streptococci (*Streptococcus mutans*, *Streptococcus mitior*, *Streptococcus milleri*, and *Streptococcus salivarius*), *Actinomyces* (*A. viscous*, *A. naeslundii*, *A. israelii*, and *Rothia dentocariosa*), and *Veilonellae* (*V. parvula* and *V. alcalescens*).

Oral biofilms can cover various surfaces throughout the oral cavity—oral mucosa, dental materials used for restoration, dental prostheses, tongue, and the tooth surfaces above (supragingival) and below (subgingival) the gingival margin.

3.2.1 Microbial biofilm causing dental caries

Dental caries is an irreversible microbial disease due to the dissolution of the inorganic and organic components of the tooth, which leads to cavitation by the acids produced by bacterial communities within dental plaque. It is a multifactorial disease that prerequisites the presence of three primary factors: (1) the host, (2) substrate, (3) microbial flora and time as an inevitable fourth factor (Fig. 3.3).

3.2.2 Microbial biofilms and its association with periodontal infections and tooth loss

Inflammatory response of the gingiva and surrounding connective tissue to the microbial plaque accumulation on teeth is mainly of two types—gingivitis and periodontitis (Fig. 3.4). Gingivitis is

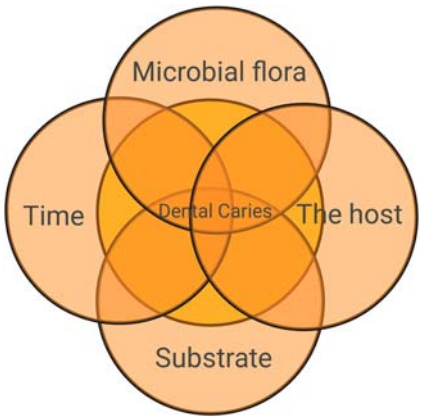

FIGURE 3.3

Contributing factors of dental caries.

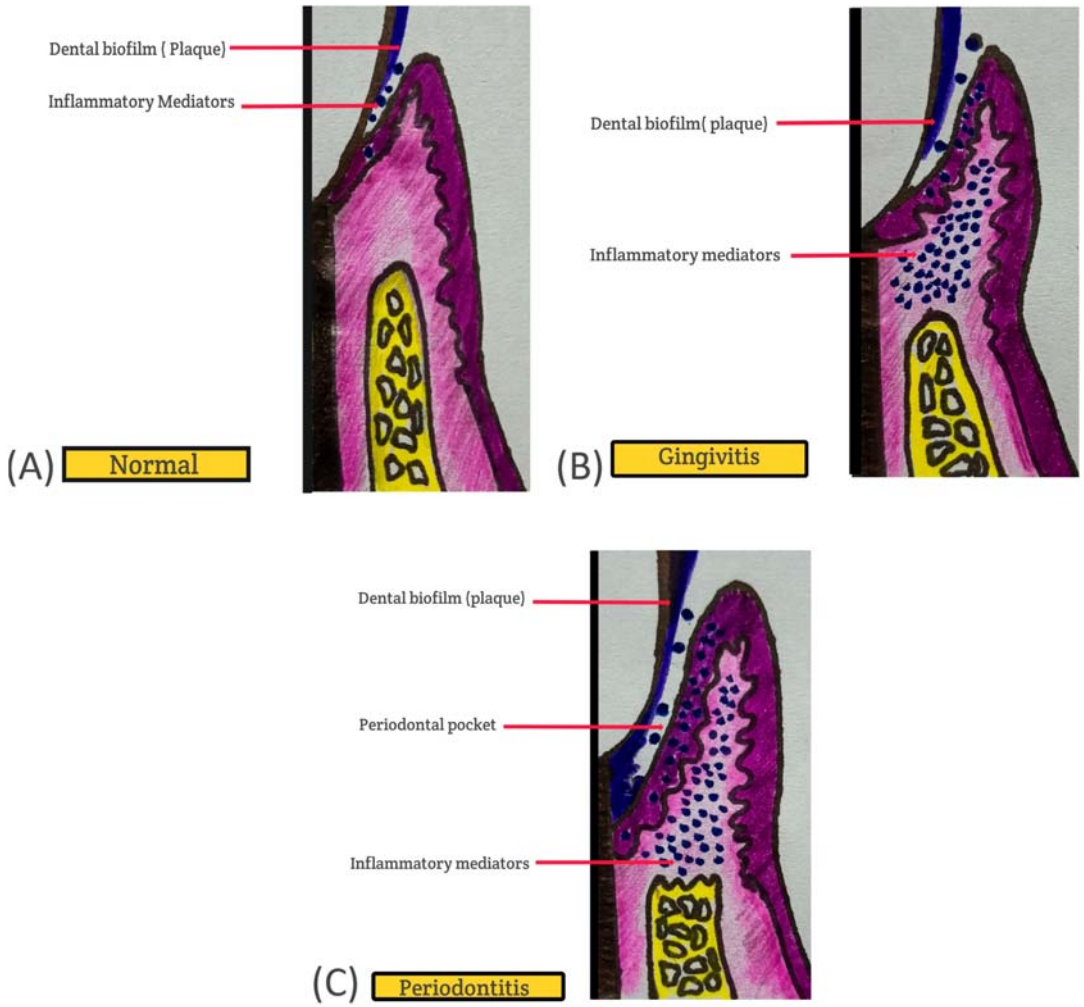

FIGURE 3.4

Development of periodontal diseases (gingivitis and periodontitis). (A) *Normal healthy gingivae*. (B) *Early or stable gingivitis*—following dental plaque accumulation. (C) *Periodontitis*—the destruction of periodontal tissues and formation of periodontal pockets.

the inflammation of the gingiva and clinically manifests as bleeding of gums whereas periodontitis occurs when the microbial plaque causes inflammation in the periodontal tissues, further leading to loss of collagen attachment of the tooth to the alveolar bone. It also causes deep periodontal pockets (spaces filled by dental plaque due to pathological loss of tissue between tooth and gingiva) and alveolar bone loss. It clinically manifests as halitosis and when teeth become lose giving discomfort during chewing.

3.2.3 Microbial biofilms and its association with prosthesis and dental implants

Apart from their direct involvement in local infection, these bacterial communities also play a major role when different medical devices are implanted in the human body as the bacterial cells immediately recognize the implant surfaces and promote bacterial colonization.

Patients with prostheses like dentures (removable or fixed) are at a higher risk of developing oral candidiasis and stomatitis (an inflammation of oral tissues). The prevalence of *Candida albicans* (a fungal pathogen causing *Candida* infection) is higher in biofilms obtained from the dentures. Similarly, dental implants due to the inherent roughness of their metallic surfaces, harbor bacteria and can form pathogenic biofilms, ultimately leading to inflammation affecting function.

3.2.4 Available agents for removal of dental plaque

There are several methods for the eradication of biofilms which include mechanical or physical methods. Antiplaque agents (1) prevent biofilm formation and (2) eradicate the established biofilms while the antimicrobial agents inhibit the growth of the associated bacteria. Commercially there are numerous plaque-controlling agents that have been formulated into oral healthcare products. Table 3.1 presents the various classes of antiplaque agents available for use.

Surfactants, also known as detergents, are important components of toothpaste and other oral hygiene-related products. They are the foam-producing agents which aid in the removal of plaque and debris, due to their surface-active properties. The main principle behind their action is the reduction of surface tension of the watery environment in the mouth. The surfactants in oral hygiene-related products also can dissolve the dental plaque biofilm to ease the tooth-cleaning process.

3.3 Biosurfactants versus synthetic surfactants

Microorganisms use carbon sources for their energy and growth. Insoluble substrates together with carbon sources facilitate intercellular diffusion and hence produce different substances. Microorganisms (bacteria, yeast, and fungi) produce a variety of biosurfactants that have different diffusing properties and structures. Microbes that produce surfactants with high emulsifying abilities, low CMC, and are less toxic are of major concern.

Table 3.1 Classes of antiplaque agents.

Class	Example
Essential oils and phenols	Thymol, hexylresorcinol triclosan
Metal ions	Zinc, copper, stannous
Natural molecules	Sanguinarine
Quaternary ammonium compounds	Cetylpyridinium chloride
Enzymes	Nuclease, glucose oxidase
Surfactants	Sodium lauryl sulfate
Bisbiguanides	Chlorhexidine

Surfactants nowadays are generally obtained from petroleum, which are very effective but are majorly toxic and difficult to degrade by microbial action. Hence, they are a big source of environmental pollution. These vulnerabilities caused by synthetic surfactants have drawn a lot of attention to the production of surfactants by microorganisms (biosurfactants). Keeping this in mind, the scientific community has been working toward achieving a class of surfactants that are easily degradable and are of utmost use. Biosurfactants hence could be helpful since they are formed by microbial action.

The study related to biosurfactants was initiated in the 1960s and now has extended their use in the recent decades. This class of surfactants is much preferred due to the following detailed properties:

1. *Tolerance to temperature, pH*: Many biosurfactants are known to be used at high pH (2−12) and temperature. They are also salt tolerant at up to 10%.
2. *Biodegradability*: Biosurfactants are used in bioremediation and waste treatment because of their high biodegradability by microorganisms in water or soil.
3. *Low toxicity*: Due to their low toxicity, biosurfactants are used in the pharmaceutical and food industries. They are also eco-friendly.
4. *Specificity*: Biosurfactants are of different types and consist of different functional groups, hence having different work actions. They are widely used in deemulsification and detoxification of pollutants.
5. Surface and interfacial activity: Effectiveness and efficiency are the two measuring parameters of biosurfactant activity. Efficiency is governed by CMC, whereas effectiveness is a measure of interfacial activity. The CMC ranges from 1 to 2000 mg/L and the surface tension of biosurfactants is 30 mN/m.

Synthetic surfactants make up around 13 million tonnes of the annual worldwide market. It is necessary to boost up the production and research about new applications for environment-friendly biosurfactants. They can hence reduce the reliability of synthetic surfactants. Research in this area unfolds that biosurfactants can possibly be applicable in various oral-related regions. Some of their properties, for example, antimicrobial action whether biocidal or biostatic, and against both Gram-positive and Gram-negative microbes, emulsion-framing capacity to make either steady or metastable microemulsions and the capacity to build bioavailability of hydrophobic mixes, which brings about a decrease of the powerful colonization of hydrophobics, makes biosurfactants possible applicants in restorative oral cleanliness products and oral-related clinical gadgets. Also, to optimize their ability, significant endeavors are needed to upgrade the nature of research.

3.4 Therapeutic properties of biosurfactants in biomedical field

3.4.1 Antimicrobial properties

It is important to understand biosurfactants antimicrobial mode of action. Synthetic surfactants have been studied to a large extent. The latter is known to compromise microbial cell surface reliability. For example, Chlorhexidine (antimicrobial surfactant) is found to damage the outer layer of the cell, it also causes the penetration of the cell wall by diffusion, leading to leakage of

intracellular constituents. Studies show that inactivation of ATPase occurs at high concentrations, which advocates that membrane disruption rather than enzymatic inactivation is associated with its mode of action [2].

Most of the biosurfactants have a common pattern of antimicrobial mode of action. The in vitro interaction between trehalose biosurfactants and antimicrobial lipopeptide surfactin has been studied. The phospholipid bilayer was studied by DSC (differential scanning calorimetry) and FTIR (fourier transform infrared spectroscopy), it was found that these interactions resulted in the absorption of trehalose into the lipid membrane. It results in deformities which lead to growth in the flexibility of the phosphatidylserine acyl chains and reduction in the hydration of certain areas of the bilayer membrane leading to conformational changes and membrane pore formation were observed upon NMR interactions between daptomycin and the lipid membrane [3].

Biosurfactants alter the hydrophobicity of the cell surface of the microorganism. This affects the interaction of the microorganism with different surfaces. Neu reviewed that microorganism−surface interaction depends on the nature of the cell surface, which requires attaching of biosurfactant by hydrophilic or hydrophobic moiety [4]. Also, depending on which part of the biosurfactant is extended to the exterior environment, biosurfactants arrange themselves on surfaces through adsorption and hence may change the surface nature. Recent reports explain, for example, the effects of *Pseudomonas aeruginosa* NBIMCC 1390 when treated with a concentration of rhamnolipids above their CMC caused an elevation in cell hydrophobicity, related to a decrease in the total lipopolysaccharides (LPS). Through the reduction in their outer membrane proteins, rhamnolipids were effective below their CMC concentration [5].

3.4.2 Antiadhesive properties

Many biosurfactants show antifungal, antibacterial, and antiviral activities, hence they are relevantly applicable in reducing different infections and diseases. Surfactins and iturins are known for their antimicrobial activity, which are produced by *Bacillus subtilis* strains, mannosyl erythritol lipids from *Candida antarctica*, rhamnolipids from *P. aeruginosa*, and biosurfactants isolated from *Streptococcus thermophilus* A and *Lactococcus lactis* 53. The use of biosurfactants as antiadhesive agents specifically against other pathogens (including Gram-positive and Gram-negative bacteria, fungi, and yeasts) in the gastrointestinal and urogenital tract, is an important application.

An evaluation of antiadhesive property of *Lactobacillus agilis* CCUG31450 was reported by Gudina et al. at 1 mg/mL concentration [6]. It exhibited a considerable antiadhesive activity against *Staphylococcus aureus* strain. Studies have also reported biosurfactants ability to reduce the pathogen adhesion to surgical implants, metallic surfaces, silicone rubber, and voice prostheses. They can hence control the adhesion of pathogenic microorganisms and result in reduced reliability of synthetic chemicals. An illustration of antiadhesive, antimicrobial, and antibiofilm activity of biosurfactants is shown in Fig. 3.5.

3.4.3 Antibiofilm properties

Antibiofilm is the property of biosurfactants to disrupt the biofilm on various substrates. Biosurfactants reduce the hydrophobicity of the bacterial cell wall selectively to reduce biofilm production. Additionally, they are also able to disrupt the preformed biofilms and down regulate the

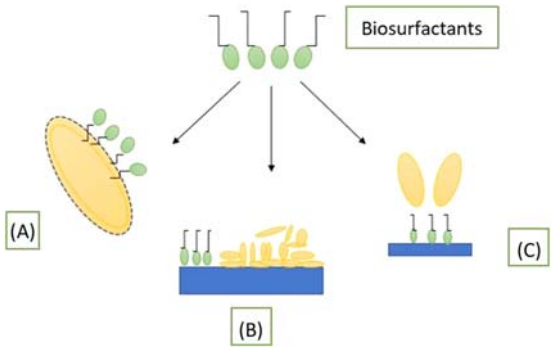

FIGURE 3.5

(A) Antibiotic activity; (B) antibiofilm activity; and (C) antiadhesion activity of biosurfactants.

virulent genes in the biofilm formers (e.g., *S. mutans*) [7–12]. Rufisan from *Candida lipolytica* UCP 0988, lipopeptide from *B. subtilis* AR 2, rhamnolipid from *Pseudomonas* have been well documented as antibiofilm agents. Table 3.2 provides a list of biosurfactants that act as antibiofilm agents.

3.4.4 Anticancer properties

The glycolipid biosurfactants such as polyol lipid, sophorose lipid, succinoyl trehalose lipid-1 and -3, mannosyl erythritol lipids (MEL-A) are reported to cause cell differentiation in human promyelocytic leukemia cell line HL60. MEL-A increased differentiation in granulocytes and succinoyl trehalose lipid-1 significantly increased differentiation in monocytes [21]. Studies also show evidence of growth arrest and physiologic apoptosis by glycolipids in malignant melanoma cells of mice [22].

3.4.5 Emulsion-forming properties

Microemulsions are formed due to the surface activeness of biosurfactants which enhance the emulsification of components to solubilize them. Emulsification increases the availability of components for microbial degradation. The high-molecular-weight compounds including polymeric substances and lipoproteins are also effective in stabilizing emulsions [23,24].

3.5 Biosurfactants from lactic acid bacteria strains

The members of lactic acid bacteria are classified as one of the potential biosurfactant producers in several studies but a key issue in these studies is the deficiency of complete characterization of the composition of the biosurfactant. Bacteria from lactic acid are well known for producing a wide variety of biosurfactants and metabolic products, having antimicrobial, antiadhesive, and

Table 3.2 Biosurfactants acting as antibiofilm agents against various biofilm-forming pathogens.

Biosurfactant as antibiofilm agents	Biosurfactant producer	Biofilm-producing pathogens	References
Di-Rhamnolipid	*Pseudomonas aeruginosa* DSVP20	*Candida albicans*	[12]
Rufisan	*Candida lipolytica* UCP 0988	*Escherichia coli*, *S. sanguinis*, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus agalactiae*, *Streptococcus oralis*, *Streptococcus aureus*, *Streptococcus pyogenes*, *Streptococcus epidermidis*, *Lactobacillus casei*, *Lactobacillus reuteri*, *Pseudomonas aeruginosa*, *Rothia dentocariosa*	[13]
Glycolipid type	*Trichosporon montevideense* CLOA72	*C. albicans*	[14]
Lipopeptide	*Bacillus tequilensis*	*E. coli*, *S. mutans*	[15]
Pseudofactin	*Pseudomonas fluorescens* BD5	*E. coli*, *C. albicans*, etc.	[16]
Lipopeptide	*Bacillus subtilis* AR 2	*C. albicans*	[17]
Glycolipid	*Lysinibacillus fusiformis* S9	*E. coli*, *S. mutans*	[18]
Uncharacterized	*Streptococcus thermophilus*	*Candida* species	[19]
Uncharacterized	*Lactobacillus fermentum*	*S. mutans*	[20]

antibiofilm activities which have imperative oral health−related advantages [25]. These bacteria form metabolic products like lactic acid, bacteriocins, and hydrogen peroxide which have antimicrobial activity. The two main reasons of why they gained popularity in dental and biomedical fields are the following: (1) they are an essential component of natural microflora [26] and (2) they have antiinfective properties [27]. *Lactobacilli* spp. possess surface protein namely the coaggregation promoting factor (Cpf) that coaggregate with the other pathogenic organisms, inhibiting the adherence of pathogens to host cells, ultimately preventing pathogenic microbial colonization [28]. Moreover, they also possess autoaggregation property promoting the formation of normal and balanced flora [29]. Table 3.3 summarizes the various studies on biosurfactants derived from lactic acid bacilli and their potential use in oral healthcare.

As observed from the table, one of the earliest studies on biosurfactants was on surfactin derived from *Lactobacillus acidophilus* RC14. Velraeds et al. studied 15 *Lactobacillus* strains to demonstrate the antiadhesive property of this biosurfactant against *Enterococcus faecalis* 1131. Subsequently, the ability of biosurfactants to inhibit the adhesion of various microbes was

Table 3.3 Lactic acid bacteria–derived biosurfactants and their major applications.

Organism producing biosurfactant	Type of biosurfactant	Major finding in the studies	References
Lactobacillus acidophilus RC14*Lactobacillus casei* 70*L. casei* subsp, rhamnosus GR-1*Lactobacillus plantarum* RC6 and RC20*L. casei* subsp, rhamnosus 36*L. acidophilus* T13, *Lactobacillus fermentum* B54*L. casei* subsp, rhamnosus 81	All 15 isolated biosurfactants are composed of protein like composition.	15 *Lactobacillus* isolates were found to be biosurfactant and B54 and RC14 prevented *Enterococcus faecalis* adhesion to glass surface.	[30]
L. acidophilus RC14	Surfactin (proteinous)	Initial adhesion of *E. faecalis* was prevented.	[31]
L. fermentum RC-14	Uncharacterized	*E. faecalis* and other pathogenic microbes were inhibited of adhesion in the presence of biosurfactants.	[32]
L. fermentum B54*Lactobacillus rhamnosus* 36*L. casei rhamnosus* ATCC 7469T	Proteinous biosurfactant	Initial adhesion of *E. faecalis* was inhibited on silicone rubber.	[33]
L. fermentum RC-14*L. rhamnosus* GR-1	Uncharacterized	Demonstrated the biosurfactant-caused inhibition of implant-related infection by *Staphylococcus aureus* in rats.	[34]
Lactococcus lactis 53 and *Streptococcus thermophilus* A	Partially characterized glycoprotein	The biosurfactants derived from *L. lactis* and *S. thermophilus*, greatly reduced the number of microbes on the voice prostheses and decreased the airflow resistance.	[35]
L. lactis 53	Partially characterized glycoprotein	Expanded the characterization approach to determine the various properties of the earlier extracted biosurfactant against microbes obtained from an explanted voice prosthesis.	[36]
Lactobacillus fermenti 126*L. acidophilus* PG 8/14*L. rhamnosus* CCM 1825	Uncharacterized	Demonstrated the antiadhesive property of the biosurfactant.	[37]
L. acidophilus	Glycoprotein type	Demonstrated the influence of biosurfactant to inhibit the adhesion and biofilm of *S. aureus* and *Streptococcus epidermis*.	[38]
Lactobacillus paracasei ssp. paracasei A20	Uncharacterized	Demonstrated the antimicrobial and antiadhesive properties.	[39]
L. paracasei ssp. paracasei A20	Uncharacterized	Expanded the approach for the characterization, that is, antimicrobial and antiadhesive activities on numerous pathogenic microbes.	[40]

(*Continued*)

Table 3.3 Lactic acid bacteria–derived biosurfactants and their major applications. ***Continued***

Organism producing biosurfactant	Type of biosurfactant	Major finding in the studies	References
L. acidophilus	Glycoprotein	Studied the crude biosurfactant—surfactin, its pH, and other properties.	[41]
Lactobacillus spp. CV8LAC	Partially characterized	Explored the biosurfactant's antiadhesive property on *Candida albicans*.	[42]
L. acidophilus DSM 20079	Uncharacterized glycoprotein type	Demonstrated the decreased expression of *gtfB* and *gtfC* genes (major contributors for biofilm formation) due to biosurfactant.	[43]
L. fermentum ATCC9338	Composition not stated glycoprotein type	Investigated the decreased effect of biosurfactant on *Streptococcus mutans* gene expression of *gtfB*, *gtfC*, and *ftf* genes.	[44]
L. rhamnosus	Crude biosurfactant	Study demonstrated the antiadhesion and antibiofilm of biosurfactant on *Escherichia coli*, *Burkholderia cepacia*, *S. aureus* biofilms.	[45]
Lactobacillus jenseniiL. rhamnosus	Uncharacterized	Study demonstrated the antimicrobial, antiadhesive, and antibiofilm properties against *S. aureus*, *E. coli*, and *Acinetobacter baumannii*.	[46]
L. casei	Glycolipids	Study demonstrated the antibacterial activities in form of simultaneous production of biosurfactants and bacteriocins by *L. casei*.	[47]
Lactobacillus helvecticus	Glycolipid (similar to xylolipids)	Studied the characterization of the biosurfactant produced from *L. helvecticus* and suggested the application of biosurfactant for oral-related aspects.	[48]
Lactobacillus agilis CCUG31450	Glycoprotein	Demonstrated antiadhesive activity against *S. aureus* and antimicrobial activity against *S. aureus*.	[49]
Lactobacillus brevis CV8LAC	Mixture of various components. Also includes sugar as a component	Study demonstrated inhibition of *C. albicans* biofilm on application of *Lactobacillus*-derived biosurfactant.	[50]

Table 3.3 Lactic acid bacteria–derived biosurfactants and their major applications. ***Continued***

Organism producing biosurfactant	Type of biosurfactant	Major finding in the studies	References
Lactobacillus pentosus	Glycolipopeptide type (with C:18 and C:16 fatty acids)	Study demonstrated the fatty acid characterization of *L. pentosus*. Higher and stable emulsion than polysorbate 20 are produced by biosurfactant derived from *L. pentosus*.	[51]
L. jensenii P_{6A} and *Lactobacillus gasseri* P_{65}	Not specified	Both biosurfactants showed reduced surface tension and antimicrobial activity against *E. coli*, *E. aerogenes*, *C. albicans*, and others.	[52]
L. acidophilus	Not specified	Demonstrated the various properties like antibacterial, antiadhesion, and antibiofilm properties on PDSM-based implants.	[53]
L. rhamnosus	(Composition not stated) glycoprotein type	Investigated the decreased effect of biosurfactant on *S. mutans* gene expression of *gtfB*, *gtfC*, and *ftf* genes.	[54]

demonstrated in an artificial throat model, on the biofilms grown on preconditioned voice prosthesis with the biosurfactants derived from *L. lactis* 53 and *S. thermophilus* A. This finding was demonstrated in 2004 by Rodrigues et al. to increase the lifespan of the voice prostheses in laryngectomized patients. The results were promising as both the biosurfactants decreased significant bacteria concentration in the biofilm on the prostheses and also reduced the resistance of airflow that occurs on the prostheses after the development of biofilm. A few years later, the team characterized the biosurfactant fractions produced by *Lactococcus lacti* and identified it to be a combination of polysaccharides and protein which perhaps contained bounded groups of phosphate. Furthermore, the isolated biosurfactant was found to be antiadhesive and antimicrobial in nature even at lower concentrations against various oral-related opportunistic microbes like yeast and other bacterial strains that were obtained from explanted voice prostheses.

As already mentioned, *S. mutans*, a Gram-positive bacterium is mainly responsible for dental biofilm formation in the oral cavity. The virulence factor of *S. mutans* is because of its ability to produce polymers like glucans from sucrose using glucosyl transferases (gtfs) and fructans from fructose moiety of sucrose using fructosyl transferase (FTF). *S. mutans* secretes gtfs (mainly gtfB and gtfC) that change the formation of biofilm and provides binding sites for either bacterial attachment to each other or bacterial colonization on dental surfaces. gtfs (particularly gtfC) are incorporated into the pellicle and on bacterial surfaces (mainly gtfB). This pioneer incorporation of gtfs on pellicle surfaces leads to rapid utilization of dietary sucrose for the synthesis of insoluble and

soluble glucan molecules which provide binding sites on the surfaces, ultimately leading to dental caries and other periodontal diseases. However, it has been suggested that for expression of the gtfs and FTF-related activities, gtfB, gtfC, and FTF genes are important. These genes have an operon-like arrangement for the encoding of these enzymes.

Tahmourespour et al. (2011) studied the decreased expression of *gtfB and gtfC* genes of *S. mutans* strain from the dental plaque in the existence of *L. acidophilus* derived biosurfactant, using RT-PCR (reverse transcription-polymerase chain reaction) quantitation method. This study demonstrated that the biosurfactant acted against *S. mutans* biofilm by the altered formation of biofilm, decreased gene expression level, reduced adhesion ability, and favorable surface tension properties. In the same context, Satpute et al. (2019) studied the antibacterial, antiadhesion and antibiofilm properties of *L. acidophilus* derived biosurfactant on polydimethylsiloxane-based (PDMS) implants. They noted a significant reduction in interfacial tension produced by the surfactant which could prevent the formation of biofilms on PDMS.

Biofouling is the adhesion of microbes or proteins to the biofilm in humans. Antibiofouling is an inexpensive and effective method to control or remove the accumulation of plaque with the help of natural products (like biosurfactants) produced by various microbes. Tahmourespour et al. (2011) explored antibiofouling nature of the biosurfactant derived from probiotic bacteria *Lactobacillus fermentum* against *S. mutans*. They successfully demonstrated reduced biofilm production and attachment of bacteria by the biosurfactant. Additionally, this study also showed a reduction in *gtfB* and *C* genes expression by *S. mutans*. Salehi et al. (2014) also demonstrated the effect of biosurfactants from another strain of *Lactobacillus*, that is, *Lactobacillus reuteri* DSM20016 culture on the gene expression profile of *glucosyltransferases (gtfs)-gftB/C* and *fructosyltransferase (ftf)* genes of *S. mutans* ATCC35668. Savabi et al. also reported decreased expression of *gtfB* and *gtfC* and *ftf* genes of *S. mutans* with the use of biosurfactant derived from *Lactobacillus casei* (ATCC39392) [55]. All of the genes had been dramatically down regulated with the application of the biosurfactant [56]. Substantial downregulation of the same genes was also reported by Tahmourespour et al. (2019) in *S. mutans* caused by *Lactobacillus rhamnosus*-derived biosurfactant. Collectively all these studies endorse the use of *Lactobacillus*-derived biosurfactants as potential candidates in newer generations of microbial-derived antiadhesive agents.

Lactobacillus brevis CV8LAC derived biosurfactant proved to inhibit the adhesion and biofilm formation of oral candidiasis causing organism, that is, *C. albicans*. The biosurfactant significantly lowered the biofilm growth and also inhibited the initial adhesion of the pathogen silicone elastomeric disk.

The minimum inhibitory concentration (MIC) for each of the pathogenic organisms varies. Moraiset et al. reported the MIC of biosurfactants derived from *Lactobacillus jensenii* P_{6A} and *Lactobacillus gasseri* P_{65} strains of lactobacilli separately against *Staphylococcus saprophyticus*, *E. coli*, *Enterobacter aerogenes*, *C. albicans*, and *Klebsiella pnemoniae*. They noted that disruption of preformed biofilms occurred at varied concentrations of biosurfactants for each of the microorganism.

Among four major species of lactobacilli (*Lactobacillus casei*, *Lactobacillus reuteri*, *Lactobacillus plantarum*, and *Lactobacillus salivarius*), *L. salivarius* showed the highest antibiofilm with antimicrobial action. All lactobacilli supernatants showed reduced gene expression of *S. mutans* and a reduction in bacterial microcolony formation under SEM (scanning electron microscopy) [57].

Lipoteichoic acid (LTA) is a vital cell wall element of Gram-positive microorganisms and is involved in the formation of biofilm [58]. It is an amphiphilic glycolipid that is linked to a

hydrophilic phosphate polymer [59]. Ahn and his coworkers noted that LTA from *L. plantarum* caused disruptions in the biofilm formation of *S. mutans* [60]. This was mediated through downregulation of exopolysaccharide (main component of biofilm) production as LTA interfered with the decomposition of sugar in *S. mutans*.

3.5.1 Cytotoxic effects of lactic acid bacteria–derived biosurfactants

Cytotoxicity of lactobacilli derivatives can be measured by the quantity of lactic dehydrogenase released and the total cell number assay. Sharma et al. demonstrated that with the increase in the concentration (6.25–25 mg/mL) of biosurfactant obtained from *Lactobacillus helvecticus*, a gradual decrease in the cell viability can occur. On the other hand, in a study, no toxicity was observed at 25–100 mg/mL but the lower level of toxicity was found at 200 mg/mL concentration by both the surfactants, derived from *L. jensenii and L. rhamnosus*, respectively [61]. There is very less literature available to confirm the cytotoxic effects of biosurfactants from *Lactobacillus* spp. which are considered potentially safe candidates for use in oral healthcare as compared to commercially available rhamnolipids [62].

3.6 Other sources of biosurfactants

3.6.1 Biosurfactants from endophytes

Amongst the wide class of biosurfactants, endophyte derivatives have antifungal and antibacterial properties as they produce lipopeptide molecules. These agents are medically used as a coating agent on medical devices, for example, catheters and dentures prostheses to avoid fungal adhesion. A study by Cochis et al. (2012) demonstrated the antiadhesive action of endophyte-derived biosurfactant against *C. albicans* [63]. A combination of biosurfactants extracted from *Robina pseudoacacia* and *Nerium oleander* significantly decreased the number of biofilm cells of *C. albicans* over silicon and denture prostheses.

3.6.2 Biosurfactants from *Candida*

C. albicans-derived biosurfactant rufisan can reduce the surface tension of water 2.5 times than the original, with a CMC of 0.03% [64]. Rufisan, isolated from *C. lipolytica* UCP 0988 in 2011 by Rufino et al., showed antimicrobial action against *Streptococcus* at a concentration higher than CMC. This biosurfactant proved out to be effective against a wide range of microorganisms found in the biofilms of the oral cavity. Overall, the results suggest that rufisan is an alternative to conventional antibiotics. However, there is no information on the toxicity levels of this biosurfactant toward human systems.

3.6.3 Biosurfactants from *Pseudomonas*

Pseudomonas is a Gram-negative bacterium. Its outer membrane act as a permeability barrier to various kinds of antibiotics as it is composed of LPS, lipoproteins, and phospholipids [65].

Rhamnolipid, which is a widely studied biosurfactant, is produced by *Pseudomonas*. Sotirova et al. (2009) showed the concentration-dependent variation in the action of rhamnolipid-biosurfactant (derivative of *Pseudomonas* sp. PS-17). At concentrations below CMC, the biosurfactant led to changes in the outer membrane protein composition, and at a concentration above CMC, it caused a reduction of cellular LPS content of the bacteria cell wall of *P. aeruginosa* [66]. Since changes in cell surface occurred at both concentrations, the biosurfactant caused an inhibitory effect on the bacterial cells without disturbing their growth and feasibility making the rhamnolipid-biosurfactant highly useful in biomedical and dental fields to eliminate a variety of pathogenic microbes.

In a study by Bonnichsen et al., disruption of *Pseudomonas fluorescens* SBW25 biofilms was studied in the presence of the lipopeptide viscosin [67]. This experiment addressed the particular role of lipopeptide viscosin, by comparing the biofilm production of *P. fluorescens* SBW25 and the viscosin deficient mutant strain SBW25DviscA in microtiter assays. Lipopeptide biosurfactant viscosin improves the dispersal of *P. fluorescens* SBW25 biofilms as confirmed by chemical complementation of the mutant biofilm.

3.6.4 Biosurfactants from streptococcus

Right after birth, *Streptococcus* becomes the first commensal of the oral cavity which plays an essential part in oral microbiota assembly. Due to their adhesive nature and ability to metabolize carbohydrates through fermentation, they are considered as chief colonizers in the mouth. Moreover, *S. mutans* is directly linked with the development of dental caries. However, certain other oral streptococci are considered useful to the host by producing molecules that can inhibit the growth of *S. mutans*.

van Hoogmoed et al. (2000) found that *Streptococcus mitis* derived biosurfactant caused a significant reduction in the growth of colonizing microbes *S. mutans* on a glass surface [68]. The biosurfactant released by *S. mitis* is a mixture of glycosidic-like residue and rhamnolipid. The same team went on to show the interaction between *S. mutans* and tooth surfaces adsorbed with the biosurfactant [69]. They recorded a significant rise in electrostatic repulsion of *S. mutans* on approaching the enamel surface of the tooth.

Several attempts have been made on lowering the adhesion of microbes to the silicone rubber in voice prostheses. Voice prostheses are artificial devices, used for speech recovery, implanted in between food pipe and windpipe in patients after laryngectomy surgery. Because of its excellent molding and other mechanical properties, these prostheses are usually made of silicone rubber. However, colonization of oral infection-causing pathogens, that is, yeast such as *Candida* and other bacteria like streptococci have been isolated in form of thick biofilm on its surface in laryngectomized patients [70]. These could block the valve of the prostheses, resulting in failure of the device. These bacteria grow on the sides of the valve of explanted prostheses, making it more difficult for removal by naturally occurring shearing forces. An interesting finding is that the number of pathogens on the prosthesis had a positive correlation with the poor periodontal status of the laryngectomized patients [71].

An early investigation by Busscher et al. (1997) was done to study the effect of a biosurfactant derived from *Streptococcus thermophiles* B on the linkage of two candida strains (*C. albicans* and *Candida tropicalis)* to silicon rubber obtained from naturally colonized voice prostheses [72]. It was found that the biosurfactant was effective in decreasing adhesion of both *Candida* strains but the preadsorbed biosurfactants only lowered the adhesion of *C. albicans*, not of *C. tropicalis*. At that stage, it was not considered feasible to release biosurfactant-coated voice prostheses.

3.7 Applications of biosurfactants in oral health

The antimicrobial, antiadhesive, antibiofilm, and bioemulsion forming traits of biosurfactants make them potential candidates for the production of oral health–related formulations creating a viable option for control of cariogenic and infection-causing pathogens. One such application in dentistry is dentifrice (toothpaste, mouthwashes, etc.) formulations for maintaining oral and overall health. These formulations mainly perform three important functions in maintaining oral and overall health: (1) detergent action to cleanse the oral cavity, (2) abrasive action for a bleaching effect on tooth, and (3) release of therapeutic compounds [73,74]. Table 3.4 summarizes the composition of toothpastes with the function of each component.

Boussida et al. (2017) proved that physicochemical properties of a lipopeptide biosurfactant derived from *B. subtilis* SPB1 were as resourceful as a chemical surfactant for antiplaque activity. Additionally, the lipopeptide showed antimicrobial activity against eight different bacteria [75].

Commercial toothpaste can be replaced by bioformulated toothpaste, as shown by Das et al. [76]. Glycolipid biosurfactant is used to produce the toothpaste along with baking soda, common salt, glycerin, and calcium carbonate. The resultant toothpaste was also tested for the following properties:

Table 3.4 Components in toothpaste and their functions.

Components in toothpaste	Functions of the components
Abrasive	It has a primary function to remove stains and plaque from the tooth surface due to their abrasive action and polish the tooth.
Binding agents	Controls the viscosity of the toothpaste. Also, acts as an emulsifier and gives the toothpaste creamy consistency.
Surfactants	They are the essential component that reduces the surface tension, allows contact between teeth and toothpaste. Also, produces foam that aids in plaque removal due to their surface action.
Water (solvent)	Act as a solvent and allows the contents to dissolve, making the paste of uniform consistency.
Humectants	They are the polyalcohol and are added to prevent the drying of toothpastes.
Flavoring and coloring agents	Flavoring agents contain essential oils that primarily give pleasant flavor and coloring agents provide attractive color to the paste.
Sweeteners	Provide a mild sweet taste.
Therapeutic agents	1. Therapeutic agents like sodium fluoride, monofluorophosphate, and stannous fluoride are added to prevent caries. 2. Antiplaque agents like triclosan that has an inhibitory effect on a wide range of microbes. 3. Metal ions like zinc (Zn^{+2}) and stannous (Sn^{+2}) are added to inhibit the glycolytic sequence in oral bacteria (anaerobic) which inhibit their growth. 4. Enzymes like glucose oxidase are also added that act as antiplaque agents.
Preservatives	For prevention of the growth of microorganisms.

1. *Abrasiveness*: Scratches produced by toothpastes (commercial and formulated) on the surface of a slide were observed under the microscope. Relative dentin abrasiveness (RDA) was measured. A value of 0−70 RDA corresponds to low abrasiveness, a value of 70−100 indicates medium abrasive, 100−150 shows highly abrasive and 150−250 is regarded as harmful limit [77]. Here, both had the same abrasiveness.
2. *Spreadability*: The commercial and formulated forms were flattened on a surface with a heavyweight and noted for the increase in diameter, to check the consistency. The spread showed a good consistency for the formulated toothpaste with no separation into solid and liquid constituents.
3. *Bacterial load*: A toothpaste is formulated to decrease bacterial growth of the oral cavity and mouth [78]. Food digestion in the oral cavity starts with the breakdown of carbohydrates, fat, vitamin, and protein. Oral bacteria help in the breakdown of carbohydrates into acids and also feed on leftover food within teeth and in the mouth. There are various kinds of oral bacteria both useful and harmful for example *Neisseria*, *Haemophilus*, *S. pneumoniae*, *Porphyromonas gingivalis*, *Diphtheroid*, *Fusobacteria*, and *Staphylococcus*. Most of these mouth bacteria cause toothache, decay, and plaque. The formulated toothpaste had reliable antibacterial properties
4. *Cleaning*: Both kinds of toothpastes had the same cleaning abilities.
5. *Removing yellowness*: Both the toothpastes had similar yellowness removing abilities.
6. *Toxicity*: Biosurfactants are biodegradable and nontoxic in nature. Hence, they are used in the formulations of toothpastes. Toxicity in cosmetic products is an important trait. Das et al., showed that the formulated toothpaste was less toxic. This can be a major criterion for the application of biosurfactants in dentistry.

An interesting study in this subject was done by Resende et al. in 2019 [79,80], where biosurfactants were derived from *Bacillus metylotrophicus*, *Candida bombicola*, and *P. aeruginosa* to develop and test six different toothpastes and mouthwashes against *S. mutans* biofilms. As compared to commercial toothpastes these formulations were found to be nontoxic, inhibited *S. mutans* biofilms, had a pH around 9 with a foaming capacity of 63%−95%, and consistency in terms of spreading capacity was 8−17 mm. The mouthwash contained biosurfactant, chitosan, and peppermint essential oils and the toxicity of the developed formulation was lesser than the commercial one, which indicated that these mouthwashes are effective and safe for controlling oral microbes.

3.8 Biosurfactants and future goals

All aspects of biosurfactants—monitoring, measurement, isolation, and applications are issues of utmost importance. They have also earned interest in both the medical as well as oral health sectors because of their various therapeutic properties and advantages over the synthetic ones. Moreover, bacterial biofilm is a major issue due to rising numbers of bacterial resistance and a higher risk rate of caries and periodontal infections in individuals. The opportunities for designing new biosurfactant-based formulations in the dental field are increasing. One area that requires further research is the characterization of biosurfactants to understand the molecular mechanism of action of each of the agents. An improved understanding of the properties of biosurfactant molecules would enable the efficient use of these agents in oral healthcare. Another area of research could be

to ascertain the specific MIC of each biosurfactant against different microbes. This could help in developing oral healthcare products with appropriate concentrations of biosurfactants to make them effective against various pathogenic microorganisms

3.9 Conclusion

Studies in this field clearly illustrate that biosurfactants can be potentially applied in cosmetic and therapeutic fields, specifically in oral-health−related areas. They are reliable because of their biodegradable and nontoxic nature as compared to synthetic surfactants. Their dentistry applicable properties include antimicrobial activity, emulsifying nature, increasing bioavailability, and some anticancer properties. Moreover, biosurfactants can also be formulated into toothpastes which work as effectively as commercial toothpaste.

However, to increase the biosurfactants applicability, attempts from researchers are needed which would improve the quality of research. The studies in this particular area need to be refined to attract industrial collaborators. To assign specific activity to biosurfactants, it is required to use a highly pure, one-molecule species to certify that the produced effects are not because of a random molecule, which may vary from one production batch to another. This could be highly therapeutic in chemically modifying the naturally available complex congenators to reduce toxicity or enhance the efficacy and also on its own. Moreover, biosurfactant sterilization methods require further evidence-based understanding.

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CHAPTER

Expansion of targeted drug-delivery systems using microbially sources biosurfactant

4

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4.1 Introduction

Microorganisms are the main producers of biosurfactants (surfactants from a biological source). This type of surfactants are amphiphilic molecules with diverse chemical structures, emulsifying characteristics and biological function [1]. The main functions of biosurfactants are the capacity in controlling the elimination and attachment of microorganisms from surfaces, biofilm construction, hydrophobic compounds bioavailability, heavy metal binding, bacterial pathogenesis, and antimicrobial properties [2]. Besides, microbial surfactants typically present higher biodegradability and lesser toxicity when compared to chemical counterparts [1,3]. Due to these properties, biosurfactants became an attractive class of biological compounds with diverse applications in the pharmaceutical and medicinal sectors [4–6].

The discovery of novel microbial surfactants with improved efficacy had a deep influence on the progress of biosurfactant-based drug-delivery systems (DDS), considering the safe drug release in the specific desired local. The micellar characteristic of biosurfactants also allows a proper preparation of stable DDS, conferrring protection to the drug, that is, preventing its degradation [7]. An ideal biosurfactant-based DDS must have an appropriated drug loading with subsequent controlled drug release. Accordingly, the biosurfactant carrier can be designed to occur in different structural forms, such as vesicular systems, micelles, nanoparticles, and microspheres [8]. Biosurfactants used in DDS are from different biosurfactants classes, namely glycolipids such as mannosylerythritol lipids (MELs), succinoyl trehalose lipids, rhamnolipids, sophorolipids, and lipopeptides [9–13].

This chapter highlights the actual status of surfactants from microorganisms, namely structure and properties of glycolipids such as rhamnolipids, MELs, sophorolipids and succinoyl trehalose lipids, and lipopeptides such as surfactin in DDS, with a special focus on liposomes, niosomes, and nanoparticles.

4.2 Microbial biosurfactants

Surfactants from microorganisms are classified into two main categories, namely low molecular weight and high molecular weight. The first category comprises polymeric biosurfactants, while the

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other category includes glycolipids, lipopeptides, lipoproteins, fatty acids, and phospholipids [14]. In this chapter, surfactants from microorganisms with application in DDS are discussed, namely glycolipids and lipopeptides.

4.2.1 Mannosylerythritol lipids

Glycolipids such as MELs contain fatty acids (hydrophobic group) and are a combination of the partially acylated derivative of 4-*O*-β-D-mannopyranosyl-D-erythritol [15]. The main differences of MELs composition are the amount and acetyl groups location on erythritol and mannose, and the fatty acid chain saturation and length [16,17]. MELs are categorized as MEL-A, -B, -C, -D. This classification is based on C-4′ and C-6′ acetylation degree of the mannopyranosyl. When the compound is diacetylated, it is named as MEL-A, on the other hand, monoacetylated at C-6′ and C-4′ compounds are named as MEL-B and MEL-C, respectively, whilst MEL-D has a completely deacetylated structure [16,17]. MELs hydrophobic portion comprises fatty acids from C2:0 to C18:1. The profiles of MELs fatty acid are of a wide variety [17]. For instance, MEL-A from *Candida pseudozyma* sp. was produced as the main mannosylerythritol, with C6:0, C12:0, C14:0, and C14:1 [18]. In another work [19], MEL-C was the main mannosylerythritol produced by *Pseudozyma hubeiensis* containing C16:2, C12:0, C10:0, and C6:0.

New structures of mannosylerythritol have been recognized as mono- and tri-acetylated, wherein mannopyranosyl C-2′, C-4′, and C-6′ are connected to OH or OAc [17]. For instance, a diastereomer type of MEL-B containing a sugar fraction was recognized as 1-*O*-β-D-mannopyranosyl-erythritol. This MEL is distinct in terms of stereochemistry when compared to traditional 4-*O*-β-D-mannopyranosyl-erythritol [20]. Additionally, a MEL constituted with the lipid mannosyl-mannitol and mannitol (as a substitute for erythritol) was reported by Morita et al. [21].

4.2.2 Succinoyl trehalose lipids

Glycolipids such as succinoyl trehalose lipids (STLs), constituted by 1 or 2 succinic acids, are attached to 2 or 3 fatty acids and connected to a trehalose [22,23]. Different STLs have been reported, being STL-1, STL-2 and STL-3 categorized as 3,4-di-*O*-palmitoyl-2,2′-di-*O*-succinoyl-α,α-trehalose; 2,3,4-di-*O*-alkanoyl-*O*-succinoyl-α,α-trehalose; and 2,3,4,2′-mono-*O*-succinoyl-tri-*O*-alkanoyl-trehalose, respectively [24]. On the other hand, the precise acyl chains in STL-2 and STL-3 position is not yet established. Nevertheless, based on STL-1 chemical structure, the location of succinic acid may be at *O*-position [24].

The production of two types of STLs (STL-1 and STL-2) by *Rhodococcus* sp. was reported by Uchida et al. [25]. Each STL presents groups of hydrophobic acyl of both STLs and have the same carbon chain length when *n*-alkane was applied as the carbon source [25]. In another work, STL-1 structural characterization shows a trehalose lipid with two residues of hexadecanoyl and succinoyl [23].

4.2.3 Sophorolipids

Sophorolipids are surfactants constituted by a long-chain hydroxy fatty acid, sophorose residue (acetylated 2-*O*-β-D-glucopyranosyl-D-glucopyranose), and 2 glucose residues connected by a β-1,2′ bond [26]. Sophorolipids differ in the number and position of *O*-substituents acetate groups in the fatty acid residues and the carbohydrate residue [27]. The acetylation in the residue containing

sophorose can be done in 6-position. The hydroxylated fatty acid terminal is the β-glycosidically attached to the sophorose [26]. C16 or C18 is usually the residue of hydroxy fatty acid with one or additional unsaturated bonds [26]. Furthermore, the carboxylic group is inside esterified at location C4 or free [27]. Sophorolipids can also be synthetized as monomeric or dimeric forms (lactones) including fatty acids [28]. *C. batistae* and *S. bombicola* produced sophorolipids with a distinct location of the hydroxylic group in the fatty acid residue, while *S. bombicola* produced different sophorolipids in the ω-1 and ω-positions [29]. Di- mono- and non-*O*-acetyl are produced by *Candida apicola* in the free-acid lactone forms [26].

4.2.4 Rhamnolipids

Glycolipids such as rhamnolipids (RLs) are biosurfactants usually produced by *Pseudomonas aeruginosa* [30]. The connection between the two rhamnose portions occurs by α-1,2-glycosidic bonds [31]. Accordingly, the authors chemically described these RLs as a glycolipid comprehending a connection between a sugar and a hydroxylated fatty acid residue. Overall, in the last decade, a substantial amount of novel RLs were recognized, and novel congeners are being described [32].

RLs may be characterized as glycosides constituted by rhamnose and lipid portions attached by *O*-glycosidic bonds. The glycon moiety is constituted by one rhamnolipid or two rhamnolipids rhamnose moieties connected by α-1,2-glycosidic bond. The moiety composed of glycon is constituted by chains of β-hydroxy fatty acid linked by an ester bond. Majorly, the carboxyl group of the β-hydroxy fatty acid is free [32]; but, in some cases, rhamnolipids present a short alkyl group esterified with this group. Differences among these homologes are essentially the variations in glycon moiety contributing to the differences among the rhamnolipids types [32].

4.2.5 Surfactin

Surfactin is the most studied cyclic lipopeptide and it is composed of a heptapeptide chiral sequence interconnected with a fatty acid chain of β-hydroxy (C12-C16 carbons), creating a ring structure of cyclic lactone [13,33]. Hydrophilic amino acids are situated on 1′ and 5′ positions [13], while hydrophobic amino acids residues are located on 2′, 3′, 4′, 6′, and 7′ positions. Surfactins S1 and S2 have two conformations, with both showing a saddle-shaped structure [34]. Additionally, both surfactins a disposed a "claw" with a polar head, opposite to a hydrophobic domain [33]. The production of three surfactins from *Bacillus subtilis* is already established [35] being two of them different from the basic structure already stated above. Later, it was found that, when incubated using the same culture medium, *B. subtilis* produced three types of surfactins. The main differences are in peptide moieties, that is, in the sequence of amino acids [36]. Moreover, as in peptide moieties, the fatty acid moiety was also identified as distinct, including different beta-hydroxy-based fatty acids [36].

4.3 Microbial biosurfactants as drug-delivery systems

DDS are an important area of drug development in pharmaceutical and medicinal sciences, and are characterized as part of a drug formulation or as a device/vehicle, which allows the controlled

administration and release of a therapeutic compound (active ingredient) into a body-specific part as increasing efficiency and security [7,8]. DDS were discovered in 1909 when Paul Ehrlich (Nobel Prize) developed a drug-delivery mechanism that directly targeted a diseased cell without destroying the adjacent normal cells [37,38]. These systems present several advantages, among them, the optimal drug loading capacity with no loss of drug, improved bioavailability and aqueous solubility; and the easier and controlled transportation of the active substance through the membranes to the desired place getting the maximum efficacy [7,8]. Using DDS, the active ingredient can be administrated into the human body through a variety of paths, depending on the effect desired and disease (Fig. 4.1).

Different carriers have been discovered and investigated to concentrate the quantity of therapeutic agents to be delivered at the desired-specific site. The most investigated carriers consist of surfactants, nanomaterials, polymers, and microspheres [39]. More recently, nanocarriers have been also reported as a good methodology in DDS with unique and interesting properties, namely protection of the active compound from degradation and enhanced permeability [4]. Therefore, these carriers can be used in DDS as vehicles to encapsulate vaccines, drugs, nucleic acids, polypeptides, proteins, among others, to provide the active compound to the target site [40]. Due to their natural origin, microbial biosurfactants as DDS offer additional attractive properties than their synthetic surfactants, since they present small toxicity and improved biodegradability. Additionally, the improved solubility of biosurfactants allows the improvements in the active compound availability. Furthermore, when compared to traditional DDS, biosurfactants-based DDS could directly deliver

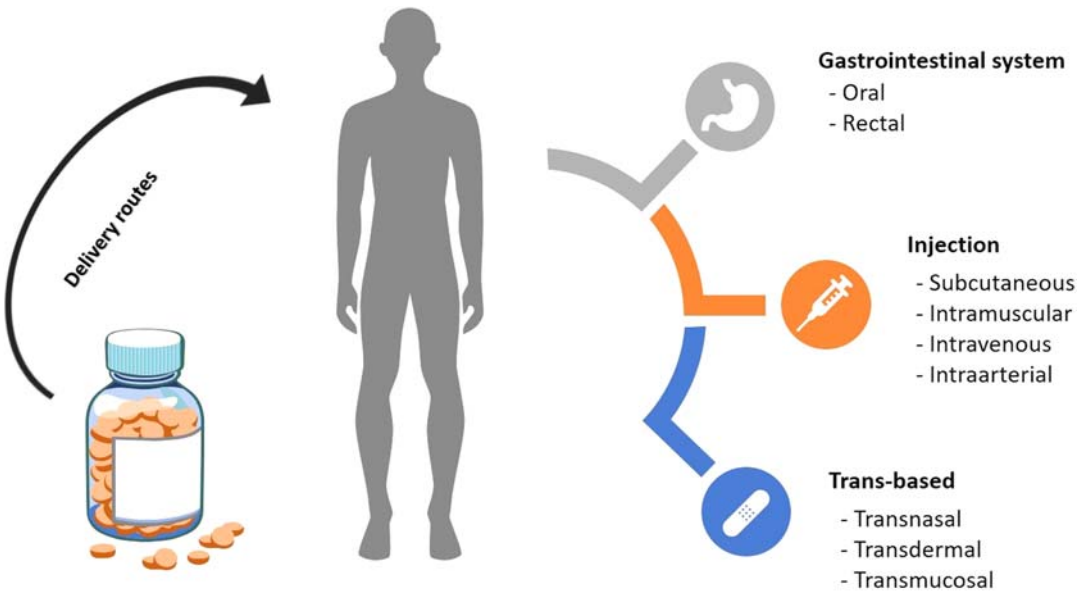

FIGURE 4.1

Main routes for biosurfactant-based drug-delivery systems (DDS). A diversity of carriers has been discovered and investigated to concentrate the quantity of therapeutic agents to be delivered at the target-specific organ/tissue.

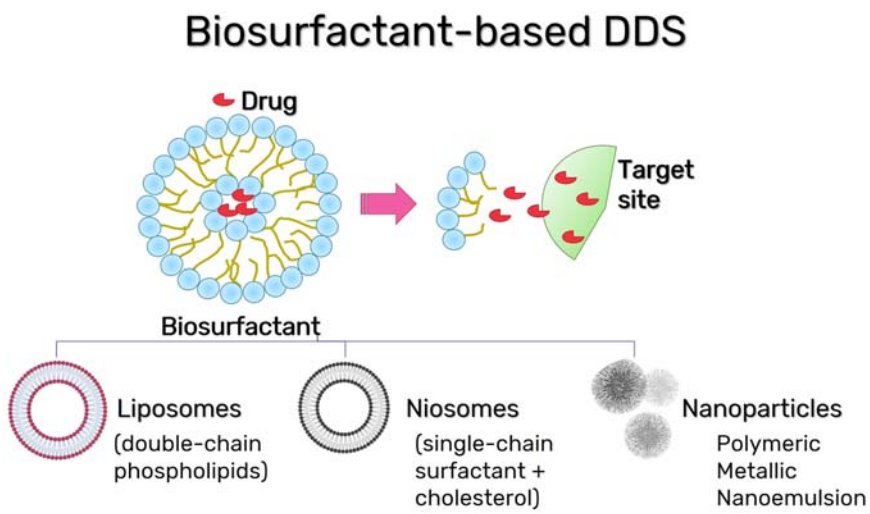

FIGURE 4.2

Types of microbial biosurfactants for drug- delivery systems (DDS).

the active compound to the site to be treated while protecting it from the severe surrounding conditions which would otherwise promote its dysfunction [41]. The ability of biosurfactants-based DDS in mediating drug delivery provides safe delivery of the active compound to the target, in addition to the natural treatment properties of biosurfactants at the site to be treated while maintaining dose proportionality. Novel DDS based on biosurfactants have been developed. They are mainly composed of nanoparticles, liposomes, and niosomes, as depicted in Fig. 4.2 and discussed below.

4.4 Types of biosurfactant-based drug-delivery system

4.4.1 Liposomes

Liposomes are (phospho)lipid-based vesicles containing extra bilayers of lipids, which surround internal aqueous sector(s), applied in DDS for a broad range of elements such as hydrophilic (internal aqueous area), lipophilic (lipid bilayer), and amphiphilic (among these two sections) [8,42–44]. As presented in Fig. 4.3, there are several types of liposomes, namely small ($\approx$100 nm) and large (200–800 nm) unilamellar (single bilayer) vesicles [45]; multilamellar vesicles (500–5000 nm with numerous concentric bilayers) [46]; long-circulating liposomes (generally surface-grafted with specific polymers) [47]; immunoliposomes (antibodies surface-bound) [48]. These nanovesicles are suitable DDS for distinct drugs, for example, antimicrobial, antiasthma, antioxidants, and cytostatics through different routes, for example, ocular and pulmonary [49].

In 2001, the nanocomplex Sit-G-liposome/DNA constituted by Tfx-20, a cationic lipid [*N*,*N*,*N*′,*N*′-tetramethyl-*N*,*N*′-bis(2-hydroxyethyl)-2,3-di(oleoyloxy)-1,4-butanediammonium iodide], L-dioleoylphosphatidylethanolamine (DOPE), 3β[*N*-(*N*′,*N*′-dimethylaminoethane)-carbamoyl]cholesterol

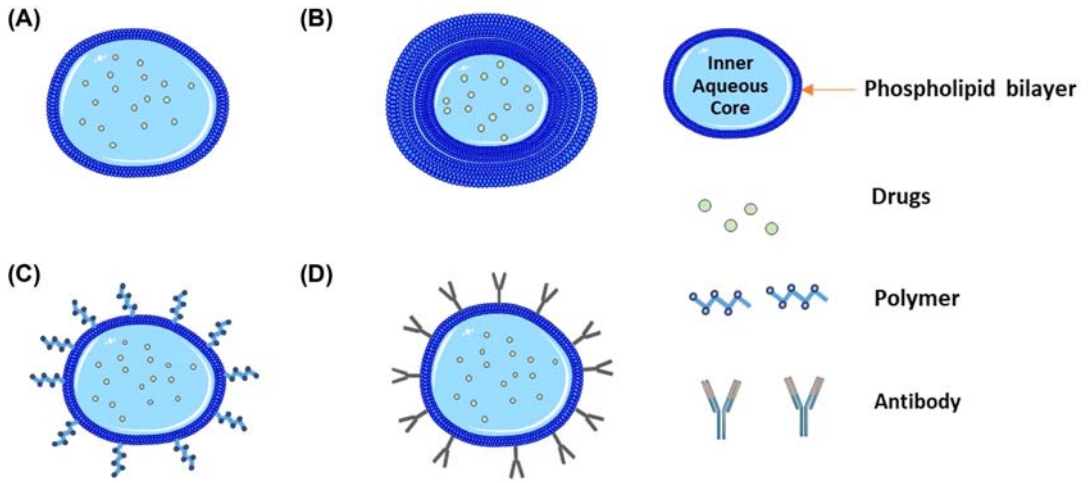

FIGURE 4.3

Illustration of liposomes types: (A) unilamellar liposomes, (B) multilamellar liposomes, (C) long-circulating liposomes, and (D) immunoliposomes as drug-delivery systems (DDS).

(DC-Chol) and a biosurfactant (β-sitosterol β-D-glucoside (Sit-G)) with plasmid DNA, exhibited enhanced luciferase marker gene transfection efficiency into human hepatoblastoma (HepG2) cells (in vitro) and liver gene expression in the following mice intravenous inoculation (in vivo) [50].

Liposomes as microbial biosurfactants, namely MEL-A, such as cholesteryl-3β-carboxyamindoethylene-*N*-hydroxyethylamine, enabled the improvement of luciferase gene transfection efficiency into target cells, such as fibroblasts (NIH-3T3 and COS-7) and human cervix carcinoma (HeLa) cell lines have been applied [51]. Actually, liposomes comprising MELs produced by *Candida antarctica* and a cationic cholesterol derivative allowed a 50- to 70-fold gene transfection efficiency enhancement [52]. In 2006, narrow liposomes [53] comprised of a biosurfactant, namely Sit-G-liposomes or MEL-A-liposomes were developed, which were subsequently complexed with DNA forming lipoplexes. Sit-G-liposomes are prospective vectors for gene therapy of herpes simplex (HSV-TK) due to their noteworthy reduced cytotoxicity, along with great luciferase gene transfection and thymidine kinase activity confirmed in HepG2 and HeLa 229 cell lines, respectively. Furthermore, HSV-TK gene therapy through recurrent intratumoral injections of Sit-G-liposomes was proposed. However, formulae and therapeutic design improvements are still required for its in vivo application [53]. As a result of previous findings, in 2009, 275 ± 88 nm cationic liposomes [41] comprising DOPE, OH-Chol, and MEL-A produced by *C. antarctica*, were demonstrated to present high gene transfection efficiency into NIH-3T3 cells through the endocytic, plus the novel membrane fusion pathways. Besides a 100-fold gene transfection efficiency enhancement (comparing with liposomes of DC-Chol without MEL-A) into B16/BL6 solid tumors in C57BL/6J mice was demonstrated, their usefulness for in vivo and in vitro gene transfection was displayed [54].

Liposomes enclosing 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (a cationic compound), plus different quantities (3, 6, and 14 mole%) of another biosurfactant, namely surfactin produced by *B. subtilis* strains, improved siRNA cellular delivery (77.4%, 76.5%, and 85.7% of the cells with a positive fluorescence signal, respectively), while keeping cell viability (84.0%) in Hela cells [55].

4.4.2 Niosomes

The use of niosomes started in the 1970s in the industry of cosmetics [56]. Niosomes are microscopic lamellar structures, bilayer vesicles formed by different nonionic surfactants presenting or not presenting cholesterol combinations or different lipids. The main properties of niosomes are biocompatibility/biodegradability, stability, reduced costs, structural flexibility and nonimmunogenicity [57]. Due to their amphiphilic characteristics, niosomes can be used for the encapsulation of hydrophilic or lipophilic active compounds, being the hydrophilic site proper for the incorporation of these drugs [57]. On the other hand, lipophilic active compounds are mostly linked in the lipid layer. The niosomes characteristics can be modified by altering the constitution of the vesicles, size, constituent concentration, surface charge, among others. Nevertheless, the stability of niosomes is dependent on the type of the microbial surfactant, type of charged compound, type of active compound encapsulation, temperature, among others [40,56,57].

Niosomes can be classified into 3 categories, which are dependent on the size of the vesicle (Fig. 4.4): (1) large unilamellar vesicles ($>0.10\ \mu m$), (2) multilamellar ($>0.05\ \mu m$), and (3) small unilamellar ($0.025-0.05\ \mu m$). Thus, depending on the size, double layer numbers, and vesicle membrane permeability, niosomes can be prepared by different techniques: sonication, micro fluidization, handshaking method, trans-membrane pH gradient, reverse phase evaporation technique, among others [56].

Some works have reported the use of niosomes in DDS. It was shown that the microbial surfactant surfactin was added into niosomes nano-formulations inside the core-shell structure (hydrophilic/hydrophobic) [13]. Additionally, rhamnolipid vesicles from *P. aeruginosa* SP4 were efficiently prepared in the presence of cholesterol addition. The rhamnolipid biosurfactant was able

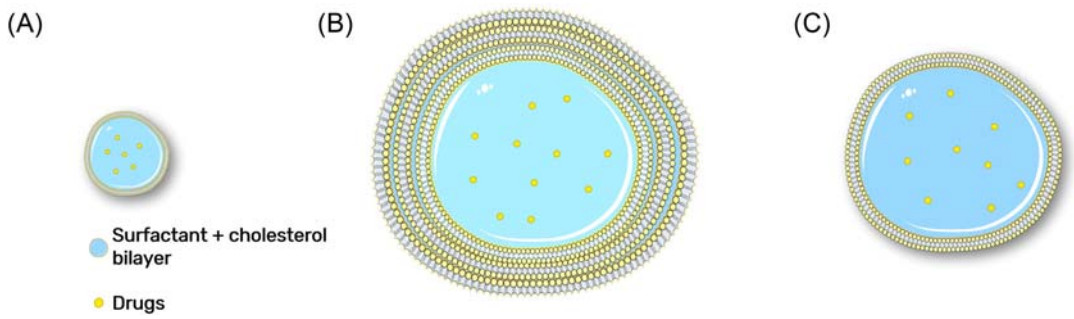

FIGURE 4.4

Types of niosomes: (A) small unilamellar niosomes, (B) multilamellar niosomes, (C) large unilamellar niosomes.

to form spherical vesicles, being the cholesterol supplementation on the bilayer membrane promoting the size reduction of the vesicle [58]. A model compound (dye) was applied as a hydrophobic constituent for the encapsulation with an improved rhamnolipid vesicles encapsulation efficiency of 90%. The entrapment capacity of these rhamnolipid vesicles indicates their potential to be used as nanocarrier systems for target DDS [58].

Niosomes based on sophorolipids were used, in a cost-effective way, for amphotericin B (AmB) delivery for the treatment of candidiasis infections by *Candida albicans* [59]. The microbial sophorolipids were produced by *S. bombicola* and the sophorolipids-AmB niosomes were prepared *in-house* with 63.20% of entrapment efficiency [59]. No cytotoxicity of sophorolipids over *C. albicans* was found since fewer hyphae were detected in the biofilm produced by *C. albicans* treated by the noisome [59]. This work proved the suitability of niosomal-based DDS for partially soluble active compounds namely AmB against fungal agents that cause infections such as candidiasis [59].

4.4.3 Nanoparticles

Nanoparticles have unique properties due to their nanometer size (between 10 and 500 nm) and consequent increase in the surface area [60]. These characteristics, together with the development of technologies that control the interaction of nanomaterials with biological structures, turned nanoparticles appealing for the development of new applications in the field of nanomedicine, creating new diagnostic and therapeutic techniques and devices. Moreover, this technology makes it possible to reach new targets, impossible to penetrate previously, and to manipulate the release and distribution of drugs [60].

With the development of new drugs, the delivery systems need innovation. The use of nanoparticles-based DDS has several advantages, namely the increased solubility of the compounds to be delivered [61]. By increasing the solubility, the bioavailability of drugs is improved, allowing to avoid the drawbacks of drugs with minimal solubility and bioavailability [62]. In addition to this advantage, nanoparticulate drugs can cross biological barriers, such as the blood-brain barrier, pulmonary system, and skin endothelial cells [61]. To achieve the perfect design for the nanoparticle-based DDS, several characteristics must be taken into account, among which are the particle size, surface properties, and drug loading, and proper release [60]. Nevertheless, the production of nanoparticles is still far from reaching its potential. The techniques used are of high cost, producing dangerous waste and unstable nanoparticles with low activity of the target [1].

The utilization of biosurfactants represents an interesting option for the preparation of metallic nanoparticles [63]. Microbial surfactants can perform a role in aggregation and stabilization procedures, by the control of the structure and size [64]. Biosurfactants can perform a role in aggregation and stabilization, controlling their size and shape. Silver nanoparticles were synthesized by Kumar et al. [65] using rhamnolipids from *P. aeruginosa* to obtain antimicrobial activity. The same biosurfactant to produce rhamnolipid-capped spherical ZnS nanoparticles [66]. *C. bombicola* synthetized sophorolipids showed good stability and these biosurfactants were applied in cobalt nanoparticles aiming the attachment of bioactive molecules for medical applications [67]. In addition to glycolipids, lipopeptides were studied in the preparation of nanoparticles. Silver and gold nanoparticles were prepared using surfactin from *B. subtilis* as a stabilizing compound [68]. The same biosurfactant was used to synthesize nanocrystalline brushite particles [69]. The group developed a novel reverse microemulsion technique based on surfactin for the synthesis. Singh and Cameotra prepared nanoparticles of cadmium sulfide using surfactin produced by *Bacillus amyloliquifaciens*, making

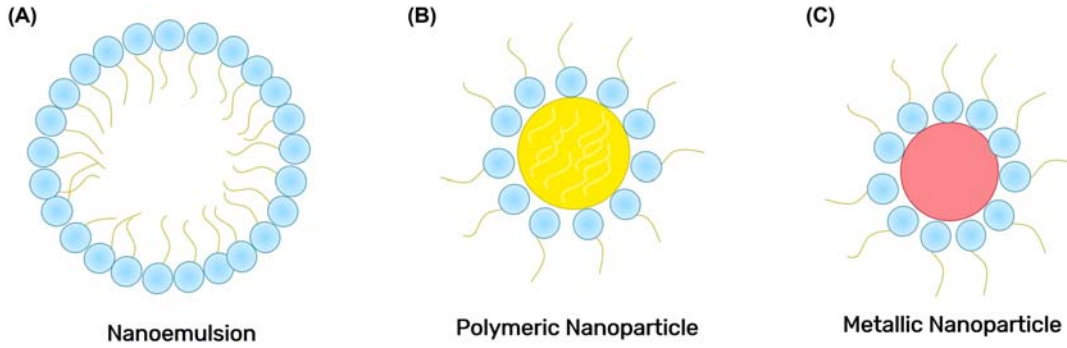

FIGURE 4.5

Biosurfactant nanoparticle types: (A) Nanoemulsion, (B) polymeric nanoparticle, (C) metallic nanoparticle as drug-delivery systems (DDS).

the nanoparticles stable for at least 6 months [6]. From a fundamental point of view, nanoparticles synthesized mediated by biosurfactants can be applied as drug-delivery vehicles in living systems, with the additional advantage of having biosurfactants that may display therapeutic properties.

Microbial biosurfactants can be used to create nanoemulsions where drugs are carried in the hydrophobic core (Fig. 4.5A). Besides, nanoparticles can also be composed of a polymeric or metallic core with a biosurfactant shell (Fig. 4.5B and C), where the drugs are carried by interacting with the biosurfactant shell. Table 4.1 presents different studies using these nanoparticles or variations of them which have been reported in DDS.

Recent studies have taken advantage of polymeric nanoparticles with biosurfactant-mediated synthesis to be applied as DDS. Hazra et al. [70] produced polystyrene-biosurfactant bionanocomposites with surfactin from *Bacillus clausii* and rhamnolipids from *P. aeruginosa*. These nanocarriers can be used for protein drug release, more specifically bovine serum albumin. The protein release in vitro assays was made mimicking characteristics of different parts of the human body, such as colon, blood, and skin. The protein release is enhanced by the increase in pH. Thus, the authors suggested the application of these nanoparticles in colon or intestine-specific controlled drug release. More recently, the same authors [71] successfully synthesized poly(methyl methacrylate)−biosurfactant nanoparticles by a modified atomized microemulsion process. In this process, three different biosurfactants were used: rhamnolipids from *P. aeruginosa* BS01, surfactin from *B. clausii* BS02 and trehalose lipids from *Rhodococcus pyridinivorans* NT2. These nanoparticles were used as a pH-responsive drug-delivery vehicle releasing ibuprofen, anthraquinone, and curcumin, with sustained drug release and without loss of colloidal stability.

Besides surfactin, rhamnolipids can also be conjugated with bioderived nanoparticles [78]. Spherical zein-rhamnolipid nanoparticles were prepared to release curcumin [78]. The addition of the biosurfactant enhanced the curcumin efficiency of encapsulation during the in vitro delivery of the simulated gastrointestinal tract. This approach indicated the good potential of nanoparticles composed of zein-rhamnolipid to deliver hydrophobic bioactive compounds. More recently, nanoparticles of zein-propylene glycol alginate-rhamnolipid were prepared and loaded with resveratrol for delivery during digestion, tested with simulated gastrointestinal juices [72]. In 180 minutes, the majority resveratrol was distributed in the intestine. Huang et al. [73] develop nanoparticles of

Table 4.1 Examples of drug-delivery systems (DDS) using nanoparticles.

Nanoparticle	Biosurfactant	Drug/nutraceutical	Reference
Polystyrene-biosurfactants nanocomposites	Rhamnolipids and surfactin	Proteins	[70]
Poly(methyl methacrylate)−biosurfactant nanoparticles	Rhamnolipids, surfactin and trehalose lipids	Ibuprofen, anthraquinone and curcumin	[71]
Zein-rhamnolipid nanoparticle	Rhamnolipid	Curcumin	[78]
Zein-propylene glycol alginate-rhamnolipid composite nanoparticles	Rhamnolipid	Resveratrol	[72]
Doxorubicin-loaded surfactin nanoparticles	Surfactin	Doxorubicin	[73]
"Mosaictype" nanoparticle system	Surfactin	Gambogic acid	[79]
Rhamnolipids nanoparticle	Rhamnolipids	Dexamethasone, and tacrolimus	[80]
Rhamnolipids nanoparticle	Rhamnolipids	Pheophorbide	[74]
Sophorolipid-coated curcumin nanoparticles	Sophorolipid	Curcumin	[75]
Graphene quantum dots conjugated with biosurfactant and folic acid	Biosurfactant	Biosurfactant and folic acid	[76]
Poly(lactic-*co*-glycolic acid) nanocapsules	Sophorolipid	Sophorolipid	[77]

doxorubicin-loaded surfactin (DOX@SUR) to be applied in breast cancer treatment. DOX@SUR was used in human breast cancer cells (MCF-7/ADR) resistant to DOX presenting higher cytotoxicity than free DOX [73]. Furthermore, these nanomaterials presented lower adverse effects and higher in vivo tumor suppression compared with the free anticancer drug. Wang et al. developed an innovative "mosaictype" nanoparticle system intended for hypoxic cancer cells composed of surfactin, heptamethine carbocyanine dye, Cy7 (a hypoxia-targeting group), and gambogic acid (GA, an anticancer drug) [79]. Deprived of particle internalization by selectively release, the anticancer drug GA-Cy7-surfactin nanoparticles represent a tool to treat hypoxic cancer cells. In vitro (human PC3 cell line) in addition to in vivo (xenograft mouse model) assays in prostate cancer cells demonstrated the selective release mode of these nanoparticles, enhancing drug distribution in tumor cells. Compared to free GA, GA-Cy7-surfactin nanoparticles showed higher antitumor when compared to angiogenesis assay and tumor growth.

Rhamnolipids can form spherical and stable nanoparticles [74,80]. These synthesized rhamnolipids nanoparticles can be applied in dermal drug delivery [80]. The materials achieve improved drug loading of 30% w/w. Rhamnolipid nanoparticles have also been used by Yi et al. [74] for in vivo drug delivery of pheophorbide (a hydrophobic photosensitizer) and photodynamic therapy. In vitro assays with SCC7 tumor cells showed a fast uptake of the nanoparticles. In in vivo assays, these rhamnolipid-pheophorbide nanoparticles were injected into SCC7 tumor-bearing mice and, with laser irradiation, it provoked complete tumor suppression.

Sophorolipids can also form nanoparticles [75]. Sophorolipids-coated curcumin nanoparticles were used to enhance curcumin bioavailability with high encapsulation efficiency and 14% of loading curcumin [75]. The in vitro bioavailability was tested using simulated gastrointestinal tract and

in vivo assays were performed with rats. Both assays demonstrated greater bioavailability when compared to the free curcumin.

Bansal et al. [76] synthesized graphene quantum dots that were coupled with a biosurfactant (from *Candida parapsilosis*) and folic acid to apply as a theranostic alternative for the treatment of cancer. These bioconjugate dots have been tested in human cells lines of breast cancer MCF-7. Graphene quantum dots-biosurfactant (2.5 μg/mL) reduced in 50% cellular viability in 24 hours and, when associated with folic acid and biosurfactant, cell viability decreased 60% within 24 hours. Nanocapsules were prepared by Haggag et al. and the poly(lactic-*co*-glycolic acid) was added to sophorolipids and used in the colon carcinoma treatment [77]. The delivery of sophorolipids was tested in vivo and in vitro using CT26 murine colon carcinoma. The nanoparticles achieve a reduction in cell viability of 80% in 72 hours and in in vivo assays, the nanoparticles provoked a cancer growth suppression of 57% with controls.

4.5 Conclusions and future challenges

Microbial biosurfactants are excellent alternatives to chemical surfactants when applied in DDS. Nevertheless, their use in this area is still recent and novel and more research must be performed. The enormity of microorganisms and their products indicates new biosurfactants could be discovered and applied in what concerns their potential of application in DDS. Despite the advantages of biosurfactants and the proven efficacy in some types of DDS, currently, limited reports are addressing the use of microbial biosurfactants in DDS, and additional research on this topic is required for the successful implementation of these delivery systems in the pharmaceutical and medicinal sectors. Moreover, further research on the interactions between cells and constituents of microbial surfactants in DDS, toxicity, systematic in vitro and in vivo assays, pharmacokinetic studies, drug release profiles, and appraisal of side-effects are still required when aiming their approval by regulatory agencies.

Acknowledgments

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CHAPTER

Inhibition of fibrin clot formation

5

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5.1 Introduction

Blood clotting or cross-linking of fibrin after hydrolysis of fibrinogen is a body defense mechanism against massive loss of blood and flow. This mechanism also intervenes during the immune response to limit the inflamed area and opposes the dispersal of pathogens, which consequently limits the infected area and tissue damage [1]. Fibrin formation and fibrin resolution are fundamental tissue repair processes in the human body. Any lesion of the vascular bed must be plugged and subsequently repaired to ensure vascular integrity [2]. Fibrinogen and fibrin play an important role in blood clotting, fibrinolysis, cellular and matrix interactions, inflammation, wound healing, angiogenesis, and neoplasia [3]. In addition, blood coagulation can be induced by a free radical reaction with fibrinogen [4].

There are more than twenty enzymes in the body that assist in the clotting of blood, while there is only one capable of breaking clots down [5]. The activation of thrombin leads to the conversion of soluble plasma protein fibrinogen to fibrin monomers thereby generating a fibrin clot [6]. The thrombin concentration influences profoundly the fibrin clot structure and its fibrinolysis [7].

The coagulation and fibrinolytic systems are highly regulated and interrelated through mechanisms that ensure balanced hemostasis [8]. However, in pathological states, blood clotting threatens seriously the health of human beings and is a major cause of morbidity and mortality [5,9]. The formation of dense fibrin networks which are relatively resistant to lysis is observed in patients with venous or arterial thromboembolism, including myocardial infarction, ischemic stroke, and venous thromboembolism [10]. Diseases associated with thrombosis development are diabetes, hyperlipidemia, and hypertension with abnormalities of the inflammatory and hemostatic systems [5].

The fibrinolysis or the process of clot dissolution is catalyzed by an enzyme system present in the blood of all mammalian species to restore blood flow through the occluded vessel [11]. Under an unbalanced situation due to some disorders, the clots are not hydrolyzed, and thus thrombosis occurs [12].

To prevent blood clot formation, many categories of medications are used. The thrombolytic or fibrinolytic agents are employed via injecting or orally administrating to lyse thrombi in blood vessels [12]. Other anticoagulant agents prevent fibrin clot formation by inhibition of vitamin K reduction [13] or inhibition of coagulation factors of final stages such as factor Xa and thrombin [14]. Nevertheless, all these anticoagulant agents are associated with a risk of bleeding complications [15–18].

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The microorganisms and their products are widely applied in biomedicine due to their therapeutic virtues. The biosurfactants which are fabricated by diverse species (bacteria, fungi, and yeast) are attracted more attention in the last few years. The biosurfactants are considered secondary metabolites and may play survival roles for their producing microorganisms in the nutrients transport, microbe-host interaction, or as biocide agents [19]. Recently, these amphiphilic molecules have several applications in various fields in particular biomedical. Despite the interesting properties of biosurfactants (surface/interfacial activity, low critical micelle concentration, lipophilic-hydrophilic balance, biocompatibility, biodegradability, low toxicity, and cost-effectiveness of production) [19–21], few studies were carried out on application as therapeutic agents especially as an inhibitor of fibrin clot formation.

The application of biosurfactants as a potential inhibitor of fibrin clot formation, green substitutes of synthetic medication, and the possible mechanisms of inhibition are discussed in this chapter.

5.2 Coagulation factors and fibrin clot formation

The hemostasis is a combination of cellular and biochemical events that function together to keep blood in the liquid state within the veins and arteries, therefore, preventing blood loss following injury through the formation of blood clot [22].

The platelets activation is the result of contact with subendothelial matrix protein, collagen, von Willebrand factor, and fibronectin, in response to vascular injury (primary hemostasis) [8,23]. It has four sequential but overlapping phases: vasoconstriction, platelet adhesion, platelet activation, and platelet aggregation [23].

Secondary hemostasis includes the two main coagulation pathways, intrinsic and extrinsic, that meet up at a point to form the common pathway. The common pathway ultimately activates fibrinogen into fibrin. The secondary hemostasis is complex and involves many different proteins: zymogens [23].

Coagulation is a complex cascade of proteolytic reactions which may be initiated either by reactions occurring between components of the blood alone, the intrinsic pathway or by reactions that also involve tissue components, termed the extrinsic pathway [24].

Fibrin formation involves sequential activation of many factors circulating in the blood. There are about 50 proteins or factors involved in coagulation which are serine protease. The majority of these factors are in an inactive form (zymogen). Their activation is done in a cascade [25].

The main factor of coagulation is a factor I or fibrinogen. It is a fibrous glycoprotein of 330 kDa synthesized in the liver. It is made up of two heteromers $(A\alpha)_2(B\beta)_2\gamma_2$ linked by disulfide bonds. In presence of factor XIIIa and calcium ions, thrombin hydrolyzes fibrinogen to give fibrinopeptides A (16 amino acids from chain $A\alpha$) and B (14 amino acids from chain $B\beta$). Insoluble fibrin polymer cross-linking is catalyzed by factor XIIIa [7,25,26].

The serine protease thrombin plays a crucial role in hemostasis; not only in the transforming of fibrinogen into fibrin clot, but also in the activation of factor V, FVIII, and FXI, and triggers platelet activation through interaction with protease-activated receptors (PARs) 1 and 4 and glycoprotein Ib-α. Thrombin also exerts an anticoagulant effect by activating protein C and thrombin-activatable fibrinolysis inhibitor [27].

Factor XIII is a 320 kDa tetramer, including two enzymatic A-subunits and two carrier B-subunits (FXIII A_2B_2). Activated FXIII (FXIIIa) catalyzes the formation of ε-(γ-glutamyl)lysyl covalent bonds between γ−γ, γ−α, and α−α chains of adjacent fibrin molecules and also cross-links the major plasmin inhibitor, α2-antiplasmin, to fibrin [26].

Zinc released from activated platelets binds fibrin(ogen) and attenuates fibrinolysis. Although zinc also affects clot formation, the mechanism and consequences are poorly understood [28].

Fibrinolysis induced by plasmin that is responsible for thrombus removal and maintaining blood flow leads to digestion of fibrin at specific lysine residues [10]. Fibrinolysis efficiency is highly influenced by clot structure, fibrinogen isoforms and polymorphisms, the rate of thrombin generation, the reactivity of thrombus-associated cells like platelets, and the overall biochemical environments [8].

5.3 Consequences of fibrin clot formation

Fibrin has a pivotal role in hemostasis and tissue repair. In normal cases, the fibrin clots are meant to be temporary structures formed to stop bleeding. After the process of wound healing has started, the ridged clot must be broken down to reduce the risk of thrombosis [29,30].

Hypercoagulability is widely linked to a variety of disease states, leading to cardiovascular complications [31]. Fibrin clot formation is a key event in the development of thrombotic disease. The formation of an occlusive clot within a blood vessel is the main thrombosis character, leading to ischemia or tissue death due to starvation and oxygen deprivation downstream of the occlusion. Thrombosis can occur in either the arterial or the venous circulation [32]. The fibrin clot structure is altered in arterial thrombotic diseases and also in venous thromboembolic diseases. The clots produced in patients with coronary artery and venous thrombosis diseases are characterized by small pores and resistance to fibrinolysis [29].

5.4 Inhibition of fibrin clot formation

5.4.1 By enzymes

Several proteolytic enzymes which have thrombolytic activity can be obtained from different sources animals [33], plants [34], and microorganisms [12].

Plasminogen or factor XII has a crucial role in fibrinolysis after its conversion into plasmin (FXIIa) which is facilitated by fibrin and fibrinogen [35]. The activation of plasminogen (zymogen) into serine protease plasmin is formed via cleaving Arg^{561}-Val^{562} bond which catalyzed by a serine protease: Urokinase-type plasminogen activator (u-PA). The plasmin is involved not only in blood clot dissolution but also in a variety of physiological and pathological processes requiring localized proteolysis [36]. It's currently produced from mammalian cell lines attempts were also made to obtain it from bacteria, fungi, and mammalian cells [35]. Prourokinase is used to treat cardiovascular diseases as a thrombolytic agent [35].

Staphylokinase is a 135 amino acid protein (comprising 45 charged amino acids, no cysteine residues nor glycosylation), secreted by *S. aureus* strains after lysogenic conversion or

transformation with bacteriophages. The Staphylokinas is a plasminogen activator, but it is immunogenic in man [37]. Nowadays, this thrombolytic agent is used for the treatment of acute myocardial infarction, acute ischemic stroke, pulmonary embolism, and other diseases [38].

Streptokinase (protein of 414 amino acid) is an extracellular nonenzymatic protein produced by various strains of b-hemolytic Streptococci. Streptokinase possesses no enzymatic activity of its own. It forms a complex with plasminogen or plasmin. The resultant activator complex is a strongly specific protease, which converts other PG molecules to proteolytically active PN [11].

Nattokinase is a fibrinolytic enzyme (275 amino acids), has intense fibrinolytic activity. It's a formidable clot-dissolving protein, obtained from *Bacillus subtilis* and used for myocardial infarction treatment [39].

Douchi fibrinolytic enzyme (DFE) was isolated from *Douchi*, a typical and popular soybean-fermented food in China, and it can dissolve fibrin directly and efficiently. A strain, *Bacillus subtilis* LD-8547 produced DFE with high thrombolytic activity has been isolated and tested its thrombolytic activity in vitro and in vivo by Yuan et al. [40].

However, these drugs have certain limitations which cause serious and sometimes fatal consequences including hemorrhage, severe anaphylactic reaction, lacked specificity, etc. Moreover, as a result of immunogenicity, multiple treatments with SK in a given patient are restricted [17]. The thrombolytic therapy for acute ischemic stroke is followed by Symptomatic intracerebral hemorrhage (SICH) which is associated with a high rate of morbidity and mortality [41].

5.4.2 By using chemical drugs

Anticoagulants are classified into four groups: vitamin K antagonists (VKAs), direct thrombin inhibitors, direct factor Xa inhibitors, and heparin. VKAs such as warfarin (coumarins) function by blocking the vitamin K-epoxide reductase, an enzyme that recycles oxidized vitamin K. The vitamin K is an activator of coagulation factors. The VKAs have an initial prothrombotic effect, by initially blocking proteins C and S, followed by a delayed antithrombotic effect, through the inhibition of coagulation factors II, VII, IX, and X [13].

Dabigatran (Pardaxa) is an oral anticoagulant that binds to and inhibits the activity of thrombin, therefore, prevent blood clot formation. It does not require frequent laboratory monitoring of clotting parameters but induces bleeding and hemorrhagic complications. However, no therapeutic agent has been accepted to reliably reverse the hemorrhagic complications of dabigatran [16].

Rivaroxaban (Xarelto) is an oral oxazolidinone-based anticoagulant agent. It inhibits not only free factor Xa with high selectivity but also prothrombinase bound and clot-associated factor Xa in a concentration-dependent manner [42]. Rivaroxaban provides practical advantages including a rapid onset of action, few drug interactions, no dietary interactions, a predictable anticoagulant effect, and no requirement for routine coagulation monitoring. The drug increases the risk of bleeding and decreases renal function in a patient with renal impairment [42,43].

Heparin is a naturally occurring polysaccharide belonging to the family of glycosaminoglycans (GAG) ubiquitously present in mast cells. It was obtained from animal tissue, most commonly the porcine intestine [44]. The active center serine of thrombin and other coagulation enzymes is inhibited by an arginine reactive center on the antithrombin molecule and heparin binds to lysine sites on antithrombin, producing a conformational change at the arginine reactive center that converts antithrombin from a slow, progressive thrombin inhibitor to a very rapid inhibitor [45]. The

nonspecific binding of heparin further leads to an unpredictable interference with inflammation pathways, microcirculation, and phagocytotic clearance of dead cells, with possible deleterious consequences for patients with sepsis and systemic inflammation [46].

5.4.3 New drugs

The efficiency and the utility of PEGylated knob peptides as potential anticoagulants were investigated by Stabenfeldt et al. [6]. These authors found that the active PEGylated has a chain length of 5 kDa and the sequence of knob peptide conjugate was GPRPFPAC [6].

5.5 Biosurfactants as drug

Biosurfactants can effectively reduce the surface tension, decrease the interfacial tension between two immiscible liquids, enhance the emulsification and increase the solubility of compounds [47]. Besides their use as surfactants or emulsification enhancers, the biosurfactants build gels, niosomes, hexosomes, and cubosomes, whose structure is directly related to lyotropic properties. These systems allow solubilization and entrapment of drugs [48].

In addition, biosurfactants have many industrial and biotechnological applications. Due to their exceptional properties, the biosurfactants have been tested and found to exhibit promising biomedical and therapeutic applications as an antimicrobial agent [49,50], antibiofilm [51], antiadhesive [52], anticancer [53], in drugs delivery [48], and immunostimulator and immunomodulator [54,55]. The use of biosurfactants as an anticoagulant is discussed below.

According to the literature, the most studied and investigated biosurfactants for their fibrinolysis and antithrombotic activity are those that belonged to the class of lipopeptides.

The surfactin is a lipopeptide microbial surfactant produced by the genera *Bacillus*. Several works proved that this biosurfactant has thrombolytic activity. Kikushi and Hasumi [56] found that the surfactin C at a concentration of 3−20 μM enhanced the activation of prourokinase which led to the activation of plasminogen and by consequent increasing the fibrinolysis in vitro and in vivo.

The in vivo application of surfactin in a rat pulmonary embolism model showed that the injection of surfactin in combination with prourokinase increased the fibrin clot lysis [57].

Kim et al. [58] investigated the effect of surfactin C produced by *Bacillus subtilis* on platelet aggregation and homotypic leukocyte aggregation. The platelet aggregation which was stimulated by thrombin and collagen was dose-dependently and strongly inhibited by surfactin C. The antiplatelet activity of surfactin C was not due to its detergent effect but by its action on the downstream signaing path [58].

Ben Ayed et al. [59] have evaluated the acute, subchronic toxicity and in vitro anticoagulant activity of a mixture of lipopeptides (A21 lipopeptides) produced by *Bacillus mojavensis* A21. These authors found that the lipopeptides A21 did not cause any change in body weight, hematological and biochemical blood parameters. They also found that these lipopeptides cause a prolongation of the thrombin time (TT), the prothrombin time (PT), and the activated partial thromboplastin time (APTT).

There are studies realized on the anticoagulant activity of biosurfactants which their structures and classes did not identify. Rabail and coworker [21] have evaluated the thrombolytic activity of biosurfactant produced by soil bacterial strain *Klebsiella* sp. KOD36. Their obtained results showed that this biosurfactant exhibited a good thrombolytic activity [21].

The fatty acid released under the action of phospholipase A_2 on membrane phospholipids is converted into inflammatory mediators (e.g., prostaglandins and leukotrienes). The resulting lysophospholipid is a precursor of platelet-activating factor. The inhibition of phospholipase A_2 by surfactin is investigated and the result obtained showed that the surfactin is a putative antiinflammatory agent due to its competitive inhibition of phospholipase A_2 (with IC_{50} of 8.5 μM and inhibition constant of Ki = 4.7 μM) [60].

Lim et al. [61] found that the surfactin produced by *Bacillus subtilis* BC1212 was able to inhibit the platelet aggregation and by consequent fibrin clot formation, in a dose-dependent manner.

Moreover, surfactin has advantages over other thrombolytic agents because it has fewer side effects and therefore it has potential for long-term use as a clot-bursting agent [62].

5.6 Conclusion

Interesting biosurfactants features have led to a wide of potential applications in the medical field, particularly in the inhibition of fibrin clot formation and activation of fibrin clot lysis. The thrombolytic activity or the enhancement of thrombolytic reactions and the inhibition of platelet aggregation of biosurfactants alone or combined with other anticoagulant agents may intervene in thromboembolic states related to pulmonary, cerebral, and myocardial disorders. These microbial surface-active molecules represent a promising alternative anticoagulant or thrombolytic agents; however, the toxicity of these compounds may limit their biomedical application. Further investigation on human cells needs to be performed to validate the use of biosurfactants in biomedical and health-related areas.

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CHAPTER

Application of biosurfactant for the management of tropical and life-threatening diseases

6

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6.1 Introduction

In academic literature, the scientific terminology of "surfactant" is loosely utilized for the variety of surface tension reducing agents/surface-active agents. Any substance that possesses the capability of reducing the surface tension at the interfaces owing to the presence of both hydrophilic and hydrophobic components is termed as surfactants [1]. Other properties of the surfactants include lubrication, detergency, solubilization, stability imparting, and foaming properties. Based on the synthesis methodology, surfactants can be broadly classified into chemically synthesized surfactants and biosurfactants (BSs). Nowadays, the utilization of synthetic surfactants is frowned upon by the scientific community as these surfactants have been known to adversely impact the environment and human health. These synthetically modified surfactants are also nonbiodegradable. The extensive and unchecked usage of these surfactants in numerous industries (such as the pharmaceutical industry, paper/printing industry, medical manufacturing industry, etc.) has led to the accumulation of these surfactants as environmental pollutants. Therefore, synthetic surfactants are not preferred for achieving applications associated with these surfactants [2]. Recently, BSs, surface-active agents extracted from biogenic organisms (particularly microorganisms), are experiencing a surge in interest from the scientific community as an efficient alternative to the conventional synthetic surfactants [3].

BSs, extracted from a variety of microorganisms, encompass numerous chemical compounds which are utilized to attain multiple applications. Chemically, the polar/hydrophilic head of the BSs has been documented to be composed of amino acids, peptides, sugars, proteins, or any other hydrophilic functional moiety such as carboxylic acids. The hydrophobic tail includes aliphatic (saturated or unsaturated), aromatic hydrocarbons, or hydroxylated fatty acids/alcohols [3,4]. The composition of the BSs is heavily dependent upon the type of the BSs involved. Knowing the fundamentals associated with the BSs is a prerequisite for utilizing the BSs for various applications.

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Use of the BSs in the medical field has been extensively documented in the academic literature [5,6]. The advantages (such as low toxicity, biodegradability, pH stability, thermal resistance, and biological activity potential, etc.) have made BSs an interesting material to be explored for biological applications. These biological activities are not observed in the case of the utilization of their synthetic counterparts. The term biological activities include several applications such as antibacterial, antimicrobial, antitumor, and immunomodulatory activities, etc. [5]. Recently, these BSs have been documented to be effective against several tropical life-threatening diseases, and presenting the generalized overview of this topic will be the focus of our discussion for this chapter. Keep that in mind, detailing the several fundamental aspects such as physicochemical properties, classifications, details of utilized microorganisms for the production of BSs lie outside the scope of this chapter and will not be given focus over here. Only essential parameters that are required for developing an understanding of the use of BSs in the treatment and management of tropical life-threatening diseases will be discussed here.

The structure of this chapter is designed in such a way that this chapter can act as a guideline for the readers entering this field of BSs and their biomedical applications. Section 6.1 provides an introduction to the topic. Section 6.2 presents a description of the generalized framework used for utilizing the BSs as a medicinal agent for treating/managing tropical and life-threatening diseases. Section 6.3 provides the necessary information on tropical and life-threatening diseases. Section 6.4 provides the account of the literature survey associated with the medicinal applications of BSs in the case of these above-stated diseases. The literature was also organized and classified based on the causative agent of the disease for presenting a comprehensive picture of the topic.

6.2 Framework of the research study

Utilization of the BSs for medical and biomedical applications against tropical and life-threatening diseases includes the following steps: (1) production of the BSs, (2) extraction of BSs, (3) confirmation assays/characterization of the BSs, (4) medicinal application of BSs against the particular disease. It is worth mentioning here that this framework is not specified and only major steps are highlighted here. Some other supplementary steps are not included in the discussion over here. These steps are as follows:

1. The molecular characterization of the microbes producing BSs (i.e., crude isolate collected from the site may contain many microbes including bacteria, fungi, or viruses. The isolation of the microorganism is followed by purification and deoxyribonucleic acid (DNA) extraction. Extracted DNA is made to undergo polymerase chain reaction (PCR) amplification and the sequences of the DNA are acquired. Comparison of the acquired sequence with the documented sequence will give information about the microbe (bacteria, fungi, or virus species) present in the medium) [7].
2. Purification of the extracted BS by utilizing the techniques of thin-layer chromatography (TLC) [8], isoelectric focusing (ICE), lyophilization or dialysis [9], etc.

6.2.1 Production of biosurfactants

Microorganisms required for the production of the BSs are collected from the sampling location. Pristine environmental mediums, stressed soils, and stressed aquatic environments are the three focal sampling locations used for the collection of microorganisms. However, the best results for the collection of the microorganisms are acquired when the compromised environment such as oilfields, oil reservoirs, dumping sites for diesel [10] and automobile industrial waste [11], etc. are used as sample collection sites. These sites contain very high hydrophobic compounds as pollutants and serve as growing sites for the production of microorganisms. Isolation of the microbe from the sampling site is performed by using the enrichment process via mineral salt media (MSM) [12] where the organic hydrophobic compounds are incorporated as the carbon source acting alongside the nutrient broth. Isolated microorganisms are then purified and subjugated to growth by providing optimal growth conditions such as the incorporation of appropriate amounts of carbon source, nitrogen source, phosphate content, mineral content, optimum temperature, optimum pH, controlled incubation period, and controlled aeration/agitation rates. All these factors influence the production of BSs in the medium.

6.2.2 Extraction of the biosurfactants

The processes involved in the extraction of the BSs are summarized in Table 6.1.

Table 6.1 Some of the basic extraction procedures utilized for the extraction of biosurfactants.

Extraction methodology	Process involved	References
Ethanol precipitation	The cell wall containing the microorganism strain was first heated at 100°C followed by the centrifugation process and the supernatant was separated. Biosurfactants (BSs) were precipitated by adding ice-cold ethanol (95% pure; 20°C) to the supernatant. The precipitated BSs were then collected via centrifugation process.	[13]
Acetone precipitation	The cell-free supernatant was treated with acetone in the v/v ratio of 1:1. After the precipitation growth period of 24 h at 4°C, the precipitates were centrifuged for separation.	[14]
Centrifugation	Acid precipitation (acquired by treating the isolate with 0.1 N HCl) was followed with the centrifugation process performed at 4°C for 30 min with a speed of 12,000 rpm. The BSs were extracted as the precipitates.	[15]
Ammonium sulfate precipitation	The cell-free supernatant was achieved by heating and centrifugation process. The supernatant was supplied with 2 N HCl fractioned with 60% saturated ammonium sulfate solution. The acquired precipitated BSs were filtered, washed, dialyzed, and lyophilized for further experimental work.	[16]
Acid precipitation	The cell-free supernatants of the strains were treated with the 2 N HCl solution. Precipitated BSs were suspended in water and were stored at −20°C.	[17]
Solvent extraction	The cell-free supernatant (200 mL) was treated against the 200 mL of methanol: chloroform (1:2) taken in the extraction funnel. The BS was separated by evaporating the solvent.	[18]

6.2.3 Biosurfactant activity tests (confirmation assays)/characterization of biosurfactants

The physiochemical properties of the BSs are utilized for the formation of various surface activity tests including hemolytic test, surface tension test, oil spreading test, etc. Similarly, various characterization techniques are also utilized for identifying the chemical and physical properties of the extracted BSs. A detailed summary of these assays and characterization techniques utilized for these BSs is provided in Table 6.2.

Table 6.2 Confirmation assays and characterization techniques utilized for the biosurfactants.

Activity tests/confirmation assays			
Biosurfactant activity test	**Procedure**	**Inference**	**References**
Drop collapse test	Few drops of oil are placed on the glass slide. 10 μL of the biosurfactant (BS) aliquot is introduced onto the drop by using a micropipette in such a way that the drop shape is not disturbed during the introduction.	Collapsing of drop within one minute indicates the BS is present in the sample	[19]
Oil spreading test	Distilled water (40 μL) is introduced into the petri dish by micropipette. Then diesel oil (20 μL) is introduced to the surface of the water which forms an oil film on the surface of the water. This step is followed by adding 10 μL of the BS containing supernatant.	Development of clearing zone in the thin film of oil is indicative of the presence of surfactant in sample	[19]
Emulsification index (%EI24)	The same amount (2 mL) of oil and the BS supernatant is introduced into the glass tube followed by mixing it with the aid of vortex for 2 min. The mixture is allowed to stand for 2 h.	A floating layer of BS is formed at the upper part. %EI24 is calculated by dividing the measured height of the BS by the total liquid. The acquired answer is multiplied by 100 for percentage values.	[20]
Surface tension test	Cultures of the strain are produced in the nutrient broth. In a 100 mL MSM, 1 mL of inoculum and 1% filtered oil are added. Control samples (containing only 100 mL MSM and 1% filtered oil) were incubated for 3 days at 30°C with a shaker speed of 150 rpm. The surface tension of the sample and control is measured by the tensiometer.	If the surface tension values of the sample are found to be lower than that of the control, then a BS is present in the medium.	[21]

Table 6.2 Confirmation assays and characterization techniques utilized for the biosurfactants. ***Continued***

Activity tests/confirmation assays			
Biosurfactant activity test	**Procedure**	**Inference**	**References**
CTAB-MB agar plate test	Agar medium containing 2% glucose, 0.5 mg/mL CTAB and 0.2 mg/mL MB was prepared. With the help of a cork broker, equidistant (4 mm) wells were prepared and 30 μL of the cell-free supernatant is added into these wells. The labeled wells were allowed to be incubated for 48−72 h at 37°C.	The formation of dark blue complexes is indicative of the presence of anionic BSs in the sample.	[22]
Characterization techniques			
Analytical technique	Purpose		References
FTIR	Information about the functional groups present in the BS is acquired by using this technique		[23]
NMR	The functional groups and the position of the respective linkages present between the lipid and carbohydrate content of BS are identified by this technique. Type and the characteristic BS are identified by using NMR.		[24]
LC-MS	This technique is heavily dependent upon the utilization of the differences in the hydrophobic content of BSs as a means to achieve purification and characterization. BSs belonging to the class of lipopolysaccharides are best identified by using this technique.		[24]
GC-MS	This technique is utilized for the identification of the BSs based on their respective molecular weights.		[25]
HPLC	The m/z based fragmentation pattern of the BS is utilized for the identification of BS.		[26]

MSM, *Mineral salt solution;* FTIR, Fourier transform infrared spectroscopy*;* MB, *methylene blue;* CTAB, *cetyl trimethylammonium bromide;* LC, *liquid chromatography;* MS, *mass spectrometry;* GC, *gas chromatography;* HPLC, *high-performance liquid chromatography;* m/z, *mass/charge number.*

6.2.4 Medicinal application of the biosurfactant

Extracted BSs can be employed in several ways to acquire medicinal applications. The most common way documented in the academic literature for this purpose was found to be focusing on adversely impacting the vectors of the diseases. The vectors are responsible for disease transmission. Controlling the larvicidal and pupicidal activities of the vectors by incorporating the BSs in the medium is a quite effective approach in this regard [27]. Furthermore, the production of the young instars is also negatively impacted by the addition of surfactant in the medium [28]. The use of BSs for this purpose revealed positive results in terms of disease control as well particularly the disease of malaria has been heavily documented to be controlled by utilizing this approach [29].

Direct cell lysis of the causative agent or cell line has also been documented as a means to treat certain diseases (particularly cancer [30] and HIV [31]). Haque et al. [32] documented the necrosis potential of the extracted BSs against the skin, lung, and breast cancer cell lines. Similarly, antiviral potential has also been documented in the case of these BSs [33]. Even for the Coronavirus diseases-2019 (COVID-19), the BSs have been documented to show enhanced potential in terms of the lysis potential against the causative virus [34]. The pictorial description of the structure of the BSs, their surface tension reduction properties, and their effectiveness against COVID-19 has been presented in Fig. 6.1.

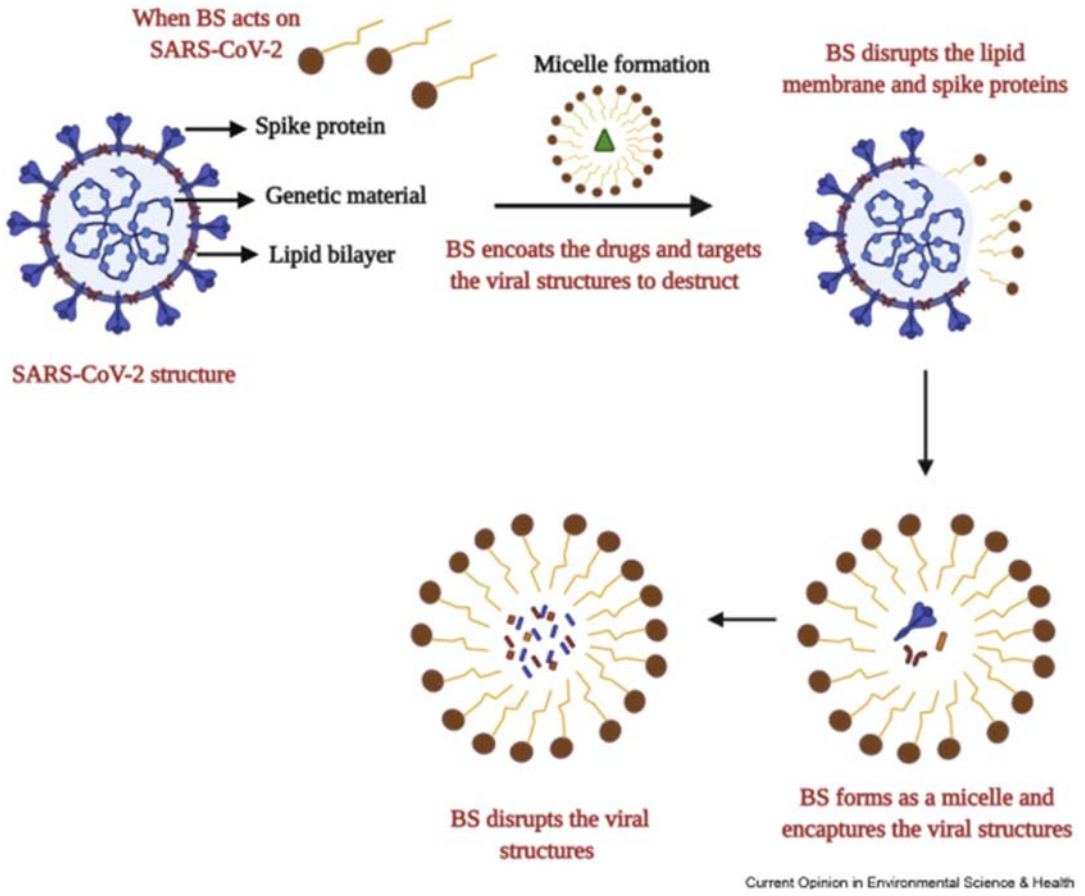

FIGURE 6.1

Antiviral potential of biosurfactants against the severe acute respiratory syndrome Coronavirus-2, Coronavirus disease 2019.

Reproduced from M.D. Subramaniam, D. Venkatesan, M. Iyer, S. Subbarayan, V. Govindasami, A. Roy, et al., Biosurfactants and antiinflammatory activity: a potential new approach towards COVID-19, Curr. Opin. Env. Sci. Health 17 (2020) 72–81 [35]

6.3 Tropical and life-threatening diseases

The diseases which are prevalent in the tropical and subtropical regions are regarded as tropical and subtropical diseases. The occurrence of these diseases in these specified areas is attributed to the fact that these regions are mostly characterized by long winter seasons. Furthermore, extended biodiversity in these regions has provided these areas with large populations of vectors of these diseases. Most often these diseases are transmitted by vectors such as mosquitoes, worms, insects, and flies, etc. to human beings through bites which introduce the pathogenic species into the subcutaneous bloodstream. Most of the diseases have already defined vaccines (virus-related diseases) or the conventional medical inputs are considered highly effective for the treatment of the diseases (worms-related diseases) making the majority of the diseases to be less life-threatening in comparison to other diseases. However, research is still required for certain life-threatening diseases and the sheer number of the cases reported for such cases certainly warrant the necessary focus these diseases commanded from the scientific community. The information regarding the respective diseases is summarized in Table 6.3.

6.4 Application of the biosurfactants against tropical and life-threatening diseases

For the sake of clarification and summarization of the surveyed academic literature, the case studies documenting the use of BSs against tropical and life-threatening diseases were classified based on the causative agents/pathogens of the diseases. Broadly, the causative agents of tropical diseases can be classified into the categories of viruses, bacteria, and parasites. Specific case studies are discussed under these subheadings. Further, the medicinal impact of the BSs in the case of these studies is presented in Table 6.4.

6.4.1 Viruses based tropical diseases

Antiviral activity of the BSs has been described for a variety of enveloped viruses in the past thirty years. Anion channel formation in viral capsids and lipid envelops, protein loss which is involved in adsorption/penetration like processes, and viral membrane fusion inhibition were the points where inhibitory properties of BSs were observed [57]. There are two major reported classes of antivirals namely directly acting agents (DAAs) and host-acting antiviral agents (HAAs). Surface active agents as remarkable antivirals have a promising role in drug discovery owing to their physicochemical properties. Their mode of action is the destruction of the lipid membranes of envelopes viruses. Surface active agents interfere interestingly with gene expression, virion assembly, and exit kind of later stages of viral life cycle thus enhancing the host's response and improved viral clearance in this regard. Surfactin is also a lipopeptide obtained from *Bacillus subtilis*. It is a well-known BS that can interact with artificial and biomembrane systems for example enveloped viruses [58]. The bacterial sources of BSs may include *Bacillus, Lactobacillus, Pseudomonas, Burkholderia, Mycobacterium, Rhodococcus, Arthrobacter, Nocardia, Gordonia*, and *Acinetobacter*

Table 6.3 Description of the tropical diseases.

Tropical viral disease	Cause	Transmission	Regions	Symptoms	Year of spread	References
Dengue	Mosquito-borne *flavivirus* *Aedes aegypti* or *Aedes albopictus*	Spread to people through the bite of an infected *Aedes* species	Common in more than 100 countries in the world	Pain in head, eyes, muscles, joints. Severe conditions involve circulatory failure and shock.	The first epidemic of clinical dengue recorded in Madras in 1780, India	[36]
Yellow Fever	Arboviral disease *Aedes* mosquitoes	Primarily through the bite of infected Aedes or Haemagogus species mosquitoes	Tropical South America and Africa	Fever, hemorrhage, often a fatal liver complication	Originated in Africa, the first epidemic reported in 1648 in Yucatan	[37]
Rotavirus	Genus of double-stranded RNA viruses in the family *Reoviridae, out of A, B, C, D, F, G, H, I and J., rotavirus A* most common among human	Viruses in children's stool, usually due to contact with the hands of children who wipe after using the toilet, contaminate anything they touch	Worldwide especially in developing countries	Watery diarrhea and vomiting primarily in children, severe dehydration among people	In 1973, in the intestinal tissue of children with diarrhea	[38]
AIDS (Acquired immunodeficiency syndrome)	The human immunodeficiency viruses (HIV)	Through body fluids like blood, semen, vaginal and rectal fluids, breast milk. Not via air or water or casual contact	Widespread in developing nations	Vary according to the phase of infection,	Along the historic trade routes of the Congo basin in the 1920s	[39]
Ebola	Ebola viruses	Spreads rapidly to those in contact with body fluids from the patient and the mortality rate is very high	Widespread in developing nations	Severe headache, backache, vomiting, diarrhea, severe hemorrhaging	First discovered in 1976 near the Ebola River, Democratic Republic of Congo	[40]
Lassa fever	Fatal hemorrhagic fever virus, some from arenavirus family	Transmitted by rodents	Widespread in developing nations	Sharp backache, headache, sore throat, fever, rashes, dehydration, general swelling, skin		[41]

				hemorrhaging, irregular heartbeat, disorientation		
Malaria	05 species of *Plasmodium* parasites *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*.	Primarily transmitted by *Anopheles* mosquitoes Other parasitic modes include from mother to unborn child, sharing of needles utilized for drugs and blood transfusion	Sub-Saharan Africa South and Southeast Asia Pacific Island Central America and Northern South America	Fever Chills Headache Nausea Vomiting Diarrhea Abdominal pain Muscle and Joint pain Fatigue Rapid breathing Rapid heart rate	Documentation of malaria is millennia old. Between 150−300 million lives have been lost in the 20th century alone because of this disease.	[28]
Leishmaniasis	20 species of the intracellular protozoan parasite (genus *Leishmania*)	The bites of infected female phlebotomine sandflies	Asia Middle East North Africa Southern Europe	Cutaneous leishmaniasis: Skin ulcers Stigma Life-long scars Visceral leishmaniasis: Irregular bouts of fever Weight loss Enlargement of spleen and liver Anemia Mucocutaneous leishmaniasis: Partial/total destruction of mucous membranes of nose, mouth, and throat	The skin infections, now known to be associated with leishmaniasis, are documented from the 16th century to onwards in the regions of the Middle East. However, William Leishman, who was a doctor serving alongside the British Army in India, discovered *Leishmania* in 1901	[42]
Trypanosomiasis	Species of protozoan parasites from genus *Trypanosoma*	Primarily by the bite of an infected tsetse fly. Other modes include mother to the unborn	East and West Africa are heavily infected (Congo is one of the most adversely affected areas)	East African trypanosomiasis (caused by *T. brucei rhodesiense*) First phase:	In 1895, the famous pathologist David Bruce discovered *T. brucei*. During the year 1896 and 1906,	[43]

(*Continued*)

Table 6.3 Description of the tropical diseases. *Continued*

Tropical viral disease	Cause	Transmission	Regions	Symptoms	Year of spread	References
	T. congolense, T. vivax, *T. brucei brucei*, *T. simiae*, *T. brucei rhodesiense*, and *T. brucei gambiense*	child, mechanical transmission via blood-sucking insects, sexual contact, and accidental transmission by pricking from the needle having infected blood		Painful red spot of bite (Chancre) Fever Headache Irritability Swollen lymph nodes Muscle and joint pain Sleepiness Second phase: Neural disorders Personality changes Progressive confusion West African trypanosomiasis (caused by *T. brucei gambiense*) same symptoms as stated above but without chancre	epidemic associated with this disease was observed in Uganda and Congo followed by three more epidemics in 1920, 1970, and the late 1990s in the different African countries.	
Schistosomiasis	Species of blood flukes (trematodes) from the genus *Schistosoma* S. *haematobium*, *S. japonicum*, *S. mansoni*, *S. mekongi, S. intercalatum*, and *S. guineensis*	Contact with contaminated freshwater that has infected snails. Penetration of the skin by *Schistosoma* parasites during bathing, swimming, or other contact places. Soil and places contaminated by the infected person's excreta.	Africa Middle East Rainy forest of Central Africa Cambodia China Philippines Indonesia Brazil	Intestinal schistosomiasis: Pain Diarrhea Blood in stool Hypertension Extreme infection: Accumulation of fluid in the peritoneal cavity Enlargement of spleen Urogenital schistosomiasis: Hematuria (Blood in urine)	In 1851, Theodor Bilharz and Carl Ernst discovered *Schistosoma*. However, this disease became the area of interest in the modern era with the development of new basins and irrigation systems leading to the increase in the prevalence of this disease (60%) in 1821.	[44]

				Fibrosis of bladder and ureter Kidney damage In women: Genital lesions Vaginal bleeding Pain during intercourse In men: pathologic effects on prostate, seminal vesicles and other organs Infertility		
Lymphatic Filariasis	03 thread-like nematodes (roundworms) from the family *Filarioidea: Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*	Transmitted via several different types of mosquitoes including *Culex* (present in urban and semiurban areas), *Anopheles* (rural areas of tropics and subtropics), and *Aedes* (endemic of Pacific region).	South America Western Pacific Caribbean Tropics and subtropics of Asia and Africa	Mostly asymptomatic Lymphedema Scrotum swelling Excessive fluid collection in genitalia, arms, breasts, and legs Patients become susceptible to bacterial and microbial infections. Severe infections can lead to pulmonary eosinophilia syndrome Eyes infections Blindness	The disease is known throughout history. At present, 200 million people are infected with lymphatic filariasis.	[45]
Cholera	*Vibrio cholerae*	The bacterium is usually get entered into the human body through contaminated water and shellfish	Numerous tropical countries South America	The microorganism produces harmful substances which leads to the destruction of cell lining the intestine and leads to the excessive secretion of water and minerals from the body	The disease was spread across the world during the 19th century	[46]

(*Continued*)

Table 6.3 Description of the tropical diseases. ***Continued***

Tropical viral disease	Cause	Transmission	Regions	Symptoms	Year of spread	References
Tuberculosis	*Mycobacterium tuberculosis*	This disease is usually transmitted through the air from an infected person to a healthy person from sneezing, spit, and coughing	China Philippines Indonesia Pakistan Bangladesh South Africa	The organs of the whole body can get infected by the disease, in the beginning, the lungs are being affected and difficulty in breathing, coughing, and weight loss	The disease was discovered in early 1880, when the disease killed one out of seven people in the US and Europe	[47]
Leprosy	*Mycobacterium leprae*	The hundred percent exact mechanism of transmission is unknown but it is thought to be transmitted via nasal secretion and skin	Africa Asia America China	It can affect the hands, feet, nose, eyes, and nerves. It can lead to paralysis and blindness	The disease has a history starting from 1873 from its discovery and it was declared as an endemic in 1999.	[48]
Escherichia coli	*E. coli*	The microorganism get to enter into the human body via intake of undercooked meat, unpasteurized milk, and fruit juices	America Japan Scotland	Causes diarrhea which may become persistent with associated malnutrition	The disease is still present in history and firstly the outbreak appeared in 1982	[23]

Table 6.4 Summary of the biosurfactants utilized for the treatment/management of tropical and life-threatening diseases.

Biosurfactant producer	Biogenic classification	Bacterial strain and cultural conditions 1. Carbon source 2. Nitrogen source 3. Temperature 4. pH	Characterization/ assays for the biosurfactants	Biosurfactants identified	Tropical diseases	Medicinal role of biosurfactant studies	Remarks	References
Bacillus subtilis A1 Pseudomonas stutzeri NA3	Bacteria	1. Crude oil reservoir (India) 2. Tryptophan (10.0 g/L); yeast extract (5.0 g/L) 3. 40°C for *B. subtilis A1* and 30°C for *P. stutzeri NA3* 4. pH = 7	Assays: Oil displacement test Characterization: FTIR GC-MS	Lipopeptide biosurfactants (BSs) FTIR: *B. subtilis A1* Hexadecanoic acid Octadecadienoic acid Octadecenoic acid GC-MS: *P. stutzeri NA3* 1-dodecanol Oleic acid Hexanoic acid Octadecyl ester	Malaria (can also be said to be against the disease of Lymphatic Filariasis as the *Anopheles* is the vector for this disease as well)	Toxicity studies of BSs on young instars of malarial vector *Anopheles stephensi* Fecundity, longevity, and development reduction tests on *A. stephensi* in the presence of BSs	Both BSs exhibited enhanced mosquitocidal action against the young instars, larvae and pupae of the *A. stephensi*. The reduction in the surface tension owing to the BS leads to oxygen deficiency ultimately leading to the lysis of the mosquito cells.	[28]
Planococcus maritimus SAMP MCC 3013	Bacteria	1. Glucose (1.5% w/v) 2. NH_4Cl (29.4 mg/100 mL) 3. 30°C 4. NR	Assays: Surface tension measurement Drop collapse test Oil displacement test Characterization: FTIR NMR TLC LC-MS	TLC: Linoleic acid Cholesterol Dipalmitin Oleic acid Tripalmitate FTIR: Class of terpenoids	Malaria Tuberculosis Cervical cancer Breast cancer Colon cancer	Antiplasmodial activity against the strain of *Plasmodium falciparum (3D7)* in the form of determining in the reduction of growth percentage values (growth inhibition test) and determination of EC_{50} values for the active BSs.	Excellent study where the acquired experimental results were theoretically explained by using computational tools such as molecular docking studies	[49]

(*Continued*)

Table 6.4 Summary of the biosurfactants utilized for the treatment/management of tropical and life-threatening diseases. *Continued*

Biosurfactant producer	Biogenic classification	Bacterial strain and cultural conditions 1. Carbon source 2. Nitrogen source 3. Temperature 4. pH	Characterization/ assays for the biosurfactants	Biosurfactants identified	Tropical diseases	Medicinal role of biosurfactant studies	Remarks	References
				NMR: Class of terpenoids		In vitro antituberculosis activity was studied against Mycobacterium tuberculosis		
				LC-MS: Glycolipids (class of sugar and lipid molecules) Rhamnolipid		Cytotoxicity assays against HeLa (cervical cancer), HCT (colon cancer) and MCF-7 (breast cancer) cell lines		
Bacillus tequilensis	Bacteria	1. Olive oil (0.5%) 2. $(NH_4)_2SO_4$ (3 g/L), $NaNO_3$ (2 g/L) and $NH_4MoO_40.2H_2O$ (15 mg/L) 3. 35°C 4. pH = 6.8	Characterization LC-Ms TLC HPLC	TLC Purification was confirmed LC-Ms Cyclic lipoproteins components HPLC Surfactin Fengycin	Malaria Lymphatic filariasis	The larvicidal activity against *Anopheles culcifacies*	LC_{50} value of 110 μg/mL was observed for the duration of 2 days	[50]
Bacillus subtilis DM-03 and DM-04 strains	Bacteria	1. Petroleum crude oil 2. NH_4NO_3 (1 g/L) 3. For strain DM-03 (45°C) and for DM-04 (55°C) 4. For strain DM-03 (pH = 8) and for DM-04 (pH = 7)	NR	Unknown	Lymphatic filariasis	The larvicidal activity against *Culex quinquefasciatus*	LC_{50} values for the BSs from DM-03 and DM-04 strains against the instar larvae was found to be 120 and 300 mg/L respectively after the 24 h of the treatment.	[51]

Bacillus licheniformis Dahb1	Bacteria	1. Glucose (20% v/v) 2. Tryptophan (10.0 g/L); yeast extract (5.0 g/L) 3. 37°C 4. pH = 7	Assays Emulsification index Characterization FTIR NMR	FTIR Polysaccharides NMR polysacchrides	Malaria Zika virus Lymphatic filariasis Bacterial infections Fungal infections Yeast infections	MIC assays against the bacterial strains (*Bacillus subtilis*, and *Bacillus pumilus*), fungus strains (*Pseudomonas aeruginosa* and *Proteus vulgaris)* and yeast strain (*Candida albicans)* Larvicidal activity against *Anopheles stephensi* and *Aedes aegypti*.	The study is significant in terms of not only documenting the numerical values but also providing information regarding the working mechanism of the BSs action against the pathogens by using stereomicroscopic images.	[13]
Bacillus pumilus	Bacterial strain isolated from the black coral *Antipathes* sp.	1. Starch of potatoes (10 g/L) 2. Yeast extract (4 gL) 3. NR 4. NR	Characterization HPLC TLC LC-MS	HPLC/LC-MS and TLC 3FI, AOT 3HAI Cyclo-(L-Phe-L-Pro) Cyclo-(L-Leu-L-Pro)	Leishmaniasis Trypanosomiasis Malaria	Antiparasitic activity against *Leishmania donovani*, *Trypanosoma cruzi* and *Plasmodium falciparum*	The identified were inactive against the diseases of malaria and Leishmaniasis. However, 3FI, AOT, and 3HAI were found to be extremely active against the *Trypanosoma cruzi*.	[52]
Pontibacter korlensis strain SBK-47	Marine bacterium	1. Glucose (2%) 2. NH_4NO_3 (0.3%) 3. 37°C 4. pH = 8	Assays Surface tension test Emulsification index test Characterization TLC HPLC FTIR GC-MS	TLC Confirms the presence of peptide and lipids spot FTIR Lipopeptide	Cholera *Vibrio parahaemolyticus* infection Dental caries *E. coli* infection Typhoid fever	Antibacterial potential against pathogenic strains (*Streptococcus mutans MTCC 890*, *Staphylococcus aureus MTCC 96*, *Escherichia coli*	Excellent antimicrobial activity against the pathogens and the nonpathogenic strains were observed in the case of the newly isolated surfactant.	[53]

(*Continued*)

Table 6.4 Summary of the biosurfactants utilized for the treatment/management of tropical and life-threatening diseases. ***Continued***

Biosurfactant producer	Biogenic classification	Bacterial strain and cultural conditions 1. Carbon source 2. Nitrogen source 3. Temperature 4. pH	Characterization/ assays for the biosurfactants	Biosurfactants identified	Tropical diseases	Medicinal role of biosurfactant studies	Remarks	References
						MTCC 443, *Salmonella typhi MTCC 733*, *S. paratyphi MTCC 735*, *Vibrio parahemolyticus MTCC 451* and *V. cholerae MTCC 3906*)		
				NMR Lipopeptide		Antibacterial potential against nonpathogenic strains (*Bacillus subtilis MTCC 619*, *Micrococcus luteus MTCC 3911* *Enterococcus faecalis MTCC 6845*, *Klebsiella pneumoniae MTCC 7162* and *K. oxytoca MTCC 303*0)		
				GC-MS/HPLC/ (MALDI-TOF/ MS) Pontifactin		Antibiofilm assay against the above specified strains		

Candida bombicola	Yeast	1. Glucose (10 g/L) 2. Liquid nitrogen 3. 30°C 4. pH = 7	Characterization (associated with cancer/HIV studies) VK-2/E6E7 cell line, Structure-activity relationship (SAR) analysis, CMC	Sophorolipid Diacetate ethyl ester derivatives	HIV/AIDS	Virucidal activity against HIV and sperm-immobilizing activity against human semen are similar to those of nonoxynol-9	Findings show that the BSs acquired were the most potent spermicidal and virucidal agent of the series of sophorolipids thus far studied	[54]
Pseudomonas aeruginosa strain 57RP	Bacteria	1. 2% (w/v) mannitol or naphthalene 2. $NaNO_3$ 0.9 g/L 3. 30°C 4. pH = 6.7	LC-MS HPLC GC-MS	Mixture of rhamnolipids	Dengue fever Urban yellow fever Chikumgunya viruses	Exhibit antimicrobial activity against competing microorganisms	The most abundant rhamnolipid produced from naphthalene contained two rhamnoses and one 3-hydroxydecanoic acid group.	[55]
Bacillus subtillis B50	Bacteria	1. 2% engine oil 2. NH_4NO_3 (0.1%) 3. 30°C 4. pH = 7	Assays Oil spreading technique Emulsification index Drop collapse method Foaming activity test Blood hemolysis Characterization FTIR	Lipopeptides	Dengue fever, urban yellow fever, Chikumgunya viruses	Larvicidal activity Biopesticide to control mosquito larvae.	An eco-friendly product for the eradication of mosquitoes with quite an effective accuracy and efficacy.	[56]

NR, *Not reported;* FTIR, *Fourier transform infrared spectroscopy;* GC, *gas chromatography;* Ms, *mass spectrometry;* LC, *liquid chromatography;* NMR, *nuclear magnetic resonance;* EC_{50}, *half-maximal effective concentration;* LC_{50}, *Lethal concentration 50;* MIC, *minimum inhibitory concentration;* 3FI, *3-formylindole;* AOT, N-*acetyl-β-oxotryptamine;* 3HAI, *3-hydroxyacetylindole;* MALDI, *matrix-assisted laser desorption/ionization,* TOF, *time of flight.*

whereas the examples of yeast and the filamentous fungi sources may include *Candida, Saccharomyces, Starmerella, Trichosporon, Pseudozyma*, and *Ustilago* [57].

Dengue, a mosquito-borne viral infection, is found in urban and semiurban areas of tropical and subtropical climates worldwide. The virus causing this infection is called the "dengue virus" and is abbreviated as "DENV". DENV is called severe dengue because it may be developed into a potentially lethal complication occasionally causing acute flu-like illness. The life cycle of the Dengue virus in the host cell has been shown in Fig. 6.2. Flaviviridae family is responsible for dengue fever which has four distinct serotypes namely DEBV-1, DENV-2, DENV-3, and DENV-4 with an alarming impact not only on humans health but also on global and national economies. Medical professionals must take serious note of day by day increasing illness and deaths in some Asian and Latin American countries. Early detection and access to proper medical care are the only possible measures to lower the fatality rates as there is no specific treatment for this deadly disease. About half of the population is at risk due to its dramatically increasing rate with an estimate of 100−400 million infectious cases each year. Some diseases are transmitted from person to person whereas a few may be transmitted via an intermediate species/organisms called vectors. Dengue is spread by a female mosquito mainly from *Aedes aegypti* species but to a lesser extent by *Aedes. albopictus*. One can observe its transmission in two ways, that is, mosquito-to-human and human-to-mosquito as well [60]. A high fever up to 40°C along with any of the two symptoms of severe headache, pain behind the eyes, nausea, vomiting, muscles and joint pain, swollen glands, and rash may lead to dengue suspect. However, one can suspect severe dengue (patients in critical phase) if the patient is ill for seven days and temperature starts dropping below 38°C along with symptoms of severe

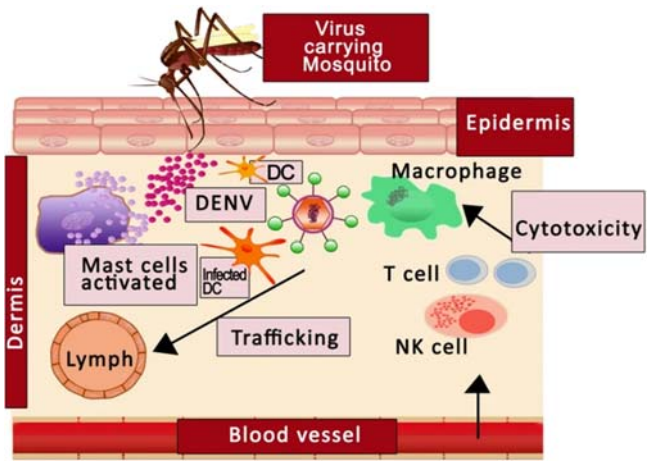

FIGURE 6.2

Mosquito bite inducing the transmission of dengue virus followed by the formation of the toxic substances to cellular components.

Reproduced fromA.N. Anoopkumar, E.M. Aneesh, Environmental epidemiology and neurological manifestations of dengue serotypes with special inference on molecular trends, virus detection, and pathogenicity, Environ. Dev. Sustain. 23 (2021) 11217–11239 [59]

abdominal pain, persistent vomiting, rapid breathing, fatigue, blooding gums, blood in vomit, or restlessness. These two major categories of dengue are designed to help health practitioners judge patient's hospital admissions for close observations to minimize health risks in time. Therefore, the prevention and control measures of dengue depend on effective vector control measures, and sustained community involvement may play an important role in substantial control efforts of dengue in this regard.

As far as the diagnostic methods are concerned, both the virological and serological methods may be followed based on the time of patient presentation. Other than community engagements and reactive vector control, some more precautions such as prevention of mosquito breeding, personal protection from mosquito bites, active mosquito, and virus surveillance may be taken to avoid this fatal tropical disease [36].

However, research is being conducted to highlight the use of BSs in curing dengue. Use of insecticides such as organochlorides, organophosphates, carbamates, and pyrethroids have been used but their toxicity and the resistance acquired by the mosquitoes over time motivates the researchers to investigate some other replacements on an earlier basis. [61]. Fusicoidan directly interacts with the dengue virus serotype 2, DENV-2 [62]. It is isolated from the marine alga *Cladosiphon okamuranus*. It interacts with the envelop glycoprotein of DENV-2, impairing its attachment and internalization to the host cell.

It's an acute viral hemorrhagic disease that is spread by the viruses from *Falviviridae* and genus *Flavivirus*. It was originated in Central Africa with its first outbreak in Tucatan peninsula, Mexico in 1648. It was transmitted by infected mosquitoes and ticks. It has three types of transmission cycles namely Sylvatic or jungle transmission, intermediate transmission, and urban transmission [37]. It's a positive-sense, single-stranded, RNA enveloped flavivirus with 50−60 nm in diameter. Blood tests (RT-PCR) sometimes are used for its detection in the early stages. However, identification via antibodies is required (ELISA and PRNT). *A. aegypti* mosquitoes are also found as primary transmitters of urban yellow fever. Rhamnolipids have been evaluated for their larvicidal and insecticidal activities against these mosquitoes. They exhibit lower toxicity and high biodegradability. Their production by renewable carbon sources and use as *A. aegypti* control make them an excellent substitute for chemical surfactants [55,63].

It was first identified as a cause of diarrhea in 1973 in infants and children and the death rate is found much greater in developing countries. It belongs to the viral family *Reoviridae*. It's a Reovirus (RNA) with 60−80 nm in size and has double-stranded RNA. It's a nonenveloped virus with a wheel like appearance (rotavirus name derived from Latin, meaning wheel) as observed by electron microscopy. The mechanism of the disease follows its replication in the gut mainly. They infect the villi enterocytes of the small intestine and produce changes in the structure and function of epithelium [38].

Vishal Shah et al. in 2005 studies the sophorolipids produces by *Candida bombicola* and its structural analogs as microbial glycolipids with antihuman immunodeficiency virus and sperm-immobilizing activities [64]. Their diacetyl ethyl ester derivatives were reported to be the most potent spermicidal and virucidal agents and their virucidal activity is comparable with those of globally well-known surfactant nonoxynol-9 [54,61]. Mycoplasmas being causative agents of serious human diseases seem to be cofactors in the pathogenesis of AIDs. Dirk et al. have observed antimycoplama properties of surfactin by using electron microscopy which indicated its mode of action. Complete and permanent inactivation of mycoplasmas in suspension cell cultures and the

mammalian monolayer was observed with the development of a fast and simple method by this research group in 1997 [65].

The main cause of this infectious disease is Ebola viruses which are diagnosed by either finding the virus, viral RNA, or antibodies in the blood. They are reported to replicate in monocytes, macrophages, dendritic cells, liver cells, fibroblasts, and many others interfering with the proper functioning of the innate immune system [40].

Various recent studies on BSs have revealed their antiviral activity mainly by their physicochemical interaction with lipidic viral envelops. Various classes of BSs namely glycolipids, phospholipids, and lipopeptides interfere with the activities of immune systems. BSs may be found on microbial cell wall surfaces. They may also be found in extracellular space by different bacteria, yeasts, and filamentous fungi. A general conception about a pandemic is the spread of an infectious disease agent to multiple borders (or continents). These outbreaks may have been caused due to some viral origin. BSs have truly presented their role in the development of therapeutics and even to meet such challenges in the future. An example of this has been the idea of the development of some suitable solution of COVID-19 in this regard. The outbreaks from air-borne RNA viruses pose high risks such as severe acute respiratory syndrome Coronavirus 2 also abbreviated as SARS-CoV-2. As a consequence, cost-effective BS based therapeutics are a necessity for currently known tropical and life-threatening viral infections including Lassa fever meningitis, Marburg virus disease, Ebola virus disease, yellow fever, and Zika virus disease [66]. BSs as innovative components are the most appropriate alternatives for vaccine development, immune system enhancers, drug delivery like therapeutics. *Candida* species are the most common microbial surfactants producing lipopeptides, glycolipids, and Mannosylerythritol lipids for pharmaceutical applications [67].

6.4.2 Bacteria based tropical diseases

BSs are biologically derived surface-active compounds that have been investigated for antimicrobial activity over a variety of bacterial strains. In 2019, Giri et al., isolated the BSs from Bacillus subtilis VSG4 and Bacillus licheniformis and found their potent antibacterial efficacy against the Gram-positive and Gram-negative bacteria. Garg and coworkers [20], derive a BS from the contaminated dairy products successfully. They determined that the isolated BS is potentially active against E. coli and S. aureus at decreased concentrations of 10 and 5 mg/mL.

Sana et al. [68] expressed the antibacterial activity of rhamnolipid produced by Pseudomonas aeruginosa C2 and a lipopeptide type BS, BS15 against two harmful bacterial strains, Staphylococcus aureus ATCC 25923 and Escherichia coli K8813. The performed experiments by the researcher determined that both the compounds exhibit a synergism and effectively work against the bacterial strains as a result of enhancement in the bacterial cell permeability.

In 2016, Balan and coworkers [53] produced a novel BS names as pontifactin. They found the BS very promising and effective against Streptococcus mutans, Micrococcus luteus, Salmonella typhi and Klebsiella oxytoca at a BS concentration ranging between 1 and 2 mg/mL. A study on the antibacterial efficacy of BS combined with sodium dodecyl sulfate in the presence of some organic acids was done by Rienzo and coresearchers in 2016 [69]. It was concluded that sophorolipids are promising BS for the disruption of biofilms formed by Gram-positive and Gram-negative bacteria and this antibacterial activity can be effectively enhanced by the addition of booster

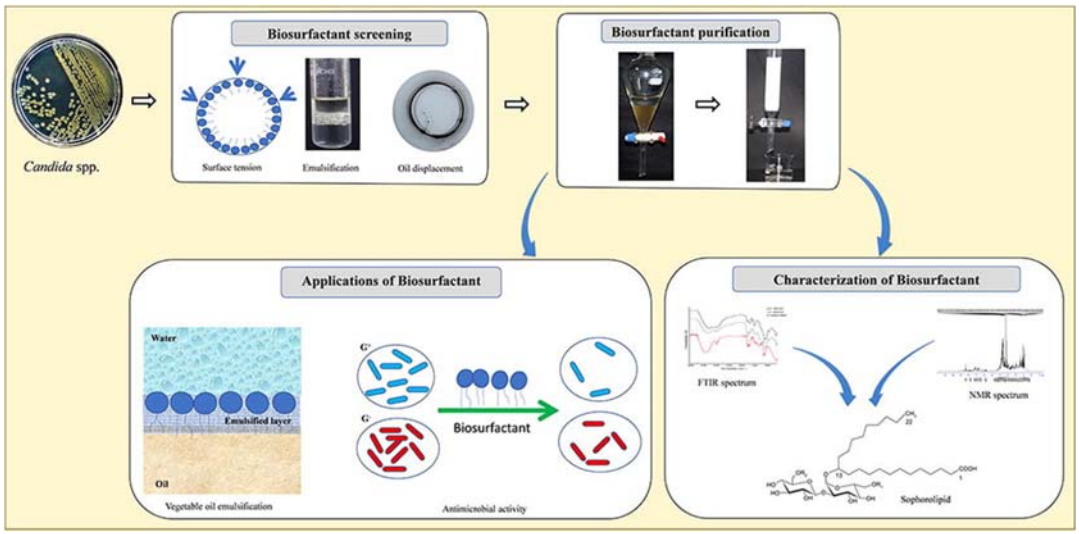

FIGURE 6.3

Research plan for the case study documenting the use of biosurfactants for antimicrobial potential.

Courtesy V.K. Gaur, R.K. Regar, N. Dhiman, K. Gautam, J.K. Srivastava, S. Patnaik, et al., Biosynthesis and characterization of sophorolipid biosurfactant by Candida spp.: application as food emulsifier and antibacterial agent, Biores. Technol. 285 (2019) 121314 [70]

compounds such as caprylic acid. Fig. 6.3 presents the graphical description of the research study documenting the use of BSs for antibacterial applications.

6.4.3 Parasites based tropical diseases

Concerning the academic survey performed, most of the case studies associated with the biosurfactants are focusing on the diseases of malaria among all other parasitic diseases. Sabarinathan et al. [11] extracted the BS of rhamnolipid from the *Pseudomonas plecoglossicida BP03* from the sample collected from rice mill waste. The BS exhibited copacetic larvicidal and pupicidal against the malarial vector of *Anopheles sunadicus*. The study also highlighted the biomedical application of BSs against the various human pathogens and several drug-resistance microbial strains (*Staphylococcus aureus*, *Bacillus subtilis*, and *Aeromonas hydrophila*). It is also worth mentioning that several species of *Anopheles* also act as vectors for the transmission of filariasis disease indicating that the documented antimalarial potential can be utilized as an antifilariasis as well. Geetha et al. [71], Ghribi et al. [72], Deepali et al. [73], and Bhosale et al. [74] have documented the use of BSs for the control/treatment of malarial disease.

Among the tropical diseases that are caused by parasites, only the disease of malaria has been found at the center of research investigations in the case of BSs. Case studies reporting the biomedical applications of BSs of the diseases of filariasis, schistosomiasis, leishmaniasis, trypanosomiasis, etc. are very scarce. Literature survey for the disease of trypanosomiasis revealed only a few

studies reporting the use of BS for achieving selective cell lysis capacity against *Trypanosoma cruzi* (the causative agent of trypanosomiasis). Luis et al. [52] utilized the isolated BSs of 3-formylindole (3FI), *N*-acetyl-β-oxotryptamine (AOT), 3-hydroxyacetylindole (3HAI) from the bacterial strain of *Bacillus pumilus* removed from the black coral surface. These three compounds exhibited the half-maximal inhibitory concentration (IC50) values of 26.9, 20.6, and 19.4 μM for the compounds of 3FI, 3HAI, and AOT, respectively. Other compounds were found to be ineffective against the strain of *T. cruzi*. Further research is still needed to be done in case of medical applications of BSs against the disease of trypanosomiasis. Zhao et al. [75] utilized recombinant bacteria (*Escherichia coli* expressing the pttABC gene cluster collected from *Photorhabdus temperata Meg1*) for the production of novel cyclic BSs. These BSs exhibited excellent antiprotozoal potential against various species including *Plasmodium falciparum* (malaria disease), *Leishmania donovani* (leishmaniasis disease), *T. cruzi* (trypanosomiasis disease) and *T. brucei rhodesiense* (trypanosomiasis disease).

Academic survey of the BSs indicated that although the use of BSs for medicinal applications has been documented for various diseases but few tropical diseases have not yet been tested. The diseases affiliated with parasitic worms have been one such domain that could be explored in the future for further research as to the best of our survey, any case study that highlights the medicinal potential of the BSs against the diseases caused by worms was not found in the academic literature. Nematodes based plant diseases have been explored [76] but the diseases in humans have not been explored. The reason behind this lack of interest in the ailments caused by warms can also be attributed to the fact that these diseases are as such not life-threatening and proper conventional medicine/treatment is available for such diseases. Similarly, the disease of the Leishmaniasis (caused by the parasitic protozoans) is also not utilized for the investigation although the study documented by Vivero et al. [77] suggested that the metabolites acquired from the bacterial strain possess excellent leishmanicidal activity with half-maximal effective concentration (EC_{50}) value of 47.7 μg/mL against the *L. braziliensis* (UA301) which is a known vector of Leishmaniasis in Colombia. However, the author utilized methanolic extract for this purpose. They didn't isolate the BS from these strains for carrying out this medicinal approach. Therefore, this disease can also be investigated further from a research expansion point of view. To the best of our knowledge, any study documenting the use of the BSs against the causative agents of schistosomasis (*Schistosoma mansoni* and *Schistosoma haematobium*) has not yet been documented. It was deduced that although it is a serious disease with the serious life-threatening implication if left unchecked but this hardly seems to be the case as the symptoms affiliated with the acute stage of this disease are very hard to ignore and patients seek treatment right away in case of these ailment resulting in the full recovery of these patients. Therefore, this disease is also not considered to be explored till now for investigating the medicinal potential of the BSs.

6.5 Conclusion

Although the BS industry had remarkably demonstrated their efficient use in combating the tropical and life-threatening diseases due to their unique surface active properties, yet their challenge as cost effective therapeutics is a major highlight in this regard. Their potential applications has helped a lot to address the public health problems such as dealing with extreme urgency, reduction and prevention of tropical viral, bacterial, parasitic and various other pathogenic diseases.

Recognition of their certain characteristics and substantial potential in pandemic management opens new horizons for planning the control measures of such life-threatening diseases. Their extensive use in development of antibiotics, vaccines and other pharmaceuticals offer treatment of both broad-spectrum and specific pathogens with significant benefits. The futures of BS based pharmaceutical industry will surely encompass their use in nanotechnology and various drone applications. Their biodegradability and environment friendly properties will help researchers in looking for new horizons to make them competitive therapeutics with respect to cost of production, developing tools for unexplored areas and more conclusive evidences for future endeavors.

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Conflict of interest

Authors do not have any conflict of interest to declare.

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CHAPTER

Application of biosurfactant for the management of *Plasmodium* parasites

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7.1 Introduction

Biosurfactants (BSs) are characterized as bacterial metabolites that are made up of amphiphilic assemblies that possess numerous biological activities such as antitumor, antimicrobial, anti-*Plasmodium*, emulsifying, and tensioactive properties. These are well known to be nonnoxious and biodegradable, making them more effective, green, cheaper, and eco-friendly when compared to the widely used artificial surfactants [1–8]. Specifically, these byproducts or metabolites are usually produced by species of *Bacillus* and *Pseudomonas* that are resourceful to several organisms as pathogens [8].

Collectively, BSs and yeasts, have been commonly utilized as agents of fermentation and generally recognized as safe (GRAS) grade that removes the hazards associated with pathogenicity and toxicity in their utilization in pharmaceutical and food manufacturing without boundaries [4,9]. Furthermore, the industrial production of BS using bioprocessing is considered beneficial in the terms of cost of production, demands, and sustainability when compared to the synthetic forms. Based on these benefits (biological and physicochemical properties), BSs are considered to be widely safe for use in the agricultural, pharmaceutical, food, medical, petroleum, and chemical industries [10–14].

Species of Anopheles mosquitoes have been documented to cause malaria in the subtropical and tropical regions of the world, which have accounted for about 198 million cases and 584,000 mortality in 2013 [15,16]. Since the year 2000, globally, the death rates from malaria have fallen by 47% and on the African continent by 54%. Nonetheless, recently, there have been fresh epidemics of malaria after the clampdown in many nations of the world [16–19].

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The biology of mosquito parasites such as species of *P. knowlesi*, *P. malariae*, *P. ovalecurtisi*, *P. ovalewallickeri*, *P. vivax*, and *P. falciparum*, affecting humans via hemoglobin, has been examined in vitro [20]. These parasites undergo about 10 replications from a sole cell to about 10,000þ cells, which differ in more than 106 animals [21–23]. Some health complications like enzyme deficiency, cytokine mutations, hemoglobinopathies, and sickle cell disease are fascinating outcomes from the pressure of these parasites, which can lead to mortality or severe morbidity in humans and animals likewise [20].

Morbidity and mortality from malaria have been an aged long issue. Teleologically, mortality from malaria below age six, been a frequent incidence for some decades and of demographical investigation and concern. However, approximately 0.2% of 438,000 clinical cases, leave the ill-fate, death as an abnormal footnote in the general biology of the whole species [24]. Common symptoms include cold or chills, anemia, fever, joint pain, headache, stomach bites, and a spectrum of other commodities [25–28].

The control and management of pathogens and parasites of malaria had been a serious challenge owing to the high resistance to insecticides and antimalarial medications. However, an efficient way to these rebounds can be the direct target on the undeveloped instars. Nonetheless, more efficient and eco-friendly control methods are needed [29–32]. Several studies have proposed the use of plants in the control of vectors of malaria [33,34] and others suggested the use of green techniques as a mixture of nanomaterials with herbal constituents [35,36]. However, it has been established recently that BS metabolites are derived from *B. thuringenesis* var. *israelensis*, *B. sphaericus*, *B. thuringiensis*, *P. fluorescens*, and *B. subtilis* have been proven to control and manage the bioactivity of mosquito larval, with little or no influence on nontarget species [6,37,38].

Therefore, the present chapter intends to examine the biomedical application of BSs in the control and management of several strains of *Plasmodium* species. The mode of action of the BSs on the parasites as well as the different methods related to their synthesis, extraction, and purification of BS have been evaluated. Additionally, the characterization of BSs utilized in the treatment of malaria, their environmental application for the management of different types of mosquitoes, and the applications in vitro and in vivo for the management of the parasites are highlighted. Furthermore, the treatment of the parasite using in vitro or in vivo techniques and the modes of action involved in the application of BS for the management of the vector and the parasites are discussed.

7.1.1 Application of biosurfactant in an in vitro and in vivo for the management of *Plasmodium falciparum* vectors

Fungi and bacteria secrete small siderophores that have a high affinity for iron (Fe) and these act as a chelating agent when combined and used with some chemicals for effective transportation across cell membranes for the control and management of diseases, parasites, pests, bacterial and fungus infections. The potential of these siderophores has been evaluated [39] in a biological in vitro study in the control and management of *P. falciparum*. The importance of Fe is important for *P. falciparum* to thrive in the red blood cells of humans has been very well discussed. Siderophores metabolites with triscatecholate or monocatecholate substituent have the potential to control its proliferation with less toxicity at 50% LD (lethal doses) on the human red blood cell.

Fascinatingly, less than 20M of Fe salts can inverse the antiparasitic influence with compounds containing triscatechol thus indicating that the antiparasitic influence may be chiefly based on restraint of the availability of cellular Fe. Conversely, the level of toxicity exerted by substances of monocatechol on *P. falciparum* was not influenced by exogenous Fe salts. The implication of this is that the antiparasitic influence of the substances is autonomous of the interface with the pool of the Fe in the intracellular contents' consequent of different mechanisms like direct interference of beneficial enzymes around the plasmid and the hemoglobin. They observed that the mode of action of the siderophores to remove the parasites was dependent on the lipophilicity and catecholate replacement of some rudimentary structures which might be also chiefly based on the Fe chelators between the derivatives of triscatechol and monocatechols. The authors suggested further investigations on the structure−activity relationships of novel synthesized BS siderophores metabolites derivatives which can be utilized for the treatment of *P. falciparum* in humans.

Parthipan et al. [16] tested and evaluated the potential of BSs manufactured by strains A1 and NA3 (*B. subtilis* and *P. stutzeri*) in the reduction of the fecundity and longevity of the vector of malaria (*Anopheles stephensi*) and induced high venoms on the undeveloped instars. The authors recounted that *Plasmodium* species were the parasites carried by a vector female Anopheles mosquito in the subtropical and tropical regions of the world. At present, the management and control of malaria is a big problem because of the resistance and rebounds of the parasites to many insecticides and antimalarial medications. The biological surfactants were evaluated against the strain of *Plasmodium* and then characterized by GC-Ms and FT-IR spectroscopic techniques which identified the lipopeptides form of the BSs. The strains A1 and NA3 were also tested against the pupae and larvae *Plasmodium* strains at 50% lethal dose values [3.51, 4.92, 5.73, 7.10, and 7.99 (larva, I-IV pupae) and 2.61, 3.68, 4.48, 5.55, and 6.99 (I-IV, pupa)], respectively. The treatment with the biological surfactants elicited different physiological variations like reduced fecundity, longevity, and oviposition timing as well as elongated pupal period. Further study was recommended by the authors to investigate novel insecticides that can combat malaria vectors.

In recent decades, the therapeutic efficacy of BSs in many areas like medicine, pharmacy, agriculture, and engineering cannot be overemphasized. In line with its immense utilization, Waghmode et al. [40] explored the pharmacological benefits of the utilization of BS originated from the strain SAMP MCC 3013 (*Planococcus maritimus*) in the management and control of cancer cells, *P. falciparum*, and *Mycobacterium tuberculosis*. The biological content of the surfactant was mixed with 1.5% w/v glucose and MSM (mineral salt medium). This was further purified and characterized as a solitary glycolipid. The glycolipid BS was proven to be an effective inhibitor against the cancer cells, *P. falciparum*, and *M. tuberculosis* at 160.8 ± 1.64 μg/mL, EC50 34.56 ± 0.26 μM and 64.11 ± 1.64 μg/mL correspondingly. More so, a cytotoxicity influence was also noticed at IC50 41.41 ± 4.21, IC50 31.233 ± 5.08, and IC50 42.79 ± 6.07 μg/mL respectively in the cell lines. The results from the glycolipid molecular docking revealed the connection of RHL (Rhamnolipid) with the enzymes InhA (reductase) of *M. tuberculosis*. There was also an observation of the correlation between the anticancer and antitubercular activities with the tubulin to form Van der Waal and hydrophobic association. The findings from the study showed that the strains had the potentials to exhibit cytotoxic, antiplasmodial, and antitubercular activities. This rated them as worthy candidates to fight several illnesses.

Silva et al. [38] characterized and tested the antiplasmodial impact of the Rhamnolipids produced by strain LBI 2A1 (*P. aeruginosa*) on the control and management of mosquito vectors and

larvae. The authors recounted that mosquitoes were the major carriers of malaria, chikungunya, yellow, and dengue viruses. These diseases have been documented to invade a larger proportion of the subtropical and tropical regions of the world. The vectors are also well known to be highly resistant to some synthetic and natural chemicals. However, eco-friendly and nontoxic Rhamnolipids from microbes and surfactants have been proven to be effective in the control and management of the negative health vectors portend. Findings from the study after in vivo exposure to concentrations (800, 900, and 1000 mg/L) of the Rhamnolipids on the vector, revealed that the BS killed all the larvae and adult metamorphic stages at the 18 hours and 100% respectively at the highest concentration (1000 mg/L). The utilization of Rhamnolipids could be employed in the eradication, combating, and repelling of ecologically related parasites. This can also be substituted for the widely used insecticides like pyrethroids and tempos that are highly resistant to these chemicals as well as harmful to the environment and human health.

Geetha et al. [41] tested and evaluated the potential of BS sourced from strain VCRC B471 (*Bacillus subtilis* subsp. *subtilis*) as an adulticidal, in the control and management of *Plasmodium* vector (*An. stephensi*) at different metamorphic stages. The outcome from the study showed that the cultured BS had adulticidal action when tested as ULV (ultra low-volume) spray in the Peet-Grady compartment. Mortality and knockdown actions were initiated to improve with an increase in the dosage of the surfactant. The outcome of the LD and KD (lethal dosage and knockdown dosage) at 50% and 90% correspondingly, were estimated to be 10.73 and 26.39 mg $m^{(-3)}$ and 16.13 and 39.21 mg $m^{(-3)}$. The mean size droplet of the BS was estimated to be 17.5 ± 1.07 μm. The finding from the study indicated that strain VCRC B471 had the bioadulticide potential against the vector *An. stephensi* transmitting malaria.

Biopesticide BSs are surface-active metabolites that consist of multifunctional properties such as antiinsecticidal, antiplasmodial, and antipesticide. Silva et al. [42] tested and evaluated the impact and efficacy of a new BS in conjunction with a fungus that is entomopathogenic on a species of economically important insect (Bemisia whitefly). The authors stated that BSs (rhamnolipid) sourced from *Beauveria bassiana* and *Cordyceps javanica* were widely used as antipesticide to combat pests of ecological concern. The result from the biological study showed that there was a dramatic improvement in the rate of recovery of the conidia about 2−5 times as when compared to the Tween 80 surfactant. The outcome of the mortality when the nymphs were sprayed with 0.1 and 0.075% (w/v) rhamnolipid, showed 100% death at day 4. The modified conidial mixture at 5×106 conidia/mL at 0.05% or 0.01%, was marked by the increase of the nymphal deaths with reduced 50% LC. It was noticed that the conidial mixture of 0.05% added rhamnolipid, was more antagonistic against the insect resulting in 87.3% death when compared to the 51.4 deaths of *C. javanica* mixture with rhamnolipid. The authors concluded by recommending the bacterium rhamnolipid as a first-tier bio-insecticidal for the treatment of any insect resistant to conventional chemicals.

Diseases like yellow fever, chikungunya, malaria, and dengue are becoming a serious threat to human existence on earth. Insects have become more resistant to many conventional chemicals like some natural ones. It has been known recently that metabolites of some strains of fungi and bacteria can be applied in the control and management of many insect pathogenic vectors. On the verge of sustainable health management, Jayasree et al. [43] tested the potential of BS sourced from *Bacillus* sp. (B50) in the control of larvae of mosquitoes. The authors stated that insect vectors proliferate in uncleaned environment and still water bodies. Commonly control involves the use of

chemicals like pesticides and insecticides which have resulted in a toxic environment and have affected nontargeted species. They stated that the BS being green, cheap, eco-friendly, and cost-effective has been a promising tool for the management and control of mosquitoes or insects, resistant to these native chemicals. Seventy-five isolates obtained from polluted petroleum soil samples were screened, characterized and the potent one was selected and identified. The characteristics of the BS sourced from the crude oil were lipopeptide. The tested larvicidal action on the insect larvae at various concentrations of the biological surfactant after 72 hours of LC 50 and 100, showed that as the concentration increased, the deaths of the insect larva follow the same pattern. The findings from the study indicated that a maximum amount of the insect larvae was exterminated with a range of concentration between 1 and −4 mg after 75 hours of the biotoxicity test. This outcome proved that a BS is a first-tier tool that could be applied for the control of mosquitoes at various metamorphic life cycle stages.

7.2 Environmental application of biosurfactant for the management of different types of mosquitoes

Many mosquitocidal BSs have been isolated from the strains of *B. amyloliquefaciens* and their kinetic activity has been examined utilizing standard microbiological techniques. The biochemical plus physiological activities were assessed and it was found that the BSs can be used to have strong control against *An. stephensi*. Several methods were employed to characterize the lipopeptide nature of the BSs such as β-hemolysis, biofilm-forming capacity, lipase activity, biochemical analysis plus thermostability. It was demonstrated that *B. amyloliquefaciens* generated mosquitocidal BS displayed a promising ability as a novel molecule mosquitocidal agent. Geetha et al. [37] revealed that BS surfactin displayed enhanced mosquito pupicidal activity in an experiment carried out to amplify the rpoB gene of pupicidal from Bacillus subtilis. They discovered that light, pH, radiation, and temperature did not affect the pupicidal properties displayed by surfactin, thus exhibiting a stable physiochemical activity.

Fenibo et al. [44] revealed that BSs are biomolecules with multidimensional capacity produced or generated biologically from a different microorganisms such (*Acinetobacter calcoaceticus*, *P. aeruginosa*, *Candida albicans*, and *B. subtilis*) species for diverse industrial activity. The authors revealed several examples of BSs like rhamnolipids, mannosylerithritol lipids, sophorolipids, surfactin, plus emulsan important for various biotechnological applications. The numerous advantages such as biodegradability, eco-friendliness, stability, low level of recalcitrant pollutants plus low toxicity level of BSs make them preferable compared to the synthetic ones though presently the synthetic ones are commercially available. The authors revealed in an experiment conducted to test the toxicity level of BS glycolipid from *P. frederiksbergensis* against *Culex pipiens* and filaria vector mosquito in Saudi Arabia which showed significant mosquitocidal activity and shrinkage of larvae cuticle of *Cx. pipiens* plus nuclear degradation of mid-gut epithelial cells in the larvae suggesting a promising agent for mosquito vector control.

Kalyani et al. [45] revealed the important role of BSs in the environment which has received increased significant attention from biomedical scientists for the control of many mosquito vectors. Many of the physiochemical properties were highlighted by the authors such as stability, diversity,

largescale production, eco-friendly, plus selectivity nature. The authors isolated rhamnolipid BS from a unique strain of *Stenotrophomonas maltophilia* as a larvicide. Deepali et al. [46] revealed that various health disease conditions are spread by mosquito vectors such as dengue fever, chikungunya viruses, malaria, urban yellow, and filariasis. Though synthetic chemicals have been used for its eradication, few results have been achieved with numerous side effects, thus the need for isolation of natural occurring BS with the larvicidal property. Silva et al. [47] revealed that resistance developed by *Aedes aegypti* against many chemical insecticides has indicated that a rigorous search for alternatives with no side effects is necessary. The authors suggested that Rhamnolipids BS have repellent, larvicidal plus insecticidal effects against *A. aegypti*.

Anitha et al. [48] revealed that synthetic insecticides have become ineffective due to the development of resistance by the malaria parasites, thus more eco-friendly, less toxic, and potent biomolecules extracted from natural sources are needed to produce effective insecticides that can be utilized for control mosquito parasite. Various natural sources have been utilized for the synthesis of BSs or metabolites with mosquitocidal or biolarvicide properties such as plant, microorganisms (*B. thuringenesis* var. *israelensis*, *B. sphaericus*, *P. aeruginosa*, *B. subtilis*, *B. sphaericus* strain, and *B. circulans*), and nanoparticles. Studies have indicated that Di-rhamnolipid, Rhamnolipid, lipopeptide, and fatty acids which are well-known BSs generated from *P. aeruginosa*, *B. subtilis*, *P. fluorescens* have been utilized as biocontrol agents for many mosquito species such as *An. stephensi*, *Aedes aegypti*, and *Cx. quinquefasciatus*. The mode of action of these biomolecules involves the reduction in interface/surface tension. Amongst different treatment approaches evaluated for environmental management of mosquito parasites, the biological method seems to be the most effective.

According to Usman et al. [49], the utilization of microbes for the bioproduction of BSs/bioemulsifiers for various environmental applications has recently gained significant attention among biomedical scientists. Many of these biomolecules have been utilized for different industrial applications such as in pharmaceutics, biotechnological, and environmental physiology. The physiochemical properties of these biomolecules make them very important for the biodegradation of toxicants and mosquito parasites. Studies have revealed that the deadliest plasmodium parasites causing widespread malaria fever among developing countries are the *An. stephensi* vector resistant to many antimalarial drugs. The authors revealed that bacterial species responsible for the production of BSs with mosquitocidal properties were *P. stutzeri* and *B. subtilis*, which have been characterized using gas chromatography and mass spectrometry plus Fourier transform infrared (FTIR) spectroscopy for their physicochemical properties. It was confirmed that the BSs generated from these microorganisms were lipopeptide in nature.

Nasr et al. [50] have observed that the mosquitoes were vectors responsible for the carrier of lymphatic filariasis, yellow fever, malaria, encephalitis, dengue fever, plus West Nile fever. The health challenges generated by mosquitoes are enormous and the eradication strategy involves the utilization of pesticides and chemical insecticides but recently it has been confirmed that most of these synthetic insecticides produce undesirable effects in the environment and also the development of resistance by these mosquitoes. Thus it was suggested by the authors that the production of natural BSs with mosquitocidal or larvicidal properties should be explored by scientists from many beneficial microorganisms such as *B. sphaericus* and *B. thuringiensis* subsp. *israelensis*.

Wirth et al. [51] have suggested that in the subtropical and tropical regions across the globe, malaria parasite infections have been the leading cause of death annually estimated to affect about

two million individuals. Also, it was estimated that the death rate among adolescents and children was around one million yearly with about 2 million-plus at risk of developing serious malaria infection. Studies have revealed that the metabolites or BSs generated from the plant like *Calotropis gigantean*, *B. sphaericus*, *Spheranthus indicus*, *Spilanthes acmella* L., *Cassia fistula*, *C. procera, Jatropha curcas* or microbes like *B. thuringiensis*, *B. sphaericus*-2362, *B. israelensis*-H14 actes as a very good source of potent filaricidal, ovicidal or mosquitocidal agents. The authors revealed that many phytochemicals and metabolites produce deleterious effects on the physiological system particularly at the malpighian tubules or gastric caeca of the different mosquito larvae like *A. arabiensis*, *An. Stephensi*, *A. aegypti*, and *Cx. quinquefasciatus*. Płaza and Achal [52] revealed that novel antimicrobial and eco-friendly substances are being developed. The authors impressed upon that BSs be considered as an important bioeconomy product with diverse industrial applications such as innovative biocides.

Murugan et al. [53] revealed that mosquito parasites such as *An. stephensi* have been responsible for millions of deaths across the globe yearly particularly in sub-Saharan Africa. Mahamuni et al. [54] pointed out that *Cx. quinquefasciatus* and *A. aegypti* being mosquito vectors are resistant to many synthetic insecticides such as organophosphate, organochlorine, carbamate plus pyrethroids and are capable of generating environmental problems. The authors utilized *P. caryophilly* and *P. fluorescence* bacteria strain to synthesize biomolecule or extracellular exotoxin with mosquitocidal ability. It was concluded that this design can be utilized for the industrial production of microbial insecticides for the malaria vector control program. Brammacharry and Paily [55] declared that about 2500 diverse mosquito species have been identified across the globe responsible for vector-borne diseases and many approaches have been developed to control the spread of these vectors and the negative environmental impact of many synthetic insecticides. The authors revealed that many microbial species were capable of synthesizing secondary metabolites, toxin protein, or delta-endotoxin which can be utilized as insecticides such as *P. pseudomallei*, *B. sphaericus*, *P. fluorescens*, *B. thuringiensis* plus *P. aeruginosa*.

7.3 Biology of *Plasmodium* species

Gruenberg et al. [56] revealed that the main characteristic feature of the *P. vivax* has been the low parasite load in the blood to make identification of infected asymptomatic patients difficult. The authors suggested that utilization of mitochondrial DNA as the multicopy template can enable an increased detection rate as compared with other approaches. Gething et al. [57] briefly demonstrated that *P. vivax* has a peculiar challenge in elimination in most parts of the endemic region particularly in the sub-Saharan countries causing serious mortality and morbidity. They revealed that tolerance to different environmental conditions and modes of transmission made it difficult for *P. vivax* elimination through utilizing many synthetic chemicals. Several parasitemia forms of *P. vivax* resident in the liver are difficult to detect which happen to be the largest reservoir of the plasmodium in an infected individual. Also, studies have revealed that it will take an average of 14 days to eliminate the liver stage *P. vivax* parasite utilizing primaquine already known to generate many adverse effects among patients with glucose6-phosphate dehydrogenase (G6PD) deficiency resulting in hemolytic anemia. Generally, detection and testing of glucose6-phosphate dehydrogenase

(G6PD) deficiency in a population is very expensive and cumbersome to undertake. Therefore, it will be unwise to prescribe primaquine to any patient whose glucose6-phosphate dehydrogenase (G6PD) status has not been confirmed. Studies have revealed that elimination and control of *P. vivax* malaria will require specific intervention such as a national, regional, and global plan with rigorous monitoring of the disease incidence. Thus, new tools and approaches against the hypnozoite reservoir through adequate research and innovations utilizing microorganisms-based lipopeptides BSs should be developed [57].

Khatoon et al. [58] revealed that *P. falciparum* and *P. vivax* genetic structure will provide a powerful understanding of their virulence, evolutionary in addition to assisting in the design and development of vaccines against these parasites particularly in Pakistan where current control and elimination strategies have not yielded any positive progress. Therefore, the authors evaluated the genetic and molecular structures of these two plasmodia to understand their mode of transmission in the malaria-endemic Bannu district of Pakistan using the merozoite surface protein. They revealed that *P. vivax* demonstrated a high level of diversity of loci with distinct allele groups, thus displaying high mixed-strain infections.

Villamil-Gómez et al. [59] reported that *P. vivax* infections were more severe with many clinical manifestations such as pulmonary, the plethora of renal, hematologic (thrombocytopenia and anemia), multiorgan, and neurologic dysfunctions compared with malaria plasmodium. Hence, it was suggested that early diagnosis and detection will help to curtail many of these clinical manifestations. Also, biomedical scientists should engage in research to quickly identify preventive strategies against the *P. vivax* parasite. Deress and Girma [60] demonstrated that in Ethiopia, *Plasmodium* species such as *P. vivax* and *P. falciparum* were the most widely distributed causative parasites for malaria resulting in the high rate of mortality and morbidity across the sub-Saharan African countries. They suggested that novel prevention strategies should be adopted and implemented for the elimination of plasmodium.

Versiani et al. [61] revealed that in the Middle East, Asia, South, western Pacific, plus Central America, *P. vivax* research has taken a central role among many biomedical scientists with the hope of finding a rapid and permanent solution toward the elimination of this plasmodium. Vaccine development against merozoite surface protein-1 has suffered tremendously due to the lack of in vitro cultures for the plasmodium. Fernandez-Becerraa et al. [62] demonstrated that the cytoadhesion of *P. vivax* in the microvasculature has received growing interest from many biomedical scientists for research. Studies have shown that these malaria parasites showed variant genes for adhesion on many organs particularly spleen fibroblasts. They evaluated and analyzed splenectomized and spleen-intact from monkeys for *P. vivax* genes and recombinant protein expression among children in Papua New Guinea. It was revealed that the spleen displayed a significant function in the expression of *P. vivax* proteins associated with cyto-adhesion, thus manifesting antigens linked with clinical protection prompting a shift in *P. vivax* physiology.

File et al. [63] briefly reported that malaria parasites such as *falciparum* and *vivax* were very endemic in many sub-Saharan regions responsible for many mortality and morbidity rate. Many of these regions have very poor sanitation practices, housing, including drainage for surface water making the environment very favorable for the growth and survival of these malaria vectors. The authors carried out a study in Adama City, Eastern Shoa Zone, Oromia, Ethiopia to understudy the transmission and pathophysiology of *vivax* and *falciparum* malaria parasites. It was revealed that *P. vivax* was responsible largely for the burden of malaria without exhibiting any form of decline

currently compounded by poverty. Costa et al. [64] discovered that *P. vivax* malaria parasite has tremendously been on the increase in Brazil with its pathogenesis poorly understood. Therefore, this necessitated rapid evaluation to develop through research a basic infrastructural approach for the identification and elimination of this plasmodium. Virginia [65] disclosed that in subtropical and tropical regions, plasmodium species responsible for malarial infections were very endemic causing over two million deaths annually. Kochar et al. [66] briefly reported that in Asia and Latin America, *P. vivax* was responsible for more than half of all malaria infection cases with notable clinical manifestation and organ dysfunction. Li et al. [67] reported that by the year 2030, there will be the total elimination of malaria parasites such as *P. malariae*, *P. ovale* spp., *P. ovale-curtisi* plus *P. vivax* across the Greater Mekong Subregion. To achieve this, adequate knowledge and understanding through rigorous characterization, molecular genetic diversity evaluation will be needed. Hence, the authors conducted a study to investigate the prevalence of these vectors in the China−Myanmar area. It was revealed that four malaria parasite species were very dominant and prevalent in this region with moderate level polymorphisms in their genes.

Gunalan et al. [68] reported that *P. vivax* cases have been unprecedentedly discovered in Africa to be responsible for malarial disease among the Duffy antigen-negative blood group as against the previously unreported cases. Thus, the authors studied the role of Duffy-negative *P. vivax* infections among African populations intending to develop in vitro cultures against *P. vivax* infections. Patel et al. [69] reported that *P. vivax* malaria infections has remained to number one source of malaria-linked mortality and morbidity. Therefore, early detection may offer a preventive strategy for control in many endemic communities. They revealed that a robust platform for scanning real-time malaria parasites called RealAmp was developed for rapid detection of *P. vivax* which has proven to be more sensitive technique in detecting *P. vivax* compared to other methods employed. Chotivanich et al. [70] investigated the rosette formation characteristics of *P. vivax* and observed that the formation was grossly dependent on the divalent cations (Ca21/Mg21) showing tremendous sensitivity to heparin and trypsin, suggesting that *P. vivax* rosette properties were antibody facilitated with both species-specific plus cross-species constituents.

Jiménez et al. [71] reported that *P. vivax* was responsible for malaria parasite infection across the United States and Israel and the report has indicated that mefloquine was the most commonly prescribed drug for its treatment. White et al. [72] experimented on the merozoite surface protein variants of *P. vivax* to understand the biology of the mosquito and its mode of transmission in Papua New Guinea and Thailand. They revealed that accurate estimation of the duration of blood-stage *P. vivax* infections may not be possible due to the dearth of the accurate mode of identification between multiple and single blood-stage infections. They suggested that future investigation on multilocus genotyping with the utilization of mathematical models will reveal more information. Arévalo-Herrera et al. [73] briefly described the epidemiological data of *P. vivax* among the developing countries with an estimated case of 80−300 million with clinical manifestation and 2.6 billion individuals at risk every year. In many parts of the world, there has been a report of increased development of drug resistance by *P. vivax* and declined effort for the development of a permanent vaccine. Recently, the growing interest of biomedical scientists to study the *P. vivax* genome and rapid development of technologies such as proteomics, metabolomics, and genomics analysis have accelerated the progress and development of candidate vaccines against *P. vivax*. Some of these vaccine candidates have already gone through Phase I clinical trials like Pvs25 and CSP.

Suratanee and Plaimas [74] demonstrated that data and integrated approaches or models like human-malaria protein can only provide the solution to the issue of malaria parasites ravaging many parts of the world. The authors revealed that the succinate dehydrogenase protein complex and the nicotinic acetylcholine receptor will offer a beneficial role in providing necessary information on the dynamics of human−malaria interactions and drug targets. de Souza-Neiras et al. [75] reported that in the Brazil amazon area, the diversity of *P. vivax* genes has been seriously evaluated over the past few years utilizing molecular markers. The phylogenetic examination of the parasite was done to understand its biology to create the vaccine for its eradication. Kenangalem et al. [76] showed that the characterization of the anemic nature of *P. vivax* and *P. falciparum* was grossly insufficient; hence a three-stage cross-sectional community survey was initiated to evaluate the proportion of anemia. It was revealed from their findings that anemia linked to *vivax* malaria could be grossly underestimated as the main contributor to indirect malaria death and developmental morbidity. de Almeida Santos et al. [77] related the rate of circum-sporozoite protein genotypes in human blood with their parasitemia nature, thus evaluating the presence of these genotypes in Anopheles in, Brazil between 2012 and 2013 utilizing restriction fragment length polymorphism and polymerase chain reaction techniques.

Bermúdez et al. [78] demonstrated that advancement in omics and high-throughput techniques will enable the characterization and identification of proteins in the pathophysiology of *P. vivax* infections. Thus, the main molecular mechanisms through which the parasite causes malaria infection such as membrane proteins remodeling can be elucidated and target drug candidates can be developed. Commons et al. [79] investigated that the efficacy of antimalarial drugs against *P. vivax* parasitemia to understand the potential benefits of elimination for all individuals with uncomplicated malaria in coendemic areas. From their findings, recurrent parasitemia was much more delayed in individuals administered with ACTs containing piperaquine or mefloquine compared with artemether-lumefantrine, thus a high risk of *P. vivax* parasitemia was more even after treating *falciparum* malaria.

7.4 Conclusion and future recommendation

This chapter has provided detailed information on the utilization of microbial metabolites in the management of *Plasmodium* parasites. The mechanisms of microbial siderophores in the removal of *Plasmodium* parasites were established to depend on the lipophilicity and catecholate replacement of some rudimentary structures which might be also chiefly based on the Fe chelators between the derivatives of triscatechol and monocatechols. The treatment of strains of *Plasmodium* parasite with biological surfactants revealed different physiological variations like reduced fecundity, longevity, and oviposition timing as well as elongated pupal period. It was established that the utilization of the metabolites generated from BSs can be considered green, eco-friendly, and cost-effective which can serve as promising tools for the management and control of mosquitoes or insects that are resistant to these native chemicals [80−89]. However, a novel synthesis of BS siderophores metabolites derivatives which can be utilized for the treatment of *P. falciparum* in humans is recommended for further study.

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CHAPTER

8

Role of biosurfactant in the destruction of pores and destabilization of the biological membrane of pathogenic microorganisms

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8.1 Introduction

Disease-causing agents like yeast, fungi, and bacteria have been fingered for a long to cause damages to plants, animals, and humans. Every year, plants are destroyed by the activities of these microorganisms leading to numerous economic, biological, and ecosystem losses [1]. The persistent advent of resistant diseases due to Gram-negative (−) and -positive (+) bacteria globally has become more worrisome in the health, agricultural, and food sectors [2]. Resilient species of yeast, fungus, and bacteria are commonly found everywhere which has led to the exacerbation of issues about antibiotic crisis responses to these pathogenic strains [3].

Recently, the use of chemicals like pesticides which have been proven to be effective in the management of pathogenic organisms in plants has been known for their toxicological impact on the health of the plant, environment, and nontarget organisms [4]. These pesticides do not degrade in nature and they have the potency to biotransform and bioaugument along the food chain thus affecting the different trophic levels, including humans at the receiving end [5,6]. This has provoked modern environmentalists to look for better and more sustainable ways to manage pesticide outbounds and plant disease outbreaks that have caused numerous environmental and health challenges [7–9].

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Currently, numerous microbial messengers and active biological particles are being investigated for their probability to reduce the growth and activities of plant pathogens, more so, to alleviate ecological stresses both abiotic and biotic in plants [10,11]. Diseases caused by phytopathogens have been one of the most emerging and important groups of the world's food security threats [12]. The metabolites produced by microorganisms to combat the effects of phytopathogens have been proven to be cost-effective, eco-friendly, nontoxic, and green unlike the traditional forms [13]. The biological association of microbes with the roots of higher plants is noted to manufacture high well-developed hydrophobic and hydrophilic moieties called biosurfactants [1,4]. Typical examples of these biosurfactants include fatty acids, neutral lipids, polysaccharide−protein multiplexes, phospholipids, glycolipids, and lipopeptides [14].

The mechanism of biosurfactants metabolites on phytopathogens like Gram (−) and (+) bacteria, the characterization of the significant differences of the membrane-bound materials as well as the chemical, physical, and structural properties of the strains have been put into considerations established by several scientists. Studies have revealed that biosurfactants utilize different physiological mechanisms to establish active antifungal, as well as antibacterial properties, and hemolytic ability such as inhibition of lactic acid bacteria (LAB) [15,16]. This chapter intends to evaluate the modes of actions involved in the destruction of pores and destabilization of the biological membrane of pathogenic microorganisms and several malaria parasites or their vectors which enable them to act as antibacterial, antifungal, and hemolytic agents.

8.2 Modes of action involved in the biological activity of biosurfactants as antipathogen agent

Kunz [15] revealed that the mechanisms of action of several biosurfactants produced from microorganisms may involve antibiosis, parasitization through stimulation of cascades of physiological signaling events, interference with the virulence of a pathogenic organism, induction of plant resistance, endophytic growth, competition for an ecological niche such as nutrients or habitats, root colonization, invertebrate pathogenicity. Sarwar et al. [16] reported that lipopeptide biosurfactants like iturin, fengycin plus surfactin generated from *Bacillus* sp. have been studied extensively for different physiological and pathological conditions. The authors utilized mass spectrometry to characterize the properties of the biosurfactants and discovered that the lipopeptide extracts showed excellent antifungal and hemolytic properties against different phytopathogens via different physiological mechanisms. Wus et al. [17] showed that surfactin generated from *Bacillus* sp. CS30 utilizing tandem Ms and spectrometry displayed strong inhibition against *M. grisea* through the production of reactive oxygen species with lipid peroxidation damage on the cell membrane.

Jiang et al. [18] briefly described that the generation of lipopeptide surfactin from *Bacillus subtilis* strain PB2-L1 which is an effective biosurfactant characterized by LC-FTICR-Ms/MS and HPLC displayed highly strong inhibitory and antifungal activities against *Micrococcus luteus*, *F. moniliforme* plus *B. pumilus* through cell membrane damage, DNA fragmentation plus leakage of protein and nucleic acids. Maget-Dana and Ptak [19] revealed that *B. subtilis* known to produce a cyclic lipopeptide referred to as surfactin is a powerful biosurfactant with strong biological property capable of tremendous interaction with lecithin vesicles in the presence of

Ca^{2+} ions. The authors demonstrated that the acyl chain length of the phospholipids influenced surfactin penetration through hydrophobic interactions and conformation alteration in the peptide cycle by calcium resulting in erythrocyte hemolysis. They revealed that surfactin can induce ion-conducting pores through ion-carrier surfactin dimers.

Ghorbanpour et al. [20] revealed that many microbial biological control agents such as lipopeptides biosurfactants interact with pathogens through some specific complex substrates capable of modulating indigenous microbiota pathogenic compositions physiologically, thus suppressing their activity. Spadaro and Droby [21] demonstrated that several biological control agents' modes of action investigation have focused mainly on the induction of resistance against the pathogen, competition, priming, antibiosis, and hyperparasitism. The authors revealed that several other mechanisms are also being utilized such as induction of hypovirulence, inactivation of enzymes or substrate involved in pathogenic infections, enzymatic degradation of pathogenic structures particular spore attachment, production of hydrolytic enzymes or metabolites against cell membrane, protease inhibitors for suppression of pathogenic enzymes and synthesis of iron-binding siderophores for nutrient or substrate competition against pathogenic organisms.

Ajouz et al. [22] revealed that extracellular glycolipids of biosurfactants such as sophorolipids, rhamnolipids, plus trehalolipids were capable of utilizing several mechanisms to combat pathogenic organisms. Fakruddin [23] examines the antimicrobial activity of biosurfactants involving the generation of substrates causing cell membrane damage, and permeability against pathogens in a similar fashion to detergents. They also showed that many extracellular glycolipids of biosurfactants can interrupt the cell cycle G1 phase, improve cell differentiation, stimulate acetylcholine esterase and inhibit the adhesion of pathogenic organisms. Köhl et al. [24] demonstrated that inducing resistance or priming seems to be the major mode of action of many microbial biological control agents against plant and animal pathogens. The authors also suggested that other mechanisms involve stimulation of antimicrobial secondary metabolites, nutrient competition, growth modulation, hyperparasitism through cascades of molecular metabolic and physiological events.

8.3 Modes of action involved in the biological activity of biosurfactants as larvicidal agents agent

Meena et al. [25] reported that lipopeptides from *Bacillus* sp. were capable to exhibit antimicrobial activities against many pathogenic organisms through the generation of small peptides associated with lipidic moiety. Due to their unique physicochemical properties, lipopeptides are presently being considered as an alternative option to many other synthetic drugs in the fight against several pathogens that are resistant to the known antibiotics, antiparasitic, antiviral, antithrombotic, antitumor, anticancer plus hemolytic activities. Studies have revealed that cyclic lipopeptides are very strong and potent larvicidal agents against *Plasmodium* parasites.

Porrini et al. [26] revealed that surfactin as an antiparasitic agent can effectively incorporate in the luminal of bee mid-gut or act on spores then competitively inhibiting NAD + and acetylated peptides uncompetitively. The authors also discovered that the mosquitocidal action of surfactin-lipopeptides was due to intraerythrocytic growth inhibition of mosquito parasites through the spores. Bélanger et al. [27] discussed the mode of action of many lipopeptides biosurfactants in detail. They

revealed that the compounds have different modes of action and not just a single action such as cell wall degradation through mycotoxins, botulinum−neurotoxin, induction of resistance or priming via signaling mechanism, utilization of specific nutrient or substrates, selection of potential antibiotic metabolite. Meena and Kanwar [28] pointed out that cyclic lipopeptide biosurfactants as mosquitocidal agents utilized diverse modes of action such as synthesis of toxic substrates against mosquitocidal proteins as antiparasitic agents to disrupt the mosquito development and growth.

8.4 The mechanism involved in the biological control of pathogenic microorganisms

The mechanism or mode of action of microorganisms like bacteria and fungi on different plant and animal substrates is usually cut across several biological processes such as antibiosis, hyperparasitism, competition, priming, induced resistance, and so on [24].

Fungi viruses of the family hypoviridae for example can be used to elicit hypovirulence in *C. parasitical* an agent of chestnut blight. The mode of action involves an antagonistic influence through the deactivation of enzymes that are involved in pathogenic contagions as well as the enzymes lectin needed to start up the degradation of the host in contact with the disease-causing organism *Magnaporthe oryzae* [29−31].

The spores generated are attached to the leaf exterior of the host, which can be broken down by special bacterium (*Chryseo bacterium* sp.) isolate metabolites [24,32]. However, 2 degrees metabolites like DAPG (2,4-diacetylphloroglucinol) can also have a direct influence as antimicrobial against the metabolite of the pathogen, nevertheless, to act as a MAMP (microbe-associated molecular pattern) [33]. Therefore, both the induced resistance and the antibiosis work simultaneously, hence influencing the DAPG in situ as a solitary mechanism. The production of Fe-chelating siderophore by the microbes can also induce resistance of the pathogen during the competition of food [34].

The nature of the mode of action of microbes as well as the interaction with the substrates is more complex to demystify because of the methodical discrimination and scientific mode to unravel [35]. However, groups of risk managers, regulators, and scientists have been able to ravel the antagonistic relationship between the host and the pathogens via biocontrol and result evaluation of their intensive mode of actions. This can be seen in *Trichoderma* sp. which is manufactured using hydrolytic organic chemicals. This organism can degrade and permeabilize the cell wall of fungi sequentially. This effective permeability is facilitated by the entry of 2 degrees antagonistic microbial metabolites [36]. Also, the strain *Trichoderma harzianum* (T39) which is initially chosen for the management of *B. cinerea*, and pathogens of *Sphaerotheca fusca*, *S. sclerotiorum*, and *Pseudoperonospora cubensis*, is generally antagonistic. These are commonly known to manufacture metabolites that are antimicrobial in nature and act through the process of hyperparasitism [37]. The strains produce proteases, an enzyme that can lock up the activity of *B. cinereal* through the reduction of cutinase, b-1,3-glucanase, chitinase, pectate lyase, pectin methylesterase, endo, and exo-polygalacturonase, which are important for the disease organism in the host infection. More experiments using strain T39 as a biocontrol entity, have shown its efficacy in the inhibition of protease which overpowers the enzymes of the host at the production period. The ability in terms of mechanism of action also cut across localized induced resistance,

ISR, and competition of nutrients that regulate the necrotrophic and biotrophic of the foliar of the disease-causing organism by the strain T39 [24,29].

The antagonist efficacy of *Pseudozyma flocculosa* on *Erysiphales* (powdery mildew) does not penetrate the cells; however, it results in the rapid mortality of the cell. These species of fungi do this by the production of glycolipid flocculosin and 6-methyl-9-heptadecanoic acid since there is no record to show the induced resistance and competitive properties in plants that are treated, which seem to be improbable antagonism in a biotrophic disease-causing organism. In this context, the mode of action is antibiosis, though, studies on gene expression have shown a nonsignificant rise in the expression of related genes at the period of the process of the antagonism [27]. However, there is currently evidence of the rise of competition for Mn and Zn (micronutrients) which play an important part in the interaction of the dedicated tritrophic; a process where the *Erysiphales* takes up the micronutrients from *P. flocculosa*, and the host plants draws these nutrients from the disease-causing organism [27,29].

A novel combination of the transcriptomic and genomic information of the microorganism has boosted the efficiency in combating certain pathogens that are highly resistant to the mode of action of a solitary application. For example, the gene expressions of *Metschnikowia fructicola* and *Clonostachys rosea*, have played antagonist influence mechanisms that have sustained vigor, are environmentally friendly, and safe [38,39]. The in situ mechanism of these microorganisms needs no risk assessment because of their ubiquitous properties, complexity, and principal physiological indications according to the data and condition provided by the Regulatory Commission 283 [35].

8.5 Modes of action involved in the application of biosurfactant as an antibacterial agent

There have been several investigations on the utilization of microbes as antibacterial tools. Fracchia et al. [40] published a review on the potential of the beneficial use of microorganisms as antibacterial biosurfactants describing their applications as therapeutic, antibiofilm, and antimicrobial in the areas of medicine and pharmacy. The mechanism of inhibition and control of microbes as antibiotics have resulted in the incidence of resistance of many strains that have also made it difficult to eliminate the formations of biofilms. Nonetheless, the utilization of biosurfactant with the potentials of antibiofilm and antimicrobial control properties, have reduced the foregoing issues of resistance. Besides, the biosurfactants have dispersal ability and inhibit the biofilms of the microbes, making them stable and nonnoxious under extreme environmental conditions. The authors suggested that the use of microbial surfactants as antimicrobial tools or adjuvant in the eradication and suppression of stubborn, resistant strains of yeast, fungal and bacterial, stands the better future chance in the biotechnology sector.

Garg et al. [41] isolated and tested the antibacterial efficacy of biosurfactants derived from *Candida parapsilosis*. The microbial biological experiment was screened in the laboratory. The activity of the hemolytic, index of emulsification, spreading of oil and collapse, drop tests, established the true nature of the strain. The biosurfactant revealed significant oil spread and emulsifying activities. The outcome from the Fourier transform infrared (FT-IR) and gas chromatography-mass spectroscopy (GC-Ms) and characterization, showed different peaks of C¼C, C¼O, N—H, and

O—H (carbon functional groups, amide, and phenol) and 13-docosenamide (337.5 g/mol) correspondingly. The findings from the studies on biosurfactants clearly indicated their strong antibacterial actions against strains of *Staphylococcus aureus* and *Escherichia coli* at the concentration levels of 5 and 10 mg/mL correspondingly. The growth pattern of the Gram (−) and (+) strains of the pathogenic organisms showed a positive prospect in the utilization of the isolated biosurfactants as the preferred antimicrobial agents.

Biosurfactants are important 2 degrees metabolites that are produced by specific yeast, fungi, and bacteria strains. The application of biosurfactants in different sectors like agriculture, environment, medicine, and pharmaceuticals, have yielded positive effects as an antimicrobial messenger. To verify some of their potential influence, Ndlovu et al. [42] tested and characterized the antimicrobial action of biosurfactants derived from *P. aeruginosa* (ST5) and *B. amyloliquefaciens* (ST34) isolates from a waste effluent treatment control plant. The authors stated that biosurfactants naturally manufacture lipopeptides and glycolipids which are found as a combined congener and boost the antimicrobial strength. The fingerprinting of the chromatography and mass spectrometry coupled; UPLC (ultra-performance liquid chromatography) and ESI−Ms (electrospray ionization mass spectrometry), respectively indicated that strain ST34 was able to form a surfactant (C13−16) referents. Strain ST5 was established to produce congeners of mono and di-rhamnolipid such as Rha−C12−C10/Rha−C10−C12, Rha−Rha−C12−C10/Rha−Rha−C10−C12, Rha−C8−C10/Rha−C10−C8, Rha−Rha−C8−C10/Rha−Rha−C10−C8, Rha−C10−C10 and Rha−Rha−C10−C10. The findings from the study showed that the biosurfactant metabolites rhamnolipid and surfactin extracts possessed strong antimicrobial actions against a wide spectrum of pathogenic and opportunistic microbes which include *C. albicans*, *E. coli*, and *S. aureus* strains.

Gomaa [43] isolated, characterized and tested the antimicrobial efficacy of biosurfactant (lipopeptide) derived from strain M104 (*B. licheniformis*) on the Whey plant. The authors employed the disc-diffusion technique against various types of Gram + bacteria (*Listeria monocytogenes*, *S. aureus*, *B. cereus*, *B. thuringiensis*, and *B. subtilis*) and Gram− bacteria (*Klebsiella pneumoniae*, *Proteous vulgaris*, *Salmonella typhimurium*, *E. coli*, and *P. aeruginosa*) as well on *C. albicans* yeast. The outcome showed clear antibacterial and antifungal actions against the confirmed microbes. The highest antimicrobial action and inhibitory influence on the intracellular structures of the biosurfactant were on strain ATCC 25928 (*S. aureus*). The antimicrobial influence of the metabolite lipopeptide formed by strain M104 was concentration and time-dependent. When it was introduced into *S. aureus* of concentration 48 μg/mL, the highest decrease of DNA, RNA, total protein was realized. Total lipids and soluble phosphorous acid noted at a 12 hours period of incubation were 48.50%, 83.295%, 53.43%, 90.47%, and 53.06%, respectively. It could be thus safely concluded from their study that the novel biosurfactant possessing potent antimicrobial activity is good for application in the area of medicine and related fields.

Yuliania et al. [44] tested the antimicrobial efficacy of biosurfactants sourced from strain C19 (*B. subtilis*). They recounted that the strain synthesizes surfactin-lipopeptides having great benefits for biopharmaceutical and biotechnological applications. The cultivated biosurfactant characterized after 300 hours was revealed to have high stability to different salinity and pH levels. It was later tested against six different human disease-causing microbes such as *C. albicans*, *Listeria monocytogenes*, *S. enterica typhi*, *P. aeruginosa*, *E. coli*, and *S. aureus*, as an antimicrobial messenger. The findings of the study demonstrated strain C19 as a strong antimicrobial messenger against human pathogens with special inhibitory effects on the growth of *C. albicans*. However, the inhibitory

effects were not observed in the Gram + and Gram− bacteria, thus, it could be endorsed as a major active antifungal messenger.

Mayri et al. [45] tested the antibacterial assets of biosurfactant metabolites (sodium dodecyl sulfate, sophorolipids, and rhamnolipids) on strain NCTC 10,400 (*B. subtilis*), strain PAO1 (*P. aeruginosa*), and strain ATCC 9144 (*S. aureus*). The results showed that the metabolites sophorolipids inhibited strain PAO1 at >5% v/v concentrations. Strain NCTC 10418 was inhibited by sodium dodecyl sulfate and sophorolipids at 0.1 and >5% v/v correspondingly. Strain NCTC 10,400 was subdued by >0.5% v/v concentrations of the three metabolites. A similar influence was observed in strain ATCC 9144. The capability of the firm grip between the biofilm formation and the surfaces of strains NCTC 10400, NCTC 10418 and PAO1, was suppressed by the action of sophorolipids at a concentration of 1% v/v under the influence of caprylic acid at a concentration of 0.8% v/v. Further study showed that strain ATCC 9144 had the best outcome when caprylic acid was introduced. From the findings, it can be denoted that the metabolite sophorolipids had promising disruptive biofilms produced by the Gram − and + bacteria especially when caprylic acid acted as a booster.

8.6 Modes of action involved in the application of biosurfactant as antiyeast and antifungal (pathogenic microorganism of medical relevance)

Amaral et al. [46] published a review on the application, production, and characterization of biosurfactants sourced from yeast. The authors recounted that the active molecules from biosurfactants were biological bases. Biological surfactant derived from fungi or yeast has grown over the years because of their significant advantages for different purposes, especially as antiyeast. They have been proven more effective over the synthetic forms because of their extreme functionality, diversity in terms of usage, low noxiousness, and high biodegradability in harsh environmental conditions. The application of biosurfactants as antiyeast will foster future industrial development and thus reduce the cost of synthetic ones.

Saravanakumari and Nirosha [47] isolated and tested the antiyeast efficiency and molecular mode of action of biosurfactant derived from *L. lactis*, extract from *C. lipolytica* in the management of *C. albicans*. The authors reported that the disease-causing organism, *C. albicans* can cause serious health issues in humans. Of recent, different drugs have been applied to control their infestation, but to no avail, failed in their antagonistic impacts. Currently, biosurfactants have been proven to be effective as an antiyeast in the control of severe pathogens in humans. The inhibitory effects of the biosurfactant revealed >50% action when compared to ketoconazole. This effect regulated the growth and development of the pathogen by reducing the internal tension. The impact of the biosurfactants established more than 6% capacity of permeability compared to the synthetic drugs (ketoconazole and fluconazole) on the pathogen cells. The influence of the biosurfactant IC50 against the pathogen showed 5 mg/mL and elicited apoptosis of the cell. Therefore, the findings of the study indicated that the biosurfactant strain showed strong antiyeast activity toward the pathogen as a result of the cellular damage and permeabilizing activities. They recommended that the present strain of fungi used as biosurfactants can apply medication for the control and management of disease caused by the understudy pathogen.

Rufino et al. [48] isolated, characterized, and tested the potential of biosurfactant sourced from strain UCP 0988 (*Candida lipolytica*) on some vegetable plants. The authors reported that the manufacturing and characterization of biosurfactants using biotechnological means have facilitated the importance of its application industrially because of the low cost of fresh materials. The biological tested organism was cultivated for 72 hours to determine its production and growth. The results showed that there was a superficial strain of the cell-broth, which was decreased from 55 to 25 MN/m. The biosurfactant yield was observed to be 8.0 g/L. The outcome from the characterization revealed a lipopeptide anion made up of 50%, 20%, and 8% protein, lipids, and carbohydrates respectively. It was also noticed that the combinational effects of the residue of the biosurfactant and glutamic acid, occasioned maximum production of biosurfactant by the yeast. There were also shreds of evidence of the reduction of the superficial strain as well as the critical micelle concentration (CMC). It could be concluded the fungi biosurfactant showed no venom to *Artemia salina*, *Lactuca sativa* L., *Solanum gilo*, and *Brassica oleracea* seeds and can be used industrially to mitigate some environment-related stresses in plants.

Sen et al. [49] characterized and tested the antiyeast efficacy of biosurfactant metabolite sophorolipid gotten from strain YS3 (*Rhodotorula babjevae*) on some pathogens (*Trichophyton rubrum*, *Corynespora cassiicola*, *Fusarium oxysporumf* sp., *Fusarium verticilliodes*, and *Colletotrichum gloeospori*). The authors stated that sophorolipids can be considered as the widely used biosurfactants gotten from glycolipids in various industrial sectors. These surfactants have strong antiyeast properties against various strains of microorganisms. The results of the biological experiment showed that there was a decrease in the superficial strain from 70 to 32.6 mN/m after 1 day in glucose concentration (10%, w/v) as the basic source of carbon. It was observed that the biosurfactant sourced from the crude oil yielded about 19.0 g/L which might be improved after the growth parameters have been optimized. The characterization of the biosurfactant by LC-Ms, FTIR, and TLC, showed that lactonic acids and different sophorolipids were the major metabolites of the biosurfactant. The sophorolipids showed excellent emulsifying and oil-spreading actions on the crude lubricant at 100% and 34.46 mm^2 respectively. The outcome from the CMC was detected to be at 130 mg/L. The solidity of the sophorolipids was assessed over a broad range of temperature, salinity, and pH of 120°C, 2%−10% NaCl, and 2−10, respectively in harsh environmental conditions. Findings showed that the sophorolipids showed promising antiyeast action against a wide group of fungi; *T. rubrum*, *C. cassiicola*, *F. oxysporumf* sp., *F. verticilliodes*, and *C. gloeospori*. It can be concluded that the biosurfactant with promising antiyeast properties is the most important fungi used in the management and control of severe diseases of humans and plants. It has proved to be effective and eco-friendly in biomedical and agricultural applications.

8.7 Treatment of the parasite using in vivo and in vitro treatments of malaria parasites

After the early diagnosis of the malaria parasite, in-vivo treatment is applied during the first 24−48 hours of the onset of malaria symptoms for the best result. This is necessary to prevent malaria from proceeding into severe and fatal forms. For effectiveness and to reduce drug resistance through the use of multiple antimalarial drugs, adherence to a complete course of treatment is

necessary using artemisinin-based combination therapies (ACT), that is, combination therapy of at least two effective antimalarial medicines with different mechanisms of action for three days. The drugs are administered at optimal dosages based on the patient's weight to provide effective concentrations of antimalarial drugs for a sufficient time to eliminate the parasites.

However, control of parasites and vectors is more effective and far better for the prevention of malaria. In vitro activity of bio, the surfactant has been seen to be effective in malaria parasite control. A study by Jayasree et al. [50] suggested that biosurfactants produced from *B. subtilis* B50 showed lipopeptide and larvicidal activity against mosquito larvae. Parthipan et al. [51] stated that biosurfactants produced by *B. subtilis* A1 and *P. stutzeri* NA3have been capable to reduce the fertility and life span of *A. stephensi*. The study also stated that the biosurfactant showed toxicity against young instars. Moreover, it has been established that strain of *B. subtilis* (VCRC B471), could produce a surfactin that inhibit *A. stephensi* which has been known as a typical example of malaria vector while *P. fluorescens* Migula (VCRC B426) could produce a metabolite that could inhibit the pupae of *A. aegypti*, *Culex quinquefasciatus*, and *A. stephensi* [52,53].

8.8 Modes of action involved in the application of biosurfactant for the management of the vector and the parasites

Malaria can be controlled by the use of insecticide-treated mosquito nets and indoor residual insecticides. Antimalarial medicines can also be used to prevent malaria through chemoprophylaxis especially for travelers. However, mosquito resistance has been reported in many of the main insecticides and insecticide-treated nets. There is also the concern of environmental and ecological pollution. Effective malaria vector control is recommended to protect people and the community at risk. Biological control using biosurfactant is effective in the control of vectors and parasite as it does not contaminate the environment and there is no resistance by *Anopheles*. They show little or no side effects on humans and other living things.

These biosurfactants isolated from strains of biological agents prove to be effective in the control of parasites and vectors as they distort or halt their growth and viability. For example, a metabolite from *B. thuringiensis* suppress late instars and outgrowing pupae at larval stages [54–56] and *B. sphaericus* destroy larval stomach by endotoxin-proteins production [57–62].

8.9 Conclusion and future recommendation

This chapter provides comprehensive information on the mechanisms involved in the destruction of pores and destabilization of the biological membrane of pathogens when subjected to the action of different biosurfactants which enable them to function as antibacterial, antifungal, and hemolytic agents. The demonstration of the influences of the chains of biosurfactant lipopeptides as an antimicrobial entity has shown that their penetrating and hydrophobic interactions can alter or induce ion-conducting pores through ion-carrier in the peptide cycle by calcium resulting in erythrocyte hemolysis in the cell of the microorganisms. The utilization of the metabolites like lipopeptides from biosurfactant microbial producing organisms (*B. thuringenesis* var. *israelensis*, *B. subtilis*, and

B. sphaericues), can be considered as an alternative option to many other synthetic drugs in the fight against several pathogens that are resistant to the known antibiotics, antiparasitic, antiviral, antithrombotic, antitumor, anticancer plus hemolytic chemicals. Some mechanisms involve in the stimulation of antimicrobial secondary metabolites, nutrient competition, growth modulation, hyperparasitism through cascades of molecular metabolic, and physiological events are recommended for further studies. The application of the biosurfactant could also have several applications in another sector including the medical sector [63–72].

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CHAPTER

Antibacterial and antifungal activities of lipopeptides

9

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9.1 Introduction

On today's ecological fob watch, there is a growing rise and the main concern of environmental deterioration caused by human activities and natural occurrences, pinpointed by environmentalists, government, and concerned stakeholders as a basic threat to sustainable development. Many chemicals such as herbicides, pesticides, and heavy metals have been used in the control of plant pathogens and vectors for over a hundred years and yet, yielded little or no effects have been documented because of the higher level of resistance and rapid rebounds of the pest and disease organisms. These chemicals cause several ecological imbalances such as health risks to humans and animals via groundwater contamination.

Environmentalists, agriculturists, and microbiologists are facing serious challenges in connection to the development of an eco-friendly and sustainable green chemical that can be applied for sustainable control and management of the incidences of pests and diseases that affect the majority of agricultural farm produce on our farmlands. The application of biosurfactants (BSs) recognized as green biotechnology, has the potential to break down environmental toxicants into less noxious, safe, and ecofriendly residues [1–4]. The precedence of economic, social, and environmental policies has necessitated the need to adopt this sustainable way to substitute the longer use of synthetic or traditional methods in the mitigation of environmental pollutants.

BSs are biologically active substances manufactured by microbes. These microbes produce metabolites or amphiphilic particles that tend to decrease interfacial stress in aqueous media. Also, BSs consist of hydrophilic and hydrophobic fractions that differ in chemical composition and properties, as well as structural and molecular sizes. Besides, they have strong antiadhesive activities against many disease-causing microbes. Other practical applications of BSs

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with a higher relevance could be found in the effective recovery of crude oil, biodegradation of pharmaceuticals and heavy metals, as well as toxic additives available in cosmetic and food products. Some biomedical utilization of BSs offer antagonistic effects on pathogenic fungi and bacteria.

One of the essential BSs metabolites produced by microbes is lipopeptide, which is applied for biomedical or clinical purposes as an antifungal and antimicrobial agent. Besides, lipopeptides are also used in the preservation, control, and management of dairy and food products in the agricultural and food sectors. In the year 2003, there was a high demand for lipopeptides; Daptomycin (CubicinR) as human antibiotics in combating many Gram-positive diseases-causing microorganisms in the United State of America. This was approved by the Food and Drug Administration (FDA) [1,5].

Historically, lipopeptides have been generally produced by the bacteria of the genus *Bacillus* and are well known to be an effective bioactive BSs molecule. This genus of bacteria has been widely approved by over 70 nations of the world, because, it has aided in higher revenue generation of over 1 billion US dollars annually [6,7]. The genus is made up of over 20 various peptides associated with different chains of fatty acids. Currently, there are over a hundred various compounds that have been characterized to belong to this group of compounds [4,8,9].

Examples of bioactive compounds such as lipopeptides are surfactin, iurins, mycocerein, plipastatin, fengycin, and kurstakins. Surfactin is derived from the supernatant of the strain *Bacillus subtilis*, which is an exocellular substrate that has been identified to be a necessity for good BS action [9,10]. The presence of this biologically active compound in this metabolite has been demonstrated to facilitate some biological activities such as hypercholesterolemic, antitumor, antibacterial, and fibrin-clot inhibitor messengers.

Iturins recognized as strong antifungal and antibacterial agents have been derived from soil *B. subtilis*. Their antagonists' influence on strains of sarcina and micrococcus has been proven super effective. Iturins in the 1970 and 1980s, were formally known by different names as mycosubtilin, bacillomycin, bacillopeptin, iturin A, iturin C and acillomycins D and F based on their related structures and compounds [10–13].

Mycocerein has been characterized as an effective antifungal lipopeptide sourced from the strain *B. cereus*. It was earlier classified to have its place in the family of the iturins [14]. Plipastatin and fengycin are known as associated lipopeptides. These are produced by *B. thuringiensis*, *B. cereus*, and *B. subtilis* strains. The strong inhibitor called phospholipase A2 has been produced by *B. cereus* [15–17]. The lipopeptides (Kurstakins) were derived from the strains of *Bacillus* sp. such as kurstaki and thuringiensis. The bioactive substance acts as an antifungal against the pathogen Stachybotryscharatum via absorption of the superficial spores [18]. This study was performed to understand the role of surfactins, iturins, and fengycins lipopeptides as effective BSs tools in the management and control of fungi and bacteria pathogens. Various aspects related to the isolation, purification, and structural elucidation of lipopeptides, using NMR, Ms, FTIR, TLC, GCMS, LCMS as well as identification of these structures using sophisticated Ms/MS experiments and software technologies have been nicely documented. Different specific examples of lipopeptides serving as antibacterial and antifungal agents derived from the strains of *Bacillus* sp. were cited. The antiparasitic, antiviral, antitumor, thrombolytic, antilarvicidal, antibiofilm, and antiadhesion activities of surfactin have been summarized. The application of lipopeptides in the food industry and their antifungal/antibacterial properties were also explained.

9.2 Specific examples of antifungal and antibacterial properties of iturins

Iturins consisting of 4 major frames as iturin A, B, C, and D contain cyclic hepata-peptides and β-amino fatty (14−17 carbons) chains which are potential lipopeptide antifungal and biopesticide tools, that are basically manufactured by *Bacillus* sp. for the management, treatment, and control of animal and human mycoses (fungal infections) because of the nonallergic influence and low noxiousness on the host species [1,19−23].

Klich et al. [24] tested and evaluated the potential of iturin A as an antifungal tool against mycopathologia. The authors stated that iturin A, manufactured by the strain *B. subtilis* is a cyclic-lipopeptide that has powerful antifungal assets as well as low noxious to humans. The biological experiment was carried with different treated concentrations of iturin A on stored cottonseed, peanuts, and corn feed grains. The results revealed that mycopathologia responded positively to the treatment of iturin A with the most effective concentration levels between 50 and 100 ppm.

Maget-Dana and Peypoux [25] characterized and evaluated the biotic properties of iturin as well as their antifungal actions on pathogens. The authors found iturin as a part of the family lipopeptides sourced from the strain *B. subtilis*. The compound is characterized by 7 rings of peptide amino acid residues, a D-Tyr′, an LDDLLDL chiral arrangement, amino and aliphatic acids. They recounted that iturin has powerful antifungal actions in a wide range of fungi and yeast, but have restricted actions on some strains of bacteria like *Micrococcus luteus*. The biological actions of iturin are controlled by the key structure of the cycle of the peptide as explained by the D-Tyr′ deposit methylation. It was noticed that there was a sudden reduction in the activity of the iturin by the upturn of the 2 head-to-head residues of Ser6-Asn′ that makes more dynamic mycosubtilin than the iturin. The antifungal action is linked to the collaboration of the lipopeptides iturin with sterols, producing lipopeptide complexes. Hence, the biologically effective structure is a triplex structure of sterol/iturin/phospholipid.

Dang et al. [26] characterized and optimized the antifungal properties of iturin sourced from strain LL3 (*B. amyloliquefaciens*) via culture and engineering settings. The authors stated that iturin, a cyclic antibiotic lipopeptide was chiefly manufactured by the genus *Bacillus* and applied for the biocontrol and biomedical management of fungal and some bacterial pathogenic strains. The strain LL3 was isolated in the laboratory and found to have a broad iturin A pathway as indicated by the genomic examination. It was also observed that iturin A cannot be formed by *B. amyloliquefaciens*, perchance ensuing from the reduced itu operon transcription level. This was corrected by inserting a powerful constituent agent C2up into the itu operon upstream to enhance the iturin transcription level. A production level of iturin reached to 37.35 mg/L. The LC-Ms (Liquid chromatography-mass spectrometry) showed that the *B. amyloliquefaciens* structured 4 homologs iturin A with a structural peak of ions at 1086, 1072, 1058 and 1044 m/z with their corresponding frames $[C17 + 2H]2+$, $[C16 + 2H]2+$, $[C15 + 2H]2+$ and $[C14 + 2H]2+$. The findings from the study showed that the iturin A demonstrated powerful inhibitory action against some common flora pathogens. However, the iturin A yield was again enhanced to 99.73 mg/L via optimization of the surface techniques of the fermentation settings. More so, a further increase (113.1 mg/L) of the iturin A, via the overexpression of the DegQ pleiotropic controller, showed a great yield. The authors concluded by suggesting that the engineering optimization by a metabolic combination of the culture settings of iturin A, may be a possible technique that will improve the formation of 2 degrees metabolites.

9.3 Specific examples of lipopeptides as antibacterial and antifungal agents

Sarwar et al. [27] characterized and tested the antifungal action of BS sourced from the genus *Bacillus*; NH-100 (*B. subtilis*), NH 217 (*Bacillus* sp.), FZB42 (*B. amyloliquefaciens*), C1225 (*Paenibacillus polymyxa*), 168 (*B. subtilis*) and 176 seconds (*B. atrophaeus*) strains, as well as their biofilm and motility formation abilities. The authors reported BS lipopeptides like fengycin, iturin, and surfactin under the genus *Bacillus*. The bacterial strain products were assayed with the aid of Ms (mass spectrometry). It was observed that strains NH-100 and NH 217 showed good surface and BS spreading actions, whereas strains C1225 and 176 seconds showed modest action, and strain *B. subtilis* showed no action. It was also observed that strains CC125, 176 seconds, NH-217, NH-100, and FZB42, exhibited good formation of biofilm. The extracts from the lipopeptide demonstrated excellent antifungal action against different diseases and phytopathogens like *Trichoderma atroviride*, *Fusarium solani*, *F. oxysporum*, and *F. moniliforme* (root or ear rot and rice bakanae diseases). Besides, the extracts from the lipopeptide also demonstrated strong hemolytic and BS potentials toward the disease strains. A posterior LC-Ms-ESI investigation revealed the presence of fengycin, iturin, and surfactin metabolites sourced from the various strains of the genus *Bacillus*. This study further indicated that the strains were excellent antifungal messengers against different Trichoderma and Fusarium species of fungi.

Different types of lipopeptides sourced from bacteria strains or species have been documented for their therapeutic and potential applications in the fields of biomedicine. Meena et al. [28] characterized and tested different lipopeptides obtained from the genus *Bacillus* as an antibiotic to different strains of pathogens. The authors stated that bacteria have some biochemical and physiochemical properties which enable them to use effectively in humans as antibiotics. They recounted that lipopeptides like fengycins, iturins, and surfactins have strong antagonistic effects against human pathogens. Fengycins, a bioactive lipopeptide BS formed by different species of *B. subtilis* acts against filamentous fungi which are very resistant to traditional antibiotics. These fungi cause life-threatening illness in humans. Besides, fengicines have been used as antibiotics but they can also be used to cure obesity and cancer cells, as well as for oil recovery enhancement. Iturins are cyclic BS lipopeptide, having 11−12 hydrophobic carbon tails and seven amino acid residues that have been effective as a powerful antifungal tool. Iturin is produced by the strain *B. subtilis* and has been utilized in the pharmaceutical, oil, and food industries. Surfactin a typical β-hydroxy fatty acid compound and a cyclic amphipathic lipopeptide exhibit strong antifungal properties. It has a high resistance to stearic, cold, and heat influences. Besides, it has low irritancy and biodegradability potentials for animals and plants when used as an antiparasitic tool. More so, its compatibility with the skin of humans when used also is excellent.

In daily agricultural activities, phytopathogens like yeast, bacteria, and fungi have caused several economic fatalities to plant produces. To combat this common menace and secure food products, Meena and Kanwar [2] tested the efficacy of lipopeptide BS from *Bacillus* strains as antibacterial and antifungal messengers in biomedicine and food therapeutics and protections. They reported that many microbes used as an antifungal and antibacterial agent have been proven nonecosystem friendly, nonbiodegradable, and highly noxious that have also extended the level of environmental contamination. The authors stressed that the bioactive extracts from the bacteria serve as

strong inhibitors to the growth and development of plant pathogens which pose serious problems to worldwide food security. The lipopeptides fengycins, iturins, and surfactins got from the species of bacteria have been shown to have strong potential for the antagonistic influence of a wide variety of oomycetes, bacteria, and fungi strains. Fengycin and iturins have been shown to possess powerful antifungal actions, whereas, surfactins show strong potential against a wide range of bacteria and larvae (antibacterial and antilarvicidal) agents. They suggested the utilization of lipopeptide metabolites obtained from the genus *Bacillus* because of being potentially biologically ecofriendly and generally acceptable for use.

Walia and Cameotra [4] in a review, looked at the application and biosynthesis of metabolites sourced from the genus *Bacillus*. They proposed that BSs being molecular amphiphilic structures tend to decrease surface strain amongst compounds. These metabolites have the firm potentials as antiviral, antitumor, antibacterial, and antifungal messengers and also are widely utilized in various sectors like pharmaceutical, biomedical, agricultural and environmental because of their nontoxic and biodegradable properties. Based on their antiviral, antitumor, antibacterial, and antifungal properties, they are now being used as chief DNA transfection, antiadhesive and immunomodulatory agents which have been proven effective in the extermination of nosocomial contagions caused by various microbes. These BS metabolites can also be used as a probiotic incorporate preparation to combat pulmonary and urogenital tract infections as immunotherapy. They concluded that these metabolites from lipopeptides are important biochemical moieties that have promising paths in the biomedical sector.

Lipopeptides are made up of metabolites that are highly structured. These metabolites are normally produced from different genera of microbes. Of recent, these metabolites have been used as a surfactant, immunosuppressant, antitumor, and antimicrobial messengers against many animals, plants, and human pathogens. In line with the continued sustainable utilization of microbes as tools of health therapeutics of various ailments, Raaijmakers et al. [29] in a review, evaluated the antibiotic effects of lipopeptide metabolites from *Pseudomonas* and *Bacillus* strains. They stressed that the use of these bacteria has gotten less attention for the past decades. The metabolites from these strains have natural functions in producing antagonistic effects against many pathogens like plant parasites, nematodes, protozoa, oomycetes, fungi, and bacteria. Their mechanism of action is based on their abilities to impede the cell motility of the parasite against the hosts. Updates on the novel discovery and detection of lipopeptide from the genes of microbes in the biosynthesis of new metabolites were proposed by the authors.

9.4 The antiparasitic and antitumor activities of surfactin

Sarwara et al. [30] characterized and tested the biocontrol potential of surfactin BS sourced from strains NH-217 and NH-100 (*Bacillus* sp.) on rice bakanae disease. The authors recounted that the genus *Bacillus* has been long-fingered as an antipathogen because of the potential to form a strong surfactin metabolite from lipopeptide. This metabolite could serve as biocontrol agents in chemical fertilizers and have proven effective and eco-friendly. The biological metabolite was assayed using HPLC and LCMS-ESI and found to contain different C12-C16 chains. The outcome from the hemolytic evaluation showed a + relationship between the halo zone and the purified surfactin

(surfactin A) produced. It was observed that the surfactin A showed excellent antifungal action against *T. reesei*, Trichoderma atroviride, *F. solani*, *F. moniliforme*, and *F. oxysporum*. The growth inhibition rate (84%) was noticed at 2000 ppm against the strain *F. moniliforme*. The purified surfactin was able to sustain the antifungal action within pH range from 5 to 9 under temperature span of 20°C, 50°C, and 120°C. The exsitu pot and hydroponic assays done to evaluate the biocontrol action of the purified surfactin and the strain SPB on the rice bakanae disease showed that the surfactin A was able to significantly decrease the pathogen to about 80%. They suggested that surfactin could effectively control the pathogen of rice and should be recommended as an effective biocontrol tool in the control and management of rising diseases.

Meena et al. [31] characterized and tested the antimicrobial and antitumoral action of surfactin gotten from strain KLP2015 (*B. subtilis*). The weight of the surfactin was evaluated with the aid of MALDI-TOF-Ms/MS and ESI-Ms. It was observed that it has strong cytotoxicity towards testing human tumor cells NIH/3T3, MCF-7, L-132, Hep2-C, and HCT-15 with the various percentages 77.84 ± 1.96, 78.91 ± 2.09, 88.56 ± 2.41, 76.09 ± 1.32, and 80.1 ± 1.92 correspondingly with reduced cytotoxicity of $31.45 \pm 2.58\%$ in the control cells. It was also observed that there was a fivefold reduction in the DNA material; L-132 cells treated with the lipopeptide and a twofold in the Hep2-C later in 20 hours, whereas, the cells Hep2-C and L-132 treated showed about 1.4 and 1. fivefold reduction in the DNA material from its preliminary stage. The antibacterial evaluation showed that the surfactin with concentration 50 μg/well, was able to inhibit *Klebsiella pnemoniae*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Escherichia coli* to a diameter of 15.0 ± 0.4, 13.0 ± 0.2, 12.0 ± 0.3, and 8.0 ± 0.7 mm respectively. The findings showed that the biofilms dislodging were noticed in all the seven strains tested exempting the strain *Shigella flexneri*. Also, the highest biofilm process formation was decreased by about 58% for strain ATCC 6538 (*S. aureus*).

Wu et al. [32] screened, characterized, and tested the antifungal efficiency of surfactin lipopeptide BS gotten from strain CS30 (*Bacillus* sp.) on cereal pathogens. The strains were isolated from a deep-sea aquifer. After screening and characterization, it was identified as strain CS30 and noticed that the strain has the potential to inhibit *M. grisea*. After chromatographic analysis and precipitation, 2 antifungal messengers were noticed and designated that belong to the surfactin family (CS30−2 and CS30−1). A posterior evaluation indicated that the antifungal action of CS30−2 was lower compared to CS30−1. However, the two surfactins induced the production of ROS (reactive oxygen species) and elicited serious cytoplasm and cell damages, therefore causing the mortality of the cell of *M. grisea*. Findings from the study indicated that there was a strong antifungal mechanism of action of the homologs surfactins CS30−2 and CS30−1thus highlighting them as promising messengers and potential biocontrol tools against plant pathogens like *M. grisea*.

Wu et al. [33] evaluated the anticancer properties of surfactins and their utilization in the delivery of nano-drugs. The authors stated that the lipopeptides of BS sourced from the genus *Bacillus*, have strong anticancer and cytotoxic properties against diseases like hepatoma, leukemia, colon and breast carcinoma, and Ehrlich ascites. This surfactin also can serve as therapeutics arrest against cell metastasis, apoptosis, cycle arrest, and cell inhibition. Owing to these effective benefits, this underlines the potential anticancer properties of surfactin. Surfactin also has amphiphilic properties which enable it to be combined with nanomixtures for effective drug delivery in biomedicine in the treatment of various forms of cancers. The immense use of surfactin has fingered it as a possible nano-BS delivery tool in the areas of biomedicine and pharmaceuticals.

Jiang et al. [34] isolated, screened, characterized, and identified new surfactin NRPS lipopeptide sourced from the modification of strain PB2-L1 (*B. subtilis*) and its antifungal action toward *Fusarium moniliforme*. The units obtained from the surfactin NRPS were SrfA-B-Leu, SrfA-B-Asp, and SrfA-A-Leu respectively. The results of the three surfactin units showed that the individual products lacked the following amino acids Leu-6 and/or Leu-3, Asp-5. LC-FTICR-Ms and HPLC were used to detect, isolate, and characterize the surfactins. The results revealed that there was a sign of toxicity decrease when surfactin [ΔLeu^6] and surfactin [ΔLeu^3] were used as compared with the traditional surfactin. Meanwhile, surfactin [ΔLeu^5] revealed powerful suppression when compared to the traditional surfactin toward the pathogens *Micrococcus luteus* and *B. pumilus*. These findings revealed that there was low inhibition concentration (50 μg/mL) of surfactin [ΔLeu^6] toward *Fusarium moniliforme*, such that the surfactin enhanced protein and nucleic acid leakages as well as cell damage and mycelium growth which could stimulate programmed cell death in *F. moniliforme*. The significant impact of the surfactins on the fungal cell has shown promising effects as an antifungal tool as well as a successful biocontrol of fungi against food contamination and portend food safety.

9.5 Synthesis, extraction, and purification of biosurfactant

Different methods are available for the isolation and concentration of biologically derived surfactants from various media. One of the most popular BSs that have been used as a promising model for most investigations is surfactin. Several studies have established several methods that could be utilized for isolation and concentration of surfactin and related BSs. One of the most frequently employed methods for the concentration of BS is the acid precipitation approach. Some of the methods that have been used as the purification systems of BSs include the ultrafiltration process using a membrane, direct partitioning of liquid, and fractionation of foam [35].

The production cost of BS formation is influenced by processes involved in the recovery as well as the concentration of the BS. In most cases, a lower concentration and amphiphilic characteristics of the BS restrict their recovery process [36]. Some of the commonly available methods reported for the isolation of BSs include ultrafiltration, solvent extraction, adsorption, acid and salt precipitation, and centrifugation at high speed [37].

9.6 Physicochemical separation parameters of biosurfactants

The role of physicochemical properties of BSs in their purification processes is very important. Different BSs have unique physical and chemical properties that make it possible to easily identify and isolate the BS from other closely related compounds that are present in the medium. BSs are known to have amphipathic properties due to the presence of hydrophilic and hydrophobic groups. For a suitable ionic strength and pH level the molecules will proceed preferentially to a region where a phase tends to be more hydrophobic than the other [38]. Like other related biological molecules, BSs are sensitive to the pH of the media. Studies have revealed that for surfactin production, *Bacillus* sp. shows a decreased activity within a pH range of 7−5.5. A similar trend has

also been documented for the ionic strength of the media. The unique tendency of a particular BS to act as either hydrophilic, hydrophobic, or a combined feature of both is useful to experts in the choice of a suitable method for the purification of the BS. In the production of BSs using fermentation, there is an accumulation of the BS at the region of the interphase of the air and the medium. Separation of this surface from the bulk of the medium shows a higher content of some and a lower of the other components. There are different methods of fractionation during which solute components of high activity at the surface layer are adsorbed selectively at the interphase within the bulk liquid and the gas phase. They also make it possible to use suitable organic solvents in the stripping of lower surface activity solutes which tend to be left in the aqueous phase [35].

9.7 Direct liquid partitioning from cell culture

Some researchers have put forward other methods aside from the acid precipitation method which has some inherent limitations most especially for the enrichment and purification of BSs. One of the unique methods is phase portioning. There have been attempts in the design of systems where two phases of liquids are coupled directly to a region for the growth and production of culture. In a related study, Drouin and Cooper employed polyethylene glycol for the creation of different aqueous phases above the region of growing culture of the bacteria, *Bacillus* sp. They were able to extract successfully the BS, surfactin, and explained that the removal of products had unique stimulating impacts. Solvent extraction can be used for the separation of the acid that is precipitated from the matrix salts.

9.8 Separation by precipitation

The precipitation method is another feasible method for the purification of BSs. A typical example is surfactin, which can easily be removed through precipitation from the used media at optimum pH. For most studies, this is done at low pH, closer to acidity. Usually, aqueous HCl (pH of 2) is used in the process. At a low pH, the BS becomes positively charged resulting in the reduction of the efficiency of the hydrophilic region bringing about aggregation. This makes the BS to be insoluble hence easily precipitated. According to Kim et al. [39], one of the limitations of this purification approach is that in some cases it can also bring about the induction of precipitation of other smaller molecules that are not highly surface-active at such at low pH. Comparably higher purity can be obtained at a pH of about 4, however, this will bring about a reduction in the overall yield. Precipitation using acids has been performed for the isolation of BSs from small-scale production studies. In some other studies, acid precipitation is used before the utilization of other complex techniques for the polishing of the BS produced.

Ammonium sulfate has been employed as a substitute for the acid in precipitation making it possible to convert the BS into a completely hydrophobic molecule. The problem connected with coprecipitation earlier identified does make it difficult to use this technique. Highly enhanced selectivity is brought through the incorporation of ultrafiltration [40].

9.9 Solvent extraction

The supernatant with the BS is usually treated with a mixture of the solvent to be used for the process of extraction. This is then followed by continuous shaking in an incubation shaker. This results in two layers of precipitate. The top layer is then discarded to obtain the residue.

9.10 Ammonium sulfate precipitation method

This is achieved by the precipitation of the supernatant using a 40% aqueous solution of ammonium sulfate (w/v) followed by incubation overnight. The collection of the precipitate is done using centrifugation at 1000 rpm for 30 minutes. It is then subjected to drying to obtain a solid residue [38].

9.11 Zinc sulfate precipitation method

In this method, the zinc sulfate solution (40%) is added gradually to the resulting supernatant to induce the precipitation of the BS produced. This is then followed by incubation for 24 hours. The centrifugation method is then used for the collection at 10000 rpm and then dried.

9.12 Acid precipitation method

In this method, acidification of the supernatant of the BS is done using HCl at pH 2 followed by incubation of the resulting mixture at 4°C for a duration of 24 hours. The precipitate realized using centrifugation at 10000 rpm for 30 minutes was dried [41].

9.13 Studies on extraction and purification of biosurfactants

A major limiting factor to the commercialization of BSs such as surfactin is the recovery and purification process. A two-step filtration process involving the use of a membrane has been put forward, in which stirred cell components and centrifugal are utilized. Measurement of the surface charge and particle sizes were used in assessing the mechanism of separation.

In a study, Cheng and Juang [42] produced surfactin, a BS through the process of fermentation using *B. subtilis*. Ammonium sulfate was used for the downstream processing of the recovery of the surfactin. They also assessed various processes such as nanofiltration and ultrafiltration as well as their blended approach. Different contents of the surfactin were used in the experiments. It was observed that the micelles of the surfactin could be destroyed effectively and macromolecules of proteins could be eliminated.

In related work, Sarachat et al. [43] produced a rhamnolipid through the cultivation of *Pseudomonas areruginosa* using palm oil and a nutrient broth. The recovery of the BS excreted was achieved using the foam method of fractionation in the free culture medium. The impact of various operating parameters on the overall performance was also investigated. The properties assessed include pore size, the flow rate of air, liquid volume, and duration of the operation. The optimum recovery of BS was 97% when obtained in 4 hours, with a flow rate of 30 mL/min and pore size of 160−250 μm. The results from the HPLC analysis further revealed that the adequate concentration of the rhamnolipid concentration was achieved using the foam fractionation method.

De Andrade et al. [44] carried out a study in which they evaluated the production of mannosyl ethritol lipids (MEL), a BS, using wastewater from the processing of cassava. The cassava wastewater acted as a hydrophilic medium and also made the provision for a low-cost substrate. The purification of the BS was achieved using ultrafiltration in a one-step process. Characterization of the MEL produced was achieved using NMR which affirmed the production of a homolog of MEL as well as a second optical isomer. The fatty acids present in the structure were confirmed using CG-Ms. The approach introduced in this study was found to be an effective and possible substitute for the conventional approach.

Zhang et al. [22] developed a highly effective approach for the removal of macromolecular impurities as well as microbial cells. They produced surfactin from a broth of fermentation generated by *Bacillus* sp. Various combinations of inorganic flocculants were tested. The most efficient was the combined use of Na_2HPO_4 and $CaCl_2$. The disruption of the surfactin was brought about by the addition of ethanol (50%) into the broth; this also promoted the ease of permeation of surfactin. Demonstration of the stability of the results obtained was further shown through the use of validation tests. This showed the feasibility of separating surfactin from the broth.

Mukherjee et al. [45] investigated the production of BSs through the action of *B. cirulans*. The purification of the crude BSs produced was done through the use of filtration chromatography. There was an optimum production at 26°C and this was found to be associated with growth. The ultraviolet-visible technique was used to ascertain the purification. The product of the filtration process was observed to show decreased critical micelle content. There was also an enhancement of the antimicrobial potential of the BS on purification.

9.14 Characterization of biosurfactant

One of the first steps during the characterization of a crude extract of a BS is drying to powder. This could then be followed by various instrumental techniques of which Fourier transform infrared (FTIR) is typical. FTIR is commonly used for the elucidation of the various functional groups that are present in the extract. Sriram et al. [46] reported that a suitable range for FTIR scanning could be 4000 to 400/cm. The sample after grounding was converted into pellets using KBr and subjected to FTIR analysis.

Chittepu [47] carried out a study in which he prepared a bacterial lipopeptide. The result from the FTIR showed that the extract was a lipopeptide. This was confirmed by the unique peaks of absorption at 2963 to 2854.68/cm and 3500 to 3200/cm. The lipopeptide extract also revealed a very high antibacterial potential at a concentration of 50 μg/mL. The critical micelle concentration (CMC) value for the BS produced was 60 mg/L and this was able to bring about the surface tension

reduction to 31.6 mN/m from the initial 71.6 mN/m. The findings from this study therefore showed the potential use of bacterial lipopeptide as a biological control agent against foodborne pathogens.

Ferreira et al. [38] synthesized a BS and employed some instrumental techniques including FTIR for the determination and elucidation of the chemical compositions of the extracted BS. The range in which the IR spectra were obtained was 450−4500/cm at a resolution of 4/cm. For characterization of the BS from *B. pseudomycoides* using FTIR, the N−H and O−H groups were indicated by a peak of 3500 to 3200/cm. The presence of chains of aliphatic hydrocarbons due to C−H vibration modes were observed in the range 2963 to 2854.68/cm. The stretching due to the CO−N bond was shown by the strong peak of absorption at 1647.26/cm. Also, the presence of C = O was confirmed by the peaks of 1452.71/cm and 1408.08/cm. The various results, therefore, support that the BS produced was a lipopeptide.

Anburajan et al. [41] in their investigation showed some additional absorption peaks in the extract of the BS produced from *B. subtilis* which was however absent in the pure surfactin. There was a strong correlation between the retention time of the standard surfactin and the extra peak that was seen in the extract tested.

In a related study carried out by Sousal et al. [48], there were similar retention peaks present in the BSs that were formed by *B. subtilis* when it was compared to the pure surfactin commercially available. The surfactin is known to be a class of compounds having cyclic lipopeptides, with, β-hydroxy fatty acids in their side chain [41]. Naturally, surfactin occurs as a mixture of different isoforms identified as A, B, C, and D, and this classification is based on the differences in the sequencing of the fatty acids. These are considered heptapeptides and are cyclic lipopeptides that have beta hydroxyl fatty acids [48].

9.15 Fourier transform infrared features of glycolipids

Astuti et al. [49] carried out an analysis on the characterization of the BS obtained from Pseudoxanthomonas sp using the blue-algae plate approach. A dark blue coloration was produced by the colony of bacteria in the media suggesting the formation of glycolipid as the BS produced. The spectra from IR reveal absorption maxima at different wavelength positions due to the various functional groups. In related work, Coates [50] reported that the presence of bands of absorption at wavelengths such as 1103, 964, 829, and 609/cm in support of the presence of the C−O functional groups which is an indication of the moieties of glycoside regions. The recorded absorption bands were due to bindings of C−H, vibrations of C−C as well as the O−H bending.

Another vital instrumental technique used in the analysis of BSs is high-performance liquid chromatography (HPLC). It involves the preparation of the sample extract through dissolution in water and connection to the device. Centrifugation is done at 13,000 rpm for 5 minutes to aid the removal of tiny particles before the injection into the port of the HPLC using a wavelength of 210 nm. In their study, Md Badrul Hisham et al. [51] performed a gradient elution using a flow rate of 1.0 mL/min and an injection volume of 500 μL/min.

In the recovery of the BS produced, the centrifugation method can be used for the removal of the dead cells from the broth at 1000 rpm for 30 minutes. Collection of the supernatant containing the BS is done to recover the rhamnolipids [52].

9.16 Fengycin

Fengycin is a lipopeptide that has unique antifungal potential usually produced by the bacteria *B. substilis*. This substance has an inhibitory property against filamentous fungi, but is not active against bacteria and yeast. The inhibition process is antagonized by the presence of oleic acid, sterols, and phospholipids whereas the antifungal effects are increased by two other unsaturated fatty acids. Two major components are present in fengycin which however differ by the presence of an amino acid exchange. Fengycin A differs from B due to the replacement of D-Ala with D-Val. There is variability in the lipid moiety of the two analogs [53].

Several studies have been done to comprehend the mechanisms of the molecular action of fengycins. As a result of the amphiphilic characteristics, fengycins are considered to be responsible for the induction of cell death due to the interaction of the cell membrane and increasing of the permeability of the cell. Fengycin has also been reported to be responsible for the ultrastructural hyphae of fungal pathogens. Also recently available researches further reveal that fengycins and iturins are involved in the antagonistic processes with the plant fungi pathogens [54].

There is very poor documentation of information related to the impact of fengycins on cells of bacteria. From some of the available studies, the fengycins are usually inserted into the biomembranes of the model as a result of its unique amphiphilic feature thus setting up a domain that is fengycin rich which makes a permeabilization site to be available resulting in a leaky target membrane [55].

Medeo et al. [56] carried out a study to investigate the impacts of fengycins on different diseasing causing bacteria using a microscopy approach. They evaluated the cytotoxic effect on fibroblast in the human lung. The pathogenic organism chosen for this purpose was *P. aeruginosa* as a phytopathogen model.

9.17 Isolation and purification of lipopeptides

In a study by Luna et al. [57] on characterization of a BS revealed the presence of a lipoprotein substance. The composition includes 8% carbohydrate, 20% lipid, and 50% protein. The liposan produced by C. lipolytica grown in the substrate of hexadecane was found to be made up of protein (17%) and carbohydrate (83%).

9.18 Conclusion and future recommendation

This chapter has provided extensive details on the biomedical applications of BS metabolites as a special type of low molecular weight antimalaria, antibacterial, antiparasitic, and antifungal remedial moieties to plant and agricultural animal diseases. The application of the metabolites-lipopeptides like the surfactins, Iturins, mycocereins, plipastatins, fengycins, and kurstakins could lead to the control and management of pest and diseases that mitigate the improvement of agricultural production and also build an environment where the plants and animals could resist abiotic stress like salinity and drought. Moreover, appropriate details on the use of novel engineered strains

of microorganisms that have the potentials to bring about a well-balanced environment, are recommended to foster sustainable agriculture [58–67].

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CHAPTER

The role of biosurfactants in the advancement of veterinary medicine 10

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10.1 Introduction

Microbial surfactants or biosurfactants are the type of biomolecules, which are structurally heterogeneous, and they possess surface tension and emulsifying activities. They are secreted by diverse microorganisms, including fungi, bacteria, and yeast. They are secondary metabolites produced during the stationary phase of microbial growth [1]. Biosurfactants are amphipathic molecules. It has both hydrophilic and hydrophobic moieties. The nonpolar part mainly comprises a hydrocarbon chain, but the polar part is amphoteric, ionic, or nonionic. The capacity of a surfactant to reduce surface tension and emulsion formation is attributed to these characteristics [2]. Microorganisms produce surfactants to enhance cell mobility, facilitate growth in the environment, and provide access to nutrients. Various agro-industrial wastes, such as waste frying oil, corn steep liquor, and glycerol are used as substrates for biosurfactant production reducing the biosurfactant production costs [3]. As estimated in 2019, the global market value of surfactants was US$39,901 million and is projected to reach US$52,417 million by 2025. However, the demand for surfactant-containing products is on the rise owing to the Coronavirus disease 2019 pandemic [4].

The use of surfactants in daily life is ubiquitous. Shampoo and conditional surfactants typically comprise anionic surfactants (e.g., sodium dodecyl sulfate) and cationic surfactants such as polyquaternium-10. In the workplace, cationic surfactant Albafix is used in carpeted floors to enhance wettability and dye fixation. Mono- and di-glycerides, propylene glycol esters, and lactic acid esters act as surfactants in ice cream [4]. However, in most cases, chemical surfactants are nonbiodegradable and have toxic effects on organisms and the environment. Furthermore, their long-term use is associated with hepatotoxicity, lung toxicity, kidney toxicity, blood toxicity, and skin irritation or damage [5]. Owing to their numerous advantages, biosurfactants are favored over chemical or synthetic surfactants. Biosurfactants are less toxic, eco-friendly, biodegradable, highly selective, and renewable sources are utillized for production. Furthermore, properties such as emulsification, wetting, foaming, corrosion-inhibition, cleaning, dispersion, and surface activity make them suitable for various biotechnological applications [6].

In the last decade, biosurfactants have been used in the development of various biomedical products. They have unique properties such as environmental friendliness, tensioactivity, structural versatility, biochemical actions (e.g., anticarcinogenic and cell-differentiation), and sustainable

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production. Furthermore, their antibacterial, antibiofilm, antiviral, and antifungal activities make them important molecules for immense therapeutic and biomedical exploitation [7]. In this chapter, we mainly focus on the use of biosurfactants in the field of veterinary science. Most recent studies have investigated biosurfactants for their potential use for various veterinary purposes.

10.2 Properties of biosurfactants

Biosurfactants contain both hydrophilic and hydrophobic moieties. When they are placed in an aqueous solution, the hydrophilic portion attracts toward the water and the hydrophobic part attracts toward hydrocarbons. The dispersion of one liquid in another is termed emulsion [8]. At low concentrations, surface-active molecules are present in the emulsion. At increased surfactant concentrations, when a critical concentration is achieved, and beyond that, interfacial properties will not be changed [8]. Micelles are formed beyond this critical concentration. Micelles are colloidal particles where polar head groups face toward the water phase and nonpolar or hydrophobic tails are positioned inside the micelle [9]. A high number of micelles lead to low surface tension. Biosurfactants decrease the repulsive forces by decreasing the interfacial tension (liquid−liquid) and surface tension (liquid−air), and allow these two dissimilar phases to mix and interact easily [10]. The critical micelle concentration (CMC) plays a vital role in the maximum reduction in surface and interfacial tension.

CMC values indicate the efficiency of the biosurfactant. It depends on the chemical structure of the biosurfactant and the self-aggregation of the molecules [3]. Furthermore, low-molecular-weight (LMW) biosurfactants efficiently reduce surface tension. However, high-molecular-weight (HMW) biosurfactants have high efficacy and efficiency. They facilitate the mixing and interaction of the two phases and the stabilization of the system [11].

The hydrophilic−lipophilic balance (HLB) value of biosurfactants is an indicator of their relation to oil-in-water or water-in-oil. The low HLB emulsifiers are lipophilic and stabilize water-in-oil emulsification, whereas high HLB value emulsifiers have the opposite effect and confer better water solubility [9]. Biosurfactants have several other properties and biological applications, such as antimicrobial, antibiofilm, antioxidant, immunomodulatory, antiinflammatory, anticarcinogenic, and heavy metal removal activities. These properties of biosurfactants favor their use in various biomedical applications [3].

10.3 Types of biosurfactants

Biosurfactants are made of hydrophilic moieties (polysaccharides, acid, peptides, cations, or anions, mono- or di-polysaccharides) and hydrophobic moieties (saturated or unsaturated hydrocarbon chains of fatty acids) [12]. Functional properties, such as the ability of surfactants to reduce the surface tension, the interfacial tension of liquids, and micelle formation, are linked with their structures. Based on the molecular mass, biosurfactants are categorized into two classes: (1) LMW biosurfactants (e.g., glycolipids), and (2) HMW biosurfactants (e.g., lipoproteins) [13]. Generally, the molecular weight of a biosurfactant ranges between 500 and 1500 Da [14]. Structurally, biosurfactants are classified into

five types: (1) lipopeptides or lipoproteins, (2) glycolipids, (3) natural lipids or fatty acids, and phospholipids, (4) polymeric surfactants, and (5) particulate biosurfactants.

Glycolipids: These LMW biosurfactants are composed of a hydrophobic portion that consists of a long-chain fatty acid in combination with a hydrophilic carbohydrate-based component (glucose, trehalose, mannose, sophorose, galactose, and rhamnose) [15]. Rhamnolipids, sophorolipids, trehalose lipids or trehalolipids, and mannosylerythritol lipids (MELs) are the most commonly used glycolipids. Rhamnolipids consist of a hydrophobic moiety with normally two molecules of hydroxyl fatty acids forming a 3-(hydroxyalkaoyloxy) alkanoic acid, connected to a hydrophilic portion comprising one or two rhamnose sugar [16]. *Pseudomonas* species are best known for their efficiency in the production of rhamolipids. *Burkholderia* sp., *Enterobacter* sp., *Acinetobacter* sp., and *Streptomyces* have also been shown to produce rhamnolipids [16]. Sophorolipids are dimeric carbohydrates (sophorose) where a di-glucose with β-1,2 bond is linked to a long chain of hydroxyl fatty acids via a β-glycosidic bond. Several *Candida* species, *Pichia anomala, Wickerhamiella domercqiae*, and *Rhodotorula bogoriensis* are used for the production of sophorolipids [17,18]. Sophorolipids are generally safe, as the producer strains are nonpathogenic. Trehalolipids consist of a trehalose hydrophilic moiety [19]. Bacteria belonging to the species *Arthrobacter*, *Corynebacterium*, *Brevibacteria*, *Dietzia*, *Mycobacterium*, *Micrococcus*, *and Nocardia* are known to produce trehalolipids [20]. Trehalose lipids are found in various forms: anionic trehalose tetraesters, mono-mycolates, di-mycolates, tri-mycolates, succinoyl trehalolipids, and nonionic acylated trehalose derivatives [21]. MELs are nonionic biosurfactants, which are produced mainly by *Ustilago* sp., *Pseudozyma* sp., and filamentous fungi [22]. MELs are classified as MEL-A, -B, -C, and -D based on the position and number of acetyl groups [15]. MELs are novel biosurfactants with excellent bioactivities.

Lipopeptides or lipoproteins: In lipopeptide biosurfactants (LBS), acyl tail(s) are linked with a short linear oligopeptide sequence. The hydrophilic head includes a peptide sequence, and the hydrophobic tail includes a hydrocarbon chain [23]. Again, LBS are categorized into various groups such as surfactin, lichenysin, polymyxin, fengycin, iturin, viscosin, and pumilacidin. *Bacillus subtilis*, *B. amyloliquefaciens*, *B. mojavensis*, *Pseudomonas fluorescens*, and *B. mojavensis* are major producers of LBS.

Phospho- or neutral-lipids, and fatty acids: Several microorganisms can produce alkanes, which are hydrophobic substrates. They secrete higher quantities of fatty acids, phospholipids, and neutral lipids to aid in the uptake of the carbon source. A few examples are *Acinetobacter* sp., *Aspergillus* sp., *Candida lepus*, *Nocardia erythropolis*, *Micrococcus* sp., *Thiobacillus thiooxidans*, *Pseudomonas* sp., and *Penicillium* sp. [24].

- *Polymeric biosurfactants*: Alasan, liposan, emulsan, lipomanan, and some polysaccharide−protein complexes are the best-studied polymeric biosurfactants. *Acinetobacter calcoaceticus* RAG-1 synthesises polymeric bioemulsifier [24]. Biodispersan and emulsan were synthesized by *A. calcoaceticus*, are the best-studied polymeric biosurfactants. They are consisting of a heteropolysaccharide backbone [25]. Polymeric biosurfactants with high molecular mass allow fine-tuning of the micellle's properties [24]. The surface activity of polymeric surfactants is mainly dependent on the distribution of monomers.
- *Particulate biosurfactants*: A microemulsion is formed from the hydrocarbon of extracellular membrane vesicle segments and they play a vital role in the uptake of alkaline by microbial cells [26]. Membrane vesicles contain about 5 times and 350 times phospholipid and polysaccharide, respectively as does the outer membrane of the same organism [26].

10.4 Toxicity of biosurfactant

Very few studies have focused on biosurfactant toxicity. Toxicity and biodegradability determine the persistence and fate of biosurfactants in terrestrial and aquatic ecosystems [27]. Surfactants often enter animals or humans via the food chain and can damage enzymatic activities. Biosurfactants are considered biocompatible and biologically degradable owing to their compositions. Oliveira et al. [27] investigated the biotoxicity of a surfactin biosurfactant produced by *B. subtilis* ICA56. Surfactin reduced the surface tension to 31.5 ± 0.1 mN/m and exhibited a low CMC value (0.015 ± 0.003 g/L). The acute toxicity tests with *Daphnia magna*, *Selenastrum capricornutum*, and *Vibrio fischeri* indicated that surfactin was less toxic than its chemical counterparts. Furthermore, the surfactin extract was degraded by *P. putida*, and a mixed microbial population was isolated from the sewage-treatment plant. The surfactin was categorized as a "readily" biodegradable compound. Hwang et al. [28] orally administered surfactin C to rats and performed a bone marrow micronuclei assay. Lipopeptide had no genotoxic effects in rats [28]. The surfactin toxicity in mice in terms of lethal dose (LD_{50}) value was greater than 100 mg/kg when administered via the intravenous route [29]. Furthermore, oral administration of the surfactant for prolonged duration did not exhibit any toxicity in mice [30]. Deravel et al. [31] investigated ecotoxicological assays in *D. magna* model. The 48 hours EC_{50} value was more than 100 mg/L, which was lower than that of other fungicides [31]. The toxicity of bacterial surfactants was examined on the bioluminescent bacterium *V. fischeri* by measuring the reduction in light emission (EC_{20}) by this microorganism [32]. Significantly lower EC_{20} values of the biosurfactants than SDS confirmed the lower toxicity of the biosurfactant to *V. fischeri*. Fei and colleagues isolated surfactin from a cell-free broth of *B. subtilis* HSO121 [33]. The results of acute oral toxicity tests ($LD_{50} > 5000$ mg/kg, $LC_{50} > 1000$ mg/kg), and skin irritation tests (PII = 0) indicated the low toxicity and nonirritant nature of the surfactin. Glycolipid and LBS isolated from *Brevisbacillus brevis* BAB-6437 and *Stenotrophomonas sp*. BAB-6435 were also found to be nontoxic [34].

A biosurfactant produced from *C. lipolytica* was not toxic to the fish *Poecilia vivipara* [35]. Furthermore, few investigations revealed the nontoxic nature of biosurfactants in fish. Biosurfactants extracted from *B. licheniformis* VS16 [36] or *Staphylococcus hominis* [37] had no adverse effects on fish. The toxicity of *B. subtilis* SPB1 LBS in male mice was investigated [38]. The LD_{50} value of SPB1 was 475 mg/kg, and the tested doses did not cause death in mice. Furthermore, the biosurfactant had no adverse effects on plasma total cholesterol, serum glucose level, bilirubin level, and aspartate aminotransferase activity in mice. In addition, a daily administration dose of less than 47.5 mg/kg of body weight had no significant adverse effects on hematological and serum biochemical activities in mice.

Acute and subchronic toxicity of LBS isolated from *B. mojavensis* A21 lipopeptides was evaluated in mice [39]. The LD_{50} of A21 lipopeptides was approximately 550 mg/kg body weight. Furthermore, 28 days subchronic toxicity study showed that LBS did not cause any noticeable changes in hematological parameters, including hematocrit value, hemoglobin levels, and white and red cell counts. Moreover, A21 lipopeptides were also found to prolong thrombin and prothrombin time. These results reveal that biosurfactants do not exhibit significant toxicity and are very promising compounds for therapeutic purposes.

10.5 Potential application of biosurfactants in veterinary field

10.5.1 Antitumor/anticancer effects

The effect of biosurfactants on human cancer cell lines has been studied in-depth, but very few studies have been conducted in veterinary animals and animal cell lines. Cancer, which is a major cause of human death, is also associated with fatal veterinary diseases. Overexpression of various membrane-bound receptors amplifies oncogenic signals in cancer cells. Glycolipids, via the association of fatty acid chains with cancerous cell membranes, can interfere with various signaling pathways, resulting in membrane destabilization [40]. MELs disturb the composition of membranes, particularly glycolipids GM3 and lactosylceramide. This causes cell differentiation and apoptosis [40].

The effect of MEL on B16 melanoma cells was quantified by measuring the amount of melanin in the culture medium and the tyrosine activity [41]. MEL treatment increased the melanin content in cells and altered their morphology [41]. Wakamatsu et al. [42] explored the antitumor mechanism of MEL in mouse pheochromocytoma PC12 cells via a series of molecular techniques. A 72 hours treatment with MEL induced the formation of neurites, as detected in nearly 80% of cells. Furthermore, they demonstrated that the mechanism of MEL-induced differentiation involved ERK/MEK kinases, along with c-Fos and c-Jun transcription [42].

The glycoprotein biosurfactant isolated from *A. indicus* M6 is thermophilic, halophytic, and acidophilic. It reduced the surface tension of water from 72.0 to 39.8 mN/m [43]. It was found that the biosurfactant at 200 μg/mL exhibited maximum antiproliferative activity against lung cancer cells (A549), leading to cell cycle arrest at the G1 phase. Furthermore, the glycoprotein had inhibitory activities against methicillin-resistant *Staphylococcus aureus* (MRSA).

N. farcinica strain-derived trehalose lipid biosurfactant is a natural potent anticancer agent [44]. Kadinov et al. [44] investigated its isometric contraction on rat mesenteric arteries. Wire myography was used for small blood vesicles to study the trehalose lipid-directed contractile responses in arteries. Trehalose lipid (75 μM) had no effect on high K^+-induced contractions. However, trehalose lipid was able to decrease the viability of cancer cells.

The LBS from *B. safensis*, an important antibacterial agent against several biofilm-forming pathogenic bacteria *Staphylococcus epidermis* S61, exhibited antitumor activity against mouse melanoma cells [45]. Thus surfactin is a good candidate for potential application in treating cancer and in preventing various diseases.

10.5.2 Biosurfactants as antimicrobial / antibiofilm agent

Biosurfactants have gained momentum as a new generation of antimicrobial and antibiofilm agents. A few examples of the antimicrobial application of biosurfactants or their derivatives are shown in Table 10.1. The biofilm formation capacity of pathogenic *S. aureus* is well documented. Silva et al. [46] evaluated *S. aureus* biofilm disruption/removal by rhamnolipids. Rhamnolipids removed up to 88.9% of the biofilms on the polystyrene surface. They demonstrated that rhamnolipids disrupted the milk-based biofilm due to the higher carbohydrate content in later ones. Those results suggest the potential application of rhamnolipids in dairy industries at low temperatures. Biosurfactants isolated from *Lactobacillus paracasei* 75FHE, *Lactobacillus plantarum* 60FHE, and *Lactobacillus paracasei* 77FHE exhibited antiviral activity against hepatitis A virus [47]. Furthermore, these biosurfactants were effective against

Table 10.1 Biosurfactants or biosurfactant-derived compounds in antimicrobial field.

Name of the biosurfactant	Application
Anidulafungin	Antifungal agent
Caspofungin	Antifungal
Daptomycin	Antibacterial
Micafungin	Antifungal
Mupirocin	Antibacterial
Oxazolidinone linezolid	Antibacterial

several gram-positive and gram-negative bacteria, but did not show any significant effect on pathogenic fungi (*Aspergillus flavus*, *A. niger*, *Penicillium sp*., and *C. albicans*). The antimicrobial activity of *the Lactobacillus* strain depends on the type of biosurfactant used. Biosurfactants usually penetrate the cell by rupturing the cell membrane, leading to cell lysis [47]. Recently, Marangon et al. [48] developed antimicrobial nanoparticles (NPs) by combining chitosan with a rhamnolipid biosurfactant. The adding of rhamnolipid minimized the polydispersity index and size of NPs which improved the stability and induced a more positive surface charge. Nanoparticles improved antibacterial activity against *Staphylococcus* strains. NPs increased the local delivery of chitosan-rhamnolipids at the cell surface. The amalgamation of chitosan and rhamnolipid increased local delivery to cell surface and improved antibacterial efficacy. The combination of rhamnolipid and chitosan offers a favorable strategy for the design of NP-based antimicrobial agents.

Ceresa et al. [49] evaluated the antibiofilm activity of three types of biosurfactants against clinically relevant pathogens (*S. aureus*, *S. epidermidis*, and *C. albicans*) using in vitro tests. All these biosurfactants were effective against dual-species biofilm formation in terms of cell viability, cell metabolic activity, total biomass, cell metabolic activity, and microstructural architecture.

Ohadi et al. [50] investigated the in vitro antimicrobial, antibiofilm, and cytotoxic effects of LBS isolated from *A. junii* B6. The biosurfactant showed activity against gram-negative (*Escherichia coli*, *Klebsiella pneumonia*, *P. aeruginosa*, and *Salmonella typhi*) and gram-positive (*Micrococcus luteus*, *S. aureus*, and *B. subtilis*) bacterial strains. Furthermore, at concentrations below the CMC, the biosurfactant had effective antibacterial activity. Moreover, the biosurfactant disrupted the biofilm of *P. aeruginosa*, *Proteus mirabilis*, and *S. aureus* at concentrations of 1250 and 2500 μg/mL. Gold NPs (G-NPs) were produced using a biosurfactant [51]. The IC_{50} of the cytotoxic activity of G-NPs against U87, A549, MCF7, and 3T3 cell lines was (μg/mL) of 89.08 ± 0.4, 646.12 ± 0.5, 3.37 ± 0.1, and 770 ± 0.15, respectively. G-NPs exhibited strong growth inhibitory activity against various bacterial strains. Das et al. [52] synthesized nontoxic silver NPs (Ag-NPs) using a surfactant extracted from *B. vallismortis* MD16. The synthesized Ag-NPs showed excellent antimicrobial activity against *E. coli*, *Listeria monocytogenes*, and *S. aureus* but not against primary mouse liver cell lines. These results indicate that LBS have potential applications in the field of biomedicine.

Kitchen waste has been used as an inexpensive substrate for the production of biosurfactants by *Wickerhamomyces anomalus* CCMA 0358 [53]. The purified biosurfactant showed high inhibitory activity against *Salmonella enteritidis*, *S. aureus*, and *E. coli*. Furthermore, it inhibited fungal growth by up to 95%. LBS from *B. subtilis* TR47II exhibited strong antibacterial activity against

the gram-negative opportunistic pathogens *Achromobacter xylosoxidans* ATCC13138, *Alcaligenes faecalis* ATCC8750, *P. putida* ATCC15175, and *P. alcaligenes* ATCC14909. Furthermore, the TR4II biosurfactant dislodged the biofilms of *P. alcaligenes*, *A. xylosoxidans*, and *A. faecalis*, was approximately 81%, 85% and 100%, respectively. Importantly, it had no cytotoxic effect on mammalian cells at the minimum inhibitory concentration (MIC). Therefore the TR4II biosurfactant may be applied synergistically to efficiently control these opportunistic pathogens. Similarly, *P. aeruginoa* SS14 derived rhamnolipid biosurfactant inhibited the biofilms formed by dermatophytic fungi *Trichophyton rubrum* and *T. mentagrophytes* [54].

Biosurfactants can block oxidative chain reactions. Zahra et al. [55] extracted surfactin and rhamnolipids from *B. amyloliquefaciens* NS6 and *P. aeruginosa* MN1, respectively. Surfactin exhibited higher antioxidant activity than rhamnolipids. However, rhamnolipid-conditioned surfaces exhibited higher antibiofilm and antiadhesive activities than surfactin-treated surfaces. Recently, Gaur et al. [56] isolated *Planococcus* rifietoensis IITR53 and *P. halotolerancs* IITR55, which could produce rhamnolipids. These rhamnolipids exhibited bactericidal activities against pathogenic bacteria. At 40 and 120 mg/mL, biosurfactants enhanced the release of DNA and protein, indicating enhanced permeabilization and distortion of the bacterial membrane. Furthermore, the release of ROS by *P. rifietoensis* IITR53 and *P. halotolerancs* IITR55aided in the killing of the bacterial population [56].

MELs exhibit antibacterial and antibiofilm potential against *S. aureus* [57] mainly by damaging the cell membrane of *S. aureus* and inducing cell apoptosis [58]. Fukuoka et al. [59] also reported that MELs cause damage to the bilayer membranes of microbial cells. These researchers investigated the antimicrobial effect of MEL-M against gram-positive bacteria, gram-negative bacteria, yeast, and filamentous fungi using the conventional agar dilution method. MEL-M was less effective than MEL-A[59]. Yeast *Pseudozyma aphidis* produce MELs which are a mixture of MEL-A, MEL-B, MELC, and MEL-D [60]. MELs effectively reduced S. aureus ATCC6538 biofilm metabolic activity Furthermore, the release of oxygen uptake pO2 and the reduction of citrate synthase activity indicated the bacteriostatic/bactericidal activity of MELs. Therefore MELs are promising antimicrobial molecules for biomedical applications.

10.5.3 Immunomodulatory role of biosurfactants

Biosurfactants have various effects on cellular and humoral immune responses. McClure and Schiller [61] demonstrated that rhamnolipids could inhibit the in vitro phagocytosis of both *Saccharomyces cerevisiae* and *P. aeruginosa* by mouse macrophages. They found that rhamnolipids might interfere with the internalization of attached particles and decline the level of lysosome-phagosome fusion of internalized targets within macrophages. The biosurfactant α-D-mannan produced by *Pseudozyma sp*. CCMB 306 exhibited an antinociceptive effect [62]. Moreover, they showed that the antinociceptive effect might be associated with its antiinflammatory action. Their study demonstrated that the activity of α-D-mannan is quite similar to that of nonsteroidal antiinflammatory and glucocorticoid drugs; however, further studies are warranted to explore the mechanism [62].

In another study, the *B. subtilis* SPB1 biosurfactant was orally administered to rats to prevent diabetic complications [63]. It was effective in reducing α-amylase activity in the plasma, and protected β-cells from damage and death, which led to a decline in blood glucose levels and

subsequently an antihyperglycaemic effect. Histological analyses also revealed the protective action on the pancreas and the efficient preservation of the liver and kidney functions of diabetic rats. These were proved by a significant reduction in alanine transaminase, aspartate transaminase, lactate dehydrogenase, and gamma-glutamyl transpeptidase activities in the plasma. Thus the SPB1 biosurfactant could be considered as a potentially strong candidate for the prevention and treatment of diabetes in veterinary animals. The SPB1 crude LBS alleviated obesity-induced complications in rats fed a high-fat–high-fructose diet (HFFD) [64]. HFFD induced hyperglycemia in rats, however, administration of LBS to rats restored α-amylase activity and blood glucose levels to normal. HFFD increased renal dysfunction, as indicated by higher serum creatinine, urea, and angiotensin I-converting enzyme (ACE) levels. Interestingly, LBS treatment significantly reduces urea and creatinine levels ($P < .001$) to nearly normal levels, in addition to inhibiting ACE activity in obese rats by 27.25%. Thus LBS presented useful hypoglycaemic and antihypertensive properties and alleviated renal lipid accumulation in rats.

The effect of surfactin on the growth rate, activities of digestive enzyme in intestine, and antioxidant performance in *Anguilla rostrate* (American eel) was evaluated in a 70 days trial [65]. Dietary surfactin increased protease and lipase activities in the eel intestine. Surfactin significantly affected the antioxidant levels, except the catalase activity ($P > .05$). They found that 25 mg/kg of dietary surfactin improved growth rate, hepatic antioxidant capacity, and intestinal enzymatic activities in *A. rostrate*. Further, dietary surfactin administration for 7 weeks promoted the growth rate, intestinal enzymatic levels, and few blood biochemical activities of fish [66].

The cationic glycopeptide bleomycin (BLM) is clinically applied to malignant tumors, but its poor cell membrane permeability limits its application. The anionic lipopeptide surfactin (LSF) has the potential to disrupt cell membranes [67]. LSF could ameliorate internalization of BLM by the cells, and the combined use of LSF and BLM remarkably enhanced the antitumor activity of BLM without affecting normal cells. Furthermore, A375 melanoma in mice treated subcutaneously with LSF enhanced the therapeutic potential of BLM family compounds in subeffective doses. Moreover, no obvious toxicity was observed in the skin or lungs [67]. Therefore LSF could a potential synergist for BLM to minimize the dose while maintaining the therapeutic potential against kind carcinoma.

Previously, we have extracted biosurfactants from *B. subtilis* VSG4 and *B. licheniformis* VS16 [68]. VSG4 and VS16 biosurfactants exhibited hydroxyl radical and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities above 63.3%. Those biosurfactants also showed significant antibacterial and antiadhesive activities against various pathogenic bacteria (e.g., *S. aureus*, *S. typhimurium*, and *B. cereus*). Therefore both biosurfactants can potentially be explored as natural antimicrobials, antioxidants, and/or antiadhesive agents for biomedical applications.

Similarly, cell-bound biosurfactants from *Lactobacillus plantarum* and *Pediococcus acidilactici* inhibited the *S. aureus* biofilm in a dose-dependent manner [69]. Furthermore, these biosurfactants affected the expression of biofilm-associated genes (icaA, cidA, agrA, dltB, sarA, and sortaseA) and interfered with the release of signaling molecules (AI-2) in quorum sensing systems, suggesting their potential against *S. aureus* biofilms.

Recently, few researchers have explored the immunomodulatory roles of microbial surfactants in fish. We evaluated the immunomodulatory role of VSG4 biosurfactants in *Labeo rohita* [70]. Intraperitoneal injection (i.p) of biosurfactant (200 mg/mL) increased serum bactericidal activity ($73.2 \pm 4.7\%$), complement pathway (ACP) activity (76.26 U/mL), lysozyme activity (36.32 U/

mL), and phagocytic activity (32.18%) ($P < .05$) in fish at 21 days post administration. Furthermore, biosurfactant administration increased the expression of cytokines IL-10, TGF-β, and IKB-α; however, TNF-α, IL-1β, NF-κB p65, IKK-β, MAPKp38, and Myd88 mRNA expression were downregulated in the treatment groups. Moreover, i.p. injection of 200 mg/mL of biosurfactant resulted in the highest postchallenge relative survival rate (67.88%) against *Aeromonas hydrophila* [70]. Even, VS16 biosurfactant increased the immune response and disease protection of *L. rohita* against fish pathogenic *A. hydrophila* infection [36]. Similarly, phospholipopeptide biosurfactants from *S. hominis* modulated the immune responses in *Oreochromis mossambicus* [37]. Biosurfactants increased the resistance of fish against diseases. These results indicate the potential of biosurfactants to modulate immune responses in fish.

Dietary supplementation of polyhydroxybutyrate for a month stimulated robust antioxidant responses and cytokine expression in the soiny mullet *Liza haematocheila* [71]. Likewise, dietary phospholipids improved the antioxidant performance, immune responses, and tight junction barrier of juvenile grass carp [72]. Previously, immunostimulatory efficacy of *B. thuringiensis* B.t.A102 derived poly-β hydroxybutyrate-hydroxyvalerate (PHB-HV) was investigated in *O. mossambicus*. Fish fed a high level of PHB-IV exhibited higher immune responses, as indicated by their lysozyme, peroxidases, and antiperoxidase activities [73].

It is worth mentioning that the results of in vitro studies may not always be the same as the results of an in vivo study. The glycolipid biosurfactant from the *Rhodococcus ruber* IEGM 231 inhibited immune parameters in mice after i.p. and intramuscular injection [74]. These researchers observed strongly in vivo suppression of antibody production, bactericidal activity, and production of pro-inflammatory cytokines by peritoneal macrophages. These results did not agree with the immunostimulatory activity of biosurfactants detected in immunocompetent cell cultures. Researchers have recently demonstrated that monoacyltrehalose biosurfactant from *R. ruber* IEGM 231 suppresses antibody production, bactericidal potential, and production of IL-1β by mouse peritoneal cells in male albino mice [75]. However, i.p. injection increased IL-10 production. These studies indicate that the results obtained in in vitro studies should be carefully evaluated in in vivo studies. Furthermore, the cell environment also contributes to these reactions and the immunotropic activity of the *R. ruber* biosurfactant.

10.5.4 Biosurfactants in wound healing

The antioxidant and protective effect of MELs on skin cells, even under H_2O_2-induced oxidative damage has been demonstrated [76]. Furthermore, MEL-B has the potential to attenuate the perspiration of the skin surface [76]. Takahashi et al. [77] analyzed the radical-scavenging activity of 1,1-diphenyl-2-picrylhydrazine. MEL-C at 10 mg/mL exhibited a scavenging activity of 50.3%. Among the MELs, only MEL-C at 10 mg/mL exhibited 60% activity. It is pertinent to mention that MELs do not have "generally regarded as safe" (GRAS) status, but microbes used in the production of MELs are considered as GRAS [78]. MELs, especially MEL-A, exhibit robust potential for microemulsion applications. In the aqueous phase, MEL-B & -C naturally from giant unilamellar vesicles. This indicates their superior molecular orientation properties and hydrophobic- hydrophilic stability [79].

The wound healing properties of LBS purified from *A. junii* B6 were evaluated in rats [80]. Treatment with 5 mg/mL LBS for 13 days decreased lesion size, increased re-epithelialization, and

decreased neutrophilic inflammation, immaturity of the wound bed, edema erythema, capillary, and necrotic tissue retention. Furthermore, LBS treatment increased glutathione (GSH) and decreased malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in rats. Thus the antioxidant potential of LBS promoted wound healing, which is comparable to that of ceramide-3 [76]. *Starmerella bombicola* derived sophorolipids exhibit antimicrobial activity against various bacterial pathogens [81]. Soporolipid-containing cream was tested in vivo using a mouse skin wound assay. The results showed that sophorolipid-containing cream did not affect the time course of wound healing, and histological examination confirmed that the healing process was parallel to that observed in control animals [81]. Sophorolipids have also been tested for dermatophytosis [82]. *R. babjevae* YS3 derived sophorolipid was effective against *T. mentagrophytes*. Studies in a mouse model of cutaneous dermatophytosis revealed that the use of sophorolipid could efficiently cure infected mice after 3 weeks of topical treatment [82]. These results suggest that sophorolipid could be used to formulate novel antifungal compounds to treat *T. mentagrophytes* infections. Therefore sophorolipids has potential for its exploitation as a constituent of antimicrobial creams against dermatophytosis and wound infection.

The emergence of antibiotic-resistant strains of *S. aureus*, made it difficult for the prevention and treatment. Giordani and coresearchers isolated a biosurfactant from *L. gasseri* BC9 [83]. BC9 was loaded in liposomes (B-L) and evaluated for its potential to prevent and eradicate the biofilm of MRSA strains [83]. The B-L was not cytotoxic and prevented the formation of MRSA biofilms. The B-L (mean diameter $<$200 nm) exhibited higher potential to inhibit and eradicate MRSA biofilm than free biosurfactant. Further, loading of B-L in lyophilized matrices resulted in its fast dissolution upon interaction with the exudate, which allowed vesicle reconstitution. The authors demonstrated the economical production of lyophilized B-L matrices for the treatment of local cutaneous infections [83].

Liu et al. [84] isolated the cyclic lipopeptide bacaucin from *B. subtilis* CAU21. Bacaucin exhibited broad-spectrum activity against Gram-positive bacterial isolates. Interestingly, a bacaucin-based ring-opened heptapeptide, that is bacaucin-1 showed strong inhibitory potential against MRSA via a membrane-damage mechanism without inducing bacterial resistance or detectable toxicity to mammalian cells. Bacaucin-1 efficiently prevented MRSA infection in both in vivo and in vitro models. Furthermore, these authors engineered a linear, short, and low-cationic peptide, bacaucin-1a [85]. Bacaucin-1a inhibited MRSA by a unique mode of action. These authors suggest that bacaucin-1a may target RNA polymerase σ factor sigB and GMP synthase [85].

Lipopeptide biosurfactant produced by *B. stratospericus* sp. A15 exhibited wound healing properties [86]. It showed tremendous antioxidant activity and notable antibacterial activity against *E. coli* and *S. aureus*. Ointment formulated using A15 biosurfactant accelerated wound closures, lessen irritation, and enhanced tissue regeneration in vivo [86]. Similarly, surfactin A from *B. subtilis* accelerated wound healing in mice by regulating cell migration, angiogenesis, and inflammatory activity [87]. Glycolipid biosurfactant from *B. licheniformis* SV1 exhibited anticipated in vitro cytocompatibility and increased the 3T3/NIH fibroblast cell proliferation [88]. The use of SV1 biosurfactant ointment exhibited wound healing activity in the skin by promoting fibroblast cell proliferation, reepitheliazation, and quick collagen deposition [88]. Therefore more investigation is necessary to improve the yield of biosurfactants and to ease the purification process for their large-scale uses in skincare or pharmaceutic applications.

10.5.5 Biosurfactants in delivery of veterinary drugs

The main challenges in microemulsion-based drug delivery systems (DDSs) are safety and effectiveness. Microemulsions have recently been used for drug delivery. A few advantages of microemulsions over chemical surfactants are lower toxicity, biodegradability, eco-friendliness, high selectivity, and effectiveness in a broad range of temperatures, pH, and salinity [89]. Stable methyl methacrylate emulsion was formulated using rhamnolipids extracted from *P. aeruginosa* PA1 [90]. Similarly, silver NPs were produced using *P. aeruginosa* BS-161R derived rhamnilipids, which exhibited satisfactory antibiotic activity against bacterial pathogens and *C. albicans* [91]. Rhamnolipid and surfactin were used in an emulsion polymerization approach to developing biodegradable methyl methacrylate /biosurfactant bionanocomposites [92]. It was revealed that the use of biosurfactants increased the dissociation rate, emulsification efficiency, and bioavailability of the therapeutic agents. Various studies have shown that glycolipids are widely used in the formulation of microemulsions. Biosurfactants could be used as a bio-source because of their tremendous self-assembly and emulsifying activity in microemulsion DDSs. Further studies using animal models are essential to validate their safety [93].

Microbial-derived glycolipids (MGLs) are attractive for biomedical applications that have been utilized in the medical field to treat diseases such as AIDS and cancer [94]. Intraperitoneal or intramuscular administration of trehalolipid from *R. ruber* IEGM231 inhibited the production of cytokines and respiratory burst dynamics in murine peritoneal cell cultures [95]. Trehalipids activate the functional activity of microphages in vitro, but the direction is different from that of the in vivo model. The high immunomodulatory activity of trehalose 6, 6-dicorynomycolates (TDM), trehalose monomycolate (TMM), and other trehalolipids produced by rhodococci are to be exploited for clinical purpose. It has been demonstrated that bacterial trehalolipids bind with receptors (e.g., Mincle and macrophage C-type lectin receptors of the lectin family). These interactions result in the production of the NF-κB transcription factor, which is associated with high immunoregulatory trehalolipid activity [96]. The molecular mechanisms behind the interaction between immune cells and trehalolipids are vital for a new immunotherapeutic approach.

Liposomes are membrane structures that can encapsulate or incorporate a broad range of bioactive agents. Liposomes are useful in the delivery of foreign DNA, oligonucleotides, antibodies, and small interfering dsRNAs into mammalian cells. The performance of liposomes and lipoplexes has been improved by incorporating microbial surfactants [97]. Cationic liposomes are an encouraging tool for gene delivery because of their low toxicity and immunogenicity, and a high degree of active targeting. However, cationic liposomes have limitations including entry into nontarget cells, avoidance of the degradation of exogenous DNA by nuclease, endosomal/lysosomal escape, and transport into the nucleus [78,97]. Biosurfactants might improve the transfection efficacy of liposomes by reducing DNA-stimulated aggregation and by decreasing their particle size [98]. They show that MEL-A stimulated the gene transfection efficiency mediated by cationic liposomes with a cationic cholesterol derivative. Liposomes with a cholesteryl-3 beta-carboxyamindoethylene-*N*-hydroxyethylamine (I) was more effective for gene transfection. Later, these researchers developed a compound consisting of MEL-A, (OH-Chol) (cationic cholesterol derivative) and L-dioleoylphosphatidyl-ethanolamine (DOPE). They demonstrated that biosurfactants facilitate gene

delivery into targeted cells through the fusion of lipoplexes to the plasma membranes [99]. MEL-A improves DNA transfection efficiency of liposomes was improved by MEL-A which induced the membrane fusion between liposomes and plasma membrane of target cells. Thus it promotes the uniform dispersal of the material in the cytosol and suppresses the expression protein with marginal cytotoxicity [100].

10.6 Future prospects and conclusion

Biosurfactants are very important due to their several essential physio-chemical and biological properties. Their diverse properties make them efficient to use as a substitute to chemical surfactants. The production of microbial biosurfactants has recently remained a major challenge. More research is necessary to improve both the quantity and quality of biosurfactants. The complete biosynthetic pathways and their regulations could be expounded using advanced tools such as transcriptomics, genomics, proteomics, and metabolomics. For example, hindering the synthesis pathway of less vital metabolites could divert the majority of energy toward biosynthetic pathways of preferred biosurfactants [7]. Effective framework is required to explore their potential for biosurfactant-based DDSs.

Various biosurfactants are promising in veterinary medicine owing to their biocompatibility and unique characteristics such as antimicrobial and antibiofilm activities, self-assembly, tensioactivity, and broad stability. High production costs hinder the wide use of microbial surfactants; therefore, the production and purification processes should be simplified. It is pertinent to mention that upgrade of lab-based processes at a profitable scale is a complex method and involves technological as well as economic valuation to assess economic viability. Considerable effort is required to find cost-effective methods to obtain biosurfactants on a large scale. In this regard, some promising results in developing fermentation media using poultry waste flour and buttermilk, agro-industrial wastes, etc. have been found.

Although a diverse range of activities has been reported, further work is needed to explore the relationship between the physicochemical properties and molecular structures of biosurfactants. This will pave the way for the improvement of biomedical and biotechnological applications of biosurfactants.

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Conflicts of interest

No conflict of interest.

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CHAPTER

Applications of surfactin and other biosurfactants in anticancer activity 11

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11.1 Introduction

The term biosurfactant connotes a heterogenous category of biological surface-active compounds which are of microbial origin (prokaryotic and eukaryotic). They are secondary metabolites produced by microorganisms and bear a pivotal role in their persistence. These compounds are amphipathic in nature. Amphiphiles inhere a hydrophilic polar group (built from proteins, peptides, sugars - mono/oligo/polysaccharides) along with nonpolar hydrophobic moiety (composed by hydroxylated fatty acids or alcohols either saturated or unsaturated). This amphipathic nature of biosurfactants enables them to improve the surface area of hydrophobic molecules, for that reason improves the bioavailability of water-insoluble molecules. These amphiphilic molecules are regarded to be endowed with various characters such as emulsifying property, dispersibility, antiadhesion, selectivity, biodegradability, nontoxic, nonhazardous, foaming. The activity of biosurfactants is invariable under harsh environmental/physiological conditions such as extreme temperature, pH, salinity. The industrial waste can be fabricated in a way that biosurfactants can be produced. This manufacturing procedure reduces the impact of industrial waste on the environment additionally, making their price affordable. Biosurfactants are increasingly employed in various sectors and are extensively investigated [1].

Biosurfactants are grouped according to their microbial origin. molecular weight, chemical composition, physicochemical properties, and mechanism of action (Fig. 11.1) [2,3]. The classification of biosurfactants is based on their microbial origin which either is an extracellular product or bound to the cell wall, based on molecular weight featuring diverse molecular properties and they are further dived into various classes and corresponds to specific microorganisms. Polar and nonpolar type of subcategory branched from chemical composition comprises of classification of biosurfactants. Table 11.1 gives the various classes of biosurfactants available and their source of microorganism.

Low molecular weight biosurfactants possess molecular weight often less than 10 KDa in contrast to high molecular weight biosurfactants. For this reason, lower molecular weight biosurfactants hold the potential to reduce surface tension attributed to water by 25−30 mN/m. whilst high molecular weight biosurfactant could reduce it in the range not less than 35−40 mN/m. The emulsifying nature mentioned above is the fundamental character of high molecular biosurfactants as they are referred to as bioemulsifiers [4].

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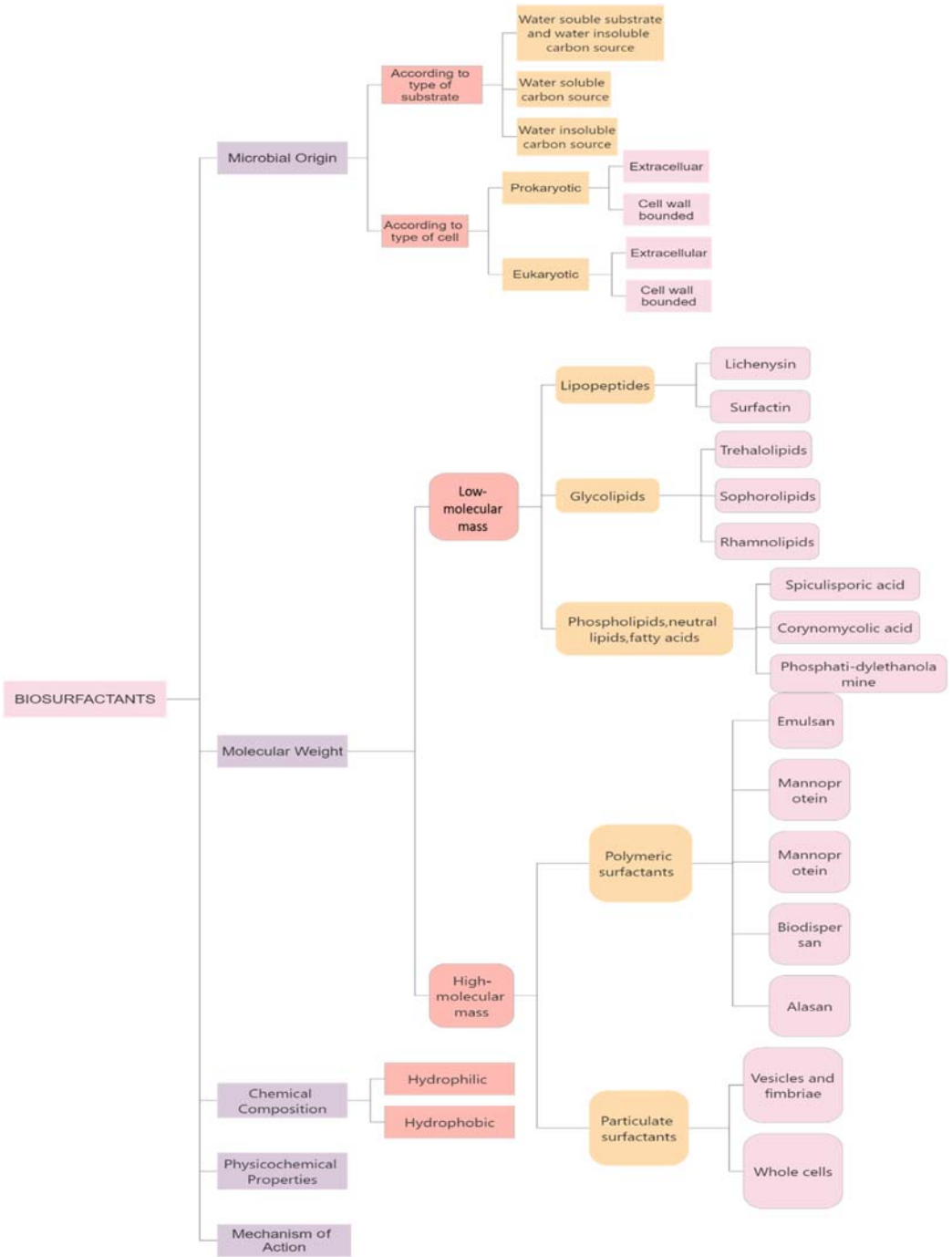

FIGURE 11.1

Classification of biosurfactants.

Table 11.1 Various class of biosurfactants available and their source of microorganism.

Class of biosurfactants	Microorganism	Reference
Rhamnolipids	*Pseudomonas sps.* (*p. aeruginosa, P. chlororaphis P. putida*) *Renibacterium salmoninarum* *Bacillus subtilis*	[1,4,3,5]
Trehalolipids	*Mycobacterium tuberculosis*, *Rhodococcus erythropolis*, *Arthrobacter sp.*, *Nocardia sp.*, *Corynebacterium* *Tsukamurella sp*	
Sophorolipids	*Torulopsis bombicola*, *Torulopsis petrophilum*, *Torulopsis apicol* *Candida bombicola* *C. apicola*	
Cellobiolipids	*U. zeae*, *U. maydis*	[2]
Corynomycolic acid	*Corynebacterium lepus*	[1,4]
Spiculisporic acid	*Penicillium spiculispor*	
Phosphati-dylethanolamine	*Acinetobacter sp.*, *Rhodococcus erythropolis* *T. thiooxidans*	
Neutral lipids	*N. erythropolis*	[2,3,4,1,5]
Peptide lipid	*B. licheniformis*	
Fatty acids	*C. lepus*	
Mannosylerythritol lipids	*Candida antartica*	
Surfactin	*Bacillus subtilis*	
Viscosin	*P. fluorescens*	
Lichenysin	*Bacillus licheniformi*	[1,3]
Emulsan	*Acinetobacter calcoaceticus RAG-1*	
Alasan	*Acinetobacter radioresistens KA-53*	
Biodispersan	*Acinetobacter calcoaceticus A2*	
Liposan	*Candida lipolytica*	
Mannoprotein	*Saccharomyces cerevisiae*	
Vesicles and fimbriae	*A. calcoaceticus*	[2]
Whole cells	*Variety of bacteria*	

11.2 Characteristics and mechanism of action of biosurfactants

11.2.1 Characteristics of biosurfactants

Compared to chemical surfactants biosurfactants are unique in various aspects:

i) Lower toxicity;

ii) Biodegradable to a large extent;
iii) Low critical micellar concentration with high surface activity;
iv) Have one or more functional moiety and one or more chirality;
v) Bulky yet sophisticated structure;
vi) Possess antitumor and antimicrobial activity.

11.2.2 Mechanism of action of biosurfactants

As stated biosurfactants can reduce the surface tension of water (from 72 to 30 mN/m). Concentration [critical micellar concentration (CMC)] of biosurfactants regulate this surface tension reduction property. CMC is the concentration at which micelles start to form. Micelles are bilayer vesicles formed at the interface of hydrophilic and hydrophobic environments. Biosurfactants are considered efficient when the desired interfacial tension is obtained at a low concentration of biosurfactant in use. Table 11.2 gives information on the CMC of biosurfactants to display their surface activity and interfacial phenomenon.

Table 11.2 Critical micellar concentration of biosurfactant to display their surface activity and interfacial phenomenon.

Biosurfactant	CMC	Surface activity	Interfacial tension	Characteristic feature	Use	Reference
Mannosylerythritol lipid	2.7×10^{-6} M	28–2 mN/M	28–2 mN/M	Excellent surface reduction at low concentration	To improve rheology of powders, Emulsifier.	[6]
Sophorolipids	40–100 mg/L	Lower to less than 40 mN/m	-	Exhibits superior hydrophilicity and water-solubility compared to other biosurfactants	Dishwashing detergent.	
Rhamnolipids (Anionic surfactant)	10^{-4} to 10^{-5} M	Lowers from 72 to 30 mN/m	From 43 to <1 mN/m	Packing property	Foaming agent, Dispersing agent, Penetrating ability, Emulsifier.	
Trehalose lipid	4 mg/L	From 72 to 36 mN/m	From 43 to 17 mN/m	Superior chemical stability (active at wide range of temperature, pH)	Sugar-based surfactants	

11.3 Applications of biosurfactants in anticancer activity

The microemulsion is a biphasic liquid dosage form with a continuous phase and a dispersed phase within. To stabilize these dosage forms of surfactants, cosurfactants are used traditionally. Biosurfactants when added form a monolayer of aggregates in between dispersed and dispersion medium thereby, improving reducing surface activity, improving the stability and shelf-life of the product [6]. It was proved that biosurfactants namely mannosylerythritol lipid has greater emulsifying action on soyabean oil and n- tetradecane than that of tweens.

Cancer is the world's worst illness, and no medication can entirely cure it. Scientists are working feverishly to find a medicine or technique that can be used to cure cancer completely and prevent it from recurring. One strategy that they investigated was the use of biosurfactants as anticancer drugs.

Biosurfactants are "detergent-like" molecules. These biosurfactants are multifunctional, nontoxic, or less toxic compounds [7]. They are stable at 60^{o}C and their pH ranges from 6 to 10 [8].

Their ability to damage the cell membrane of malignant cells has been proposed as a function of its physicochemical properties. The breakdown or disruption of cancer cells' cell membrane is the first and most important stage in their destruction. When the cell membrane of malignant cells is disrupted, anticancer agents can easily penetrate it and impair its function, resulting in cancer cell death. To carry out such membrane breakdown, various microbial-derived biosurfactants were tested to see which one had the most influence on cell death. In research comparing biosurfactants such as 95% rhamnolipids, 90% rhamnolipids, surfactin, and lactonic sophorolipid, lactonic sophorolipid is the most cytotoxic and harmful to healthy cells in low concentrations. Furthermore, focusing on cancer cells is the next approach in reducing toxicity to normal, healthy cells [9].

Another research looked at the usage of biosurfactants in breast cancer to see how they affected cell growth and viability. T47D and MDA-MB-231 breast cancer cell lines were employed in the study. Surfactin and a glycoprotein generated by Lactobacillus paracasei (BioEG) were chosen as anticancer biosurfactants.

11.4 Applications of surfactin in anticancer activity

Surfactin, a lipopeptide, is the most frequently studied biosurfactant. It was discovered to reduce the viability of both breast cancer cell types investigated. Surfactins, according to the researchers, act in a dose-dependent manner and trigger apoptosis via the mitochondrial caspase pathway driven by ROS/JNK [10]. One major drawback of surfactin is that when used in higher concentrations it imparts hemolytic effect (above 0.05 g/L). To overcome this drawback many scientists have used different ways like modifying surfactin molecules, incorporating them into a nanoparticle, etc. Surfactin has been successfully incorporated into several nanoforms such as polymeric micelles, liposomes, nanoemulsions, and others to mitigate its toxicity [11]. Both surfactants caused a G1 arrest and reduced DNA synthesis, indicating that they can impact cell cycle progression and consequently restrict cell growth. BioEG was shown to be more successful than surfactin in treating breast cancer because it had stronger anticancer activity and showed no damage to normal healthy cells [12].

Surfactin works primarily by triggering apoptosis, which causes malignant cells to die, and so serves as a cancer therapy method. Surfactin possesses anticancer, apoptotic, antiproliferative, and antimetastatic properties in addition to its anticancer properties [13].

Surfactin is a *Bacillus subtilis* derived biosurfactant that has amphiphilic properties. It has many distinctive features apart from having biosurfactant activity, like antitumor, antiinflammatory and antiviral properties. In this study, surfactin was loaded with doxorubicin-loaded nanoparticles (Fig. 11.2) which is an anticancer drug having chemotherapeutic properties and is mainly used for breast cancer treatment. This combination is synthesized through a solvent emulsion mechanism.

Aqueous solution of surfactin + solution of doxorubicin (hydrophilic)
Doxorubicin gets accumulated in the hydrophobic part of surfactin nanoparticle
DOX@SUR nanoparticles are formed.

It is seen that multidrug resistance (MDR) is causing an obstacle to the smooth treatment of various cancers. Mechanism of MDR is identified to cause efflux of anticancer agents due to overexpression of ATP binding P-glycoprotein. Surfactin loaded with doxorubicin is combined with a nanoparticle as it leads to better drug delivery, helps inhibit P-glycoprotein thus reversing MDR, increases drug accumulation and the permeation and retention of the drug are enhanced. In this study, MCF-7 human breast cells and MCF-7/ADR cells (Adriamycin resistant) were injected into the mice to induce breast cancer. Many experiments were carried out on the mice and many findings were concluded based on various parameters.

Different mass ratios of surfactin and doxorubicin were experimented, like 5:1,2:1,1:1 and 1:2. However, it was seen that the size of the nanoparticle at 1:1 ratio was seen to be the smallest. The drug release profile of DOX@SUR nanoparticles were seen to release 65% doxorubicin even after 48 hours, unlike free doxorubicin that showed a burst release spike within an hour. Experiments include free doxorubicin, blank surfactin, and DOX@SUR nanoparticles, out of which DOX@SUR nanoparticles have shown greater suppression, inhibiting tumor to further grow. When compared to all the experimented tumor tissues, the weight of the tumor that was treated with DOX@SUR nanoparticles has seen to be significantly low. Moreover, it was studied that though free doxorubicin exhibited some systemic toxicity, this nanoparticle combination did not show any toxicity.

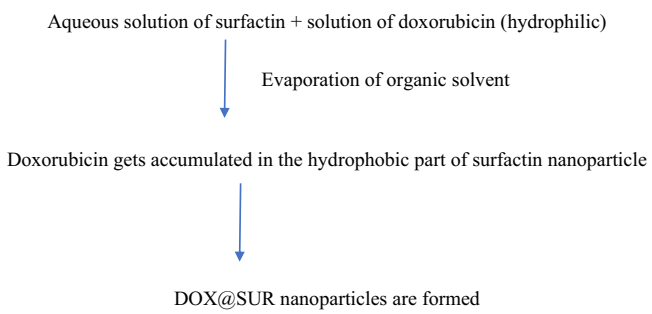

FIGURE 11.2

Preparation of doxorubicin-loaded nanoparticles (DOX@SUR).

Thus, it was concluded that DOX@SUR nanoparticles can inhibit MDR by increased cellular uptake. They even eliminate any toxicity or reduce any adverse effects, which in turn proves to be an effective anticancer agent for breast tumors [14].

Surfactin is seen to have low CMC along with high emulsification activity. Thus, an innovative technique is used for anticancer delivery that includes nanoemulsions for targeted anticancer drug delivery.

Anticancer drugs when administered in higher concentrations are seen to be affecting normal cells. Hence there is a need for drugs that must be administered in lower concentrations and give sustained release by eliminating toxicity in normal cells. A nanoemulsion with a combination of eucalyptus oil, surfactin, Tween-20 and water was prepared in which doxorubicin was loaded. The surfactant to water ratio was taken to be 1:2. Eucalyptus, the essential oil, is known to have antimicrobial, antioxidant, antifungal, and antiinflammatory properties. This emulsion was an O/W type nanoemulsion in which, surfactin inhibits the growth of cancer cells. (Fig. 11.3) This doxorubicin nanoemulsion was evaluated for various parameters like ionic stability, temperature, pH, external shear, and thermal stability, before the conduction of the experiment.

It was seen that these nanoemulsions depicted antibacterial properties against *E. coli* and *E. hirae*. Moreover, doxorubicin-loaded nanoemulsions showed anticancer properties by proving synergistic effects of doxorubicin, surfactin, and essential oil.

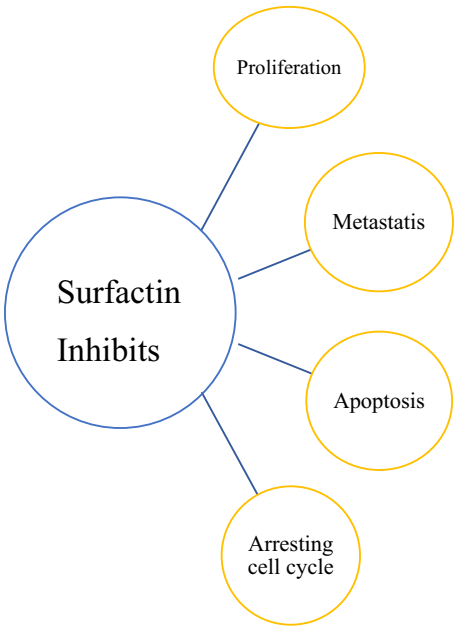

FIGURE 11.3

Surfactin inhibits proliferation, metastasis, apoptosis, and arrests cell cycle, thus, in turn, inhibiting the growth of cancer.

Thus it was concluded that doxorubicin-loaded nanoemulsion was highly effective as an anticancer agent which has potential targeted drug delivery [15]. Eucalyptus oil was seen to depict anticancer activity through the antiproliferative mechanism.

Biosurfactant surfactin is produced during the stationary phase of the growth cycle of bacillus sps. Its production can be improved when fermented along with previously fermented soybean paste. Surfactin exhibits anticancer activity against various cancer types and on different cell lines. There was superior anticancer activity displayed by the product obtained when bacillus was cofermented with soybean paste which was 2.5 to fivefold greater than that obtained from fermenting bacillus alone. It was demonstrated that surfactin was active against breast cancer, colon cancer, cervical cancer, leukemia. Surfactin in plenty of ways inhibited the growth of tumor cells. Surfactin could inhibit extensive replication (characteristic nature of cancer cells), induce apoptosis, and prevent metastasis. This cytotoxic effect varied with cancer type and the concentration required to stimulate the effect also varied (Table 11.3).

Apoptosis is stimulated by various pathways namely ROS/JNK mediated /Ca + mediated mitochondrial cascades. Surfactin initiated either of the pathways and resulted in cell death and aided in cancer therapy. 47% of the cell were subjected to apoptosis after 48hrs of administration or treatment with surfactin. Upregulating metalloproteinase−9 surfactin inhibited the invasion of adjacent noncancerous cells by cancer cells. Metastasis stimulated by 12-O-tetradecanoylphorbol13-acetate (TPA) is inhibited by surfactin. Overall, the anticancer effect inhibited the cell growth, and the cell was arrested at the G0/M phase of the development cycle in case of breast cancer MCF 7 cell lines, G0/G1phase of LoVo colon cancer cells

Table 11.3 IC50 of surfactin required to the curb growth of cancer cells.

Cancer cell lines and cancer type	Bacterial strain	Concentration of surfactin (IC50)	Effect	Reference
MCF 7 (Breast cancer cells)	*B. subtilis* CSY 191	9.65 μM	Apoptotic, Antiproliferative, Inhibits cell growth at G0/M phase	[16]
	B. subtilis 573	193 μM		
	B. subtilis Hs0121	29 ± 2.4 μM		
	B. subtilis TK-1	86.2 μM		
LoVo colon cancercells	-	26 μM	Inhibits cell growth at G0/G1 phase	
Leukemia	*B. subtilis* natto T-2	2−26 μM	Inhibit cell growth and arrest cell cycle at various phases	
	B. subtilis natto K562	10−20 μM		
Hepatocellular Cancer	*B. subtilis* HSO121	35 ± 12 μM	Growth inhibition	
HeLa cell line (Cervical Cancer)	-	37 ± 4.5 μM	Antiproliferative, Reduce viability of cancer cells	

Surfactin could attenuate localization of natural factor-kappa B activated by TPA [16].

Amphophilic surfactin is seen to have various properties like anticancer and antibiofilm. The structure of surfactin includes fatty acid and phospholipids, which efficient interact with each other hydrophobically to produce anticancer activity. Moreover, the peptide moiety bids with cancer cells and regulates proliferation through polar heads.

This paper mainly talks about the efficiency of surfactin that are produced from marine source, actinomycete *Micromonospora marina*, in the treatment of breast cancer. A human MCF-7 cell line was used in this study. Cytotoxicity activity was studied of surfactin and triton 100. The results obtained depicted that MCF 7 cell line showed high cytotoxic activity when the concentration of surfactin and triton 100 was increased. There was no cytotoxicity seen in the normal healthy human cells. There were cell alterations or abnormalities in the tumor cells due to the effect of surfactin and Triton 100. Alterations included cell rounding up, shrinking, membrane blebbing, and lots of floating dead cells were seen. There are even evidences that surfactin can be used for the effective treatment of various cancers like breast, colin, hepatoma and leukemia. It was concluded that surfactin derived from coral mucus of actinobacterium M. marina was seen to be potentially acting against cancer by optimum cytotoxicity in cancer affected MCF cells. They may induce ROS generation in mitochondria and may induce apoptosis which in turn inhibits cancer growth, but the this mechanism is less effective than other commercially available anticancer agents [17].

11.5 Applications of other biosurfactants in cancer therapy

There have been evidences proving that a lot of potent anticancer agents show significantly low toxicity along with higher efficacy. They can be classified under microbial amphiphiles. They are easily biodegradable and can be further classified into glycolipids and lipopeptides. These amphiphiles are seen to possess huge therapeutic applications.

A few examples of microbial amphiphides are mentioned below in following sections.

11.5.1 Iturin

It is derived from marine source named *B. megaterium*., and is mainly found in Mexico. Iturin A is a lipopeptide with seven amino acid chain (cyclic peptide) connected to C-16 chain Hallobacilin and Mixirins are classified under the class of ituin, which have anticancer properties. An oncogenic protein Akt is responsible for extensive replication, angiogenesis, inhibits apoptosis, also regulate the expression of GSK3b, FoxO3a. This oncogenic protein was downregulated by Iturin. They are seen to show cytotoxic activity in human colon cancer cells HCT-116 [16].

11.5.2 Fengycin

It is a lipopeptide class of amphiphile that is derived from *B. subtilis*. The peptide chain contains 10 amino acids and the lipidic chain is composed of 16−19 carbon b-hydroxy fatty acids. They exhibit anticancer as well as antifungal properties. Fengycin exserts its anticancer activity by its antiproliferative effect (observed on human colon adenocarcinoma cell line LoVo cells). Through

induction of apoptosis and arrest cells at different cell cycle phases to control cancer cell replication. They show cytotoxicity on human colon cell HCT-15 AND ht-29. The antimicrobial effect is achieved by improving cells plasma membrane permeability at the target site. At CMC Fengycin localize within the lipid bilayer, which later is self-assembled within and cause membrane disruption and exhibit an antimicrobial effect. It has potent activity against filamentous microorganisms. A remarkable increase in the number of apoptotic factors was observed when Fengycin was used in the treatment of fungal diseases [16].

11.5.3 Somocystinamide A

They show effective anticancer properties through their cytotoxic activity. They are classified under lipopeptide class, which depicts anticancer activities and act against breast, lung, prostate carcinoma and leukemia. Somocystinamide A induces apoptosis in leukemia cells.

11.5.4 Fellutamides

Fellutamide A and B are derived from fungi named *Penicillium fellutanum*, which is found in fish. Fellutamide C and D are derived from Metulocladosporiella and is seen to depict cytotoxic activity on the skin, colon, CNS, lung, and ovarian cancer cells. Fellutamides are said to have a unique anticancer fighting ability.

11.5.5 Pseudofactin

They are derived from *P. fluorescens* BDS. They are further classified into Pseudofactin I and II. Pseudofactin II is seen to be effective against melanoma cells. Moreover, no toxicity is seen in normal cells

11.5.6 Rakicidin

They are derived from marine bacteria sources namely *Micromonospora* bacteria. They can be further classified into lipopeptides like Rakicidin A and B. Rakicidin A is seen to a versatile hypoxia selective cytotoxicity, that is present in various cancer cells. Rakicidin B inhibits the growth of various cancer cells. Moreover, Rakicidin Cand D do not exhibit cytotoxicity, but Rakicidin D is seen to show an antiinvasive property on breast cancer cells.

11.5.7 Apratoxin

They are derived from a marine cyanobacterium. Various class of Apratoxin is seen, in which Apratoxin A induces apoptosis and Apratoxin F and G show cytotoxic activity on lung and colon cancer cells. They are proved to be promising against cancer as their IC50 values are greater than the standards of chemotherapeutic agents.

11.6 Conclusion

Surfactin works primarily by triggering apoptosis, which causes malignant cells to die, and so serves as a cancer therapy method. Surfactin possesses anticancer, apoptotic, antiproliferative, and antimetastatic properties in addition to its anticancer properties. Its action is studied in a variety of cancer cell lines to see how successful it is in treating certain cancer types. Various cancer cell lines on which surfactin or surfactin-like biosurfactants have been investigated are Ehrlich ascites carcinoma, breast cancer, colon cancer, hepatocellular carcinoma, leukemia, cervical cancer, and many more. Biosurfactants, when utilized in their crude form, were found to be significantly less cytotoxic against all of the aforementioned cancer cell types. Apart from these cancer types, biosurfactants are also been investigated on other cancer cell lines such as human oral, epidermoid carcinoma, pancreatic, and rat melanoma cancer. In conclusion, biosurfactants have huge potential in anticancer activity because they primarily focus on anticancer activities and have shown good cytotoxicity towards cancer cells and at the same time are less harmful to healthy cells in low concentrations. However, to excel in the application of biosurfactants in cancer therapy, more detailed study along with more clinical data is necessary.

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CHAPTER

Inhibitory activity of biosurfactants against H^+-K^+ ATPases and defense against gastric ulcers

12

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12.1 Introduction

Gastroduodenal ulcers also called gastric ulcers have emerged as an alarming problem worldwide. About 2.4% of the inhabitants of western countries are affected by peptic ulcers [1] and the annual occurrence ranges from 0.10% to 0.19% [2]. Gastric ulcer is an acid-induced sore of the digestive tract and is characterized by denuded mucosa with the erosion invading into the submucosa or muscularispropria [3]. Though the main factor for the genesis of gastric ulcer is the bacteria, *Helicobacter pylori* but other contributing factors such as excessive acid secretion, unregulated consumption of nonsteroidal antiinflammatory drugs (NSAIDs), frequent and excessive drinking, anxiety, stress, etc. are also accountable for the development of ulcer [4]. The principal etiological factor responsible for the development of gastric ulceration is the secretion of gastric acid. The presence of ion-channel pumps like gastric hydrogen-potassium ATPase (H^+/K^+-ATPase) proton pump is fundamentally liable for the release of acid by the oxyntic cells located in the gastric mucosa. Therefore, inhibiting the H^+/K^+-ATPase activity to lessen gastric acid release, emerges as a significant pharmacological strategy to treat not only gastric ulcers but also duodenal ulcers and also gastroesophageal reflux infection [5]. At present, medications such as omeprazole and histamine blockers that cause suppression of proton pumps are used to comfort patients suffering from gastric ulcers. But, sadly their long-term usage in patients with a stomach ulcer is restricted due to their hazardous side effects [6]. Hence, it is important to develop effective and safe alternatives to treat the gastric ulcer.

Biosurfactants are amphiphilic microbial compounds that display pronounced emulsifying, wetting, solubilizing, detergent, and phase-dispersing activities. These compounds comprise various ranges of chemical structures and are classified accordingly. The biosurfactants can be fundamentally classified as glycolipids, phospholipids, polymeric biosurfactants, and lipopeptides (surfactin). Biosurfactants are less toxic and possess higher biodegradability compared to their chemically synthesized counterparts. Various reports have elucidated the implementation of biosurfactants in the biomedical as well as the medicinal field. Their antimicrobial properties designate them as potent

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molecules that are capable of preventing numerous diseases [7]. Although an experimental study has demonstrated [8], but, as of today, no profound knowledge has been accessible on the use of biosurfactants for the recovery from gastric ulcers. In this chapter, we attempted to unravel the efficacy of biosurfactants to treat gastric ulcers by suppression of H^+/K^+-ATPase proton pump.

12.2 Biosurfactants: potential application as a therapeutic target

Years of commercial researches have shown the efficacy of various biosurfactants as antibacterial and antifungal which have made them items of extensive research so that they can be exploited as therapeutic agents to combat various diseases. Fascinating highlights of biosurfactants have propelled researchers to carry out diverse applications not only in the clinical field but also in the field of agriculture, industry, and the petroleum sectors. Various microorganisms give rise to several useful biosurfactants. By nature, these compounds are amphiphilic and they may be released either outside the cell or are sometimes produced when the microorganisms are cultured on water-immiscible substrates [9,10]. When the microbes grow on water-immiscible substrates, biosurfactants promote their growth by minimizing the interfacial tension, thereby increasing the accessibility to insoluble substrates for intake and absorption. The microbial biosurfactants comprise various biochemical structures ranging from fatty acids to phospholipids to lipopeptides to polysaccharide-protein complexes etc. [7]. Therefore, multifaceted properties and physiological attributes of different families of biosurfactants are quite apparent. Microorganisms like *Pseudomonas aeruginosa*, different species of *Bacillus*, and different species of *Streptococcus, Candida antartica*, *Lactobacillus, Lactococcus*, and others produce diverse biosurfactants which have immense potential in therapeutics [11,12]. Moreover, according to the need, these molecules can be customized by altering the growth medium or the specifications required for growth [13]. Amongst several other interesting characteristic features, one important feature is the amphipathic nature of the biosurfactants that allow them to interact with both hydrophilic and hydrophobic groups, especially at the boundary between the fluid phases. This property has made them indispensable for environmental applications [14]. On the other hand, despite their microbial origin and microorganism-mediated degradation, few researches have been conducted to understand their efficacy in therapeutics [7]. But researchers surely point out the significance of biosurfactants as a promising substitute to synthetic medications and antimicrobial agents. Biosurfactants are substantially more superior compared to their conventional chemical counterparts as they are lesser toxic, have higher biodegradability, and are tolerant to a wide range of temperature or pH [10,15]. They are also reported to be biocompatible and digestible, thereby making them suitable for application in different industries such as pharmaceuticals, food, and cosmetics [16].

Iturin biosurfactants are secreted by the strains of *B. subtilis* [17]. They manifest potent antifungal properties. Iturin A was found to traverse the cell barrier, forming ion escorting pores in the cytoplasmic membrane of the yeast cells leading to increased permeability of potassium ions and thereby the death of yeast cells [18]. *Pseudomonas aeruginosa* secretes another biosurfactant, Rhamnolipid, that has potential industrial and environmental applications [19]. They are used as cosmetic additives in the cosmetic industry because of their antiallergic traits. Moreover, they exhibit microbicidal properties against *Mycobacterium tuberculosis*. Several immunological and

antimicrobial properties have been attributed to the glycolipid biosurfactant, mannosylerythritol lipid (MEL), produced by a variety of yeast and fungal strains [20]. Two types of MEL, tri-acylated MEL termed as MEL-A and di-acylated MEL termed as MEL-B, exhibited bactericidal properties, specifically against Gram-positive bacteria [21]. When skin cancer cell line models like murine melanoma B16 cells were exposed to surging concentrations of MELs, a significant subG_0/G_1 arrest was noticed in their cell cycles, thereby indicating a possible apoptotic population in the cell line. This claim was also confirmed by further studies indicating that a significant percentage of the population was in early and late apoptotic stages. Moreover, in the B16 cells, other signature events of apoptosis such as genomic DNA fragmentation and chromatin condensation were also observed [22].

Surfactins are the best-studied lipopeptide that have been applied in several medical conditions. They are cyclic lipopeptide biosurfactants produced by various strains of *Bacillus* genus. Surfactins exhibit a broad range of activities against both the types of Gram bacteria [23,24], while pumilacidins have antiviral [8] and antibacterial activities. Studies have reported various biological properties for surfactin including inhibition of fibrin clot formation, H^+/K^+-ATPase activity, hampering activity of 100 Kda cytosolic phospholipase A2 of spleen and platelet, and stimulation of ion channel formation in plasma membranes [25,26]. They display antiviral activity against many viruses, including herpes simplex virus (HSV), simian immunodeficiency virus, Semliki Forest virus, etc. [27]. Cytotoxic activity of surfactin has also been reported against different cancer types, most importantly in blood cancer, in cancers associated with females like breast cancer and also in case of colon cancer [28,29]. Administration of surfactin not only inhibited the cell cycle of cancer cells by causing the arrest, but also promoted cancer cells to undergo programmed cell death events like apoptosis. Recent investigations have also confirmed that surfactin is associated with the promotion of several antimetastatic events stalling the epithelial−mesenchymal migrations. The amphiphilic nature of surfactin allows them to get easily incorporated into different types of nanoformulations [30]. An interesting nano-emulsion of surfactin prepared from *B. subtilis* clearly showed results confirming the fact that the biocompatibility of the surfactants increases many times in such nano-emulsions for the biomedical and pharmaceutical interventions. These newly designed bio-emulsions also showed the potential to act as multifunctional, custom-designed systems which might have interesting and path-breaking roles as the future of the delivery system. Within the encapsulation of these nano-formulations, surfactin can be delivered to the desired targeted organ, thus serving as improved anticancer therapeutics. Wu et al. [31]. Surfactins also exhibit antiinflammatory activity, thus making them useful not only for treating diseases as a therapeutic agent but can also be used as probiotic agents [32].

12.3 Function of H^+/K^+-ATPase in gastric ulcer formation

Gastric ulcer is a major gastrointestinal disorder characterized by lesions of the digestive tract. One of the interesting and important host defensive arsenals against invading pathogens is the secretion of gastric acid, responsible for the generation of gastric ulcer. In 1981 Sachs et al. reported that secretion of the gastric acid occurs because of H^+/K^+-ATPase pump, the activity of this pump is reliant on ATP hydrolysis [33]. A basal level of acid is always produced by the stomach oxyntic

cells (parietal cells), but the amount of acid released during this time is low. At resting state, a minimum number of H^+/K^+-ATPase is present on the surface of parietal cells, but when the cells receive a certain stimulus, a large number of H^+/K^+-ATPase are recruited onto the membrane of oxyntic cells [34]. The parietal cells harbor the enzyme gastric H + /K + -ATPase. A trace amount of this proton pump is also found in the renal medulla. In the resting unstimulated state, the enzyme is found in cytoplasmic membranous structures. When there is gastric acid secretion mediated stimulation, the enzymes undergo morphological transformation leading to their shifting to the microvilli of the extended canaliculus of the parietal cells [35]. Once the enzyme relocates to the canaliculus, it becomes associated with K^+ and Cl − conductance and release acid on account of the interchange of cytoplasmic hydronium ion (H^+) with extracellular potassium ion (K^+). This exchange is coupled with cytoplasmic ATP hydrolysis. Histamine is the paracrine stimulator of the proton pump. After binding to its cognate histamine H2 receptors, it stimulates the activity of the proton pump [36]. Acetylcholine and gastrin, are the other two stimulators that have been reported to regulate gastric acid secretion [37]. Moreover, this exocytosis was partly observed to be associated with the functioning of the SNARE proteins expressed in the gastric glands. Proteins like SNAP-23, SNAP-25, Syntaxin were observed to be predominantly expressed in the enterochromaffin-like cells (ECL) of the gastric gland compared to the parietal cells. On the other hand, Rab 11a, a few of the clathrin components and dynamin were found to be overexpressed in the parietal cells. SNARE protein expressions were low in parietal cells yet they were expressed in ECL indicated towards the fact that the products of those genes were functional. Also, the overexpressions of clatherin and dynamin in the parietal cells suggested a cycle that goes on with inverted tubulo-cisternae and the everted microvilli causing a fusion mechanism restricted enough and different to some extent from general exocytosis.

This H^+/K^+-ATPase catalyzed enzymatic reaction is the decisive factor governing gastric acid secretion [38]. Surplus secretion of gastric acid results in different kinds of gastric ulcer-related diseases. The integrity of the gastric mucosal barrier gets damaged on account of gastric ulcer formation.

12.4 Efficiency of proton pump inhibitors to treat gastric ulcers

The H^+/K^+-ATPase plays an indispensable part in the final common phase of acid release, indicative that inhibition of this proton pump, would be a reliable method in curbing secretion of gastric acid. Proton pump inhibitors (PPIs) are a group of medications that irreversibly get attached to the H^+/K^+-ATPase, hence preventing the hydrogen ions to get escorted into the stomach lumen [39]. Timoprazole became the first effective medicine and was introduced in clinical use in the year 1975. Timoprazole was succeeded by intravenous omeprazole, and lastly by the S-enantiomer form of omeprazole [40]. Gastric H^+/K^+-ATPase is inhibited by these drugs through irreversible covalent binding with the proton pump [41]. PPIs, weak bases in nature can accumulate in the acidic space located in the secretary portion of the parietal cell which gets stimulated for acid secretion. During the secretion of the acid, when the pH falls to somewhere around 1.1, the concentration of the PPIs in an acid-space-dependent manner is one of the primary exigent properties which is responsible for the determination of the therapeutic index of the drug. Generally, the PPIs in the luminal surface of the pump create a concentration that is about a thousand times more than that in blood.

After the initial concentration increase, the pH-dependent formation of the activated drug occurs through a subsequent protonation forming disulfides with readily accessible cysteines of the H^+K-ATPase. Amongst all the PPI's available, tenatoprazole is the most stable PPI.

The PPIs discussed above have demonstrated significant clinical achievements and are presently the backbone of treatment of all acid-related ailments of the gastrointestinal tract. Although these drugs are effective but considerable incidence of side effects limit their clinical administration [42]. Administration of PPIs for a long-term period or with a high dose may cause downregulation of stomach acid generation, which serves as a potent host defensive arsenal. This suppression of stomach acid may augment the possibility of gastrointestinal infection. Many other adverse effects of PPIs have also been reported with prolonged intake, such as the formation of polyps and impaired absorption of certain trace elements like magnesium, calcium, and vitamin B12 [43]. Hence, natural products with better effectiveness and safe profiles are needed as alternatives to chemical medications.

12.5 Pumilacidin: its role in the control of gastric ulcer

In 1989, Nobuaki Naruse and his coworkers reported that a bacterial strain M937-B1 from a soil sample near Lake Yamanaka was isolated; the strain was unraveled to produce a complex of new acylpeptide antibiotics, pumilacidin [8]. By taxonomical studies, the producing strain was identified as *Bacillus pumilus*, an aerobic, Gram-positive, spore-forming oblong bacterium. The antibody component was extracted and was separated into the seven different components of pumilacidin by HPLC chromatographic technique. Pumilacidin components were isolated as amorphous white powders and all of the seven components are soluble in lower alcohols, chloroform, acetone, ethyl acetate, and dimethyl sulfoxide, but insoluble in hexane and water. Chemical and spectral analysis of their structure revealed cyclic acyl heptapeptide antibiotics that are composed of a β-hydroxy fatty acid and certain amino acid residues. Pumilacidins A and B exhibited antiviral activity against HSV-1 (herpes simplex virus type1) as assessed by dye uptake assay and plaque reduction assay.

To assess H^+/K^+-ATPase inhibitory activity, gastric H^+/K^+-ATPase was prepared from hog stomachs. 7-nitrophenylphosphate (PNPP), which is known to be hydrolyzed by gastric H^+/K^+-ATPase was used as a substrate. Four known acylpeptide antibiotics and two known H^+/K^+-ATPase inhibitors, omeprazole and SCH28080, were comparatively tested along with pumilacidins. Both pumilacidins A and B inhibited hog gastric H^+/K^+-ATPase in a concentration-dependent manner. Pumilacidins A and B showed much more potent inhibitory activity than the known acylpeptide antibiotics. Moreover, these A and B variants were approximately four times more effective than either omeprazole or SCH28080.

The antigastric ulcer activity of pumilacidin B was evaluated in the Shay rat model of gastric ulcer. Male Wister rats of approximately 200 g weight were made to fast for 24 hours before the experiment. Under anesthetic condition, the abdomen of the rat was incised and the pylorus was ligated. After 10 hours, the animals were sacrificed and the forestomach was examined for ulcer formation. Soon after pylorus ligation, the test compound was administered subcutaneously in a volume of 0.1 mL/100 g bodyweight of the rats. 1 mL of 1% Brilliant Blue 6B was administered intravenously 10 minutes before killing the rats to stain the ulcer area. Shay ulcers were examined

to be significantly inhibited by 68% when 100 mg/kg of pumilacidin B was administered to ulcerated rats. Thus, the bio-surfactant pumilacidin was not only found to suppress gastric H^+/K^+-ATPase activity but also prevented gastric ulcer formation in in vivo ulcerated rat models [8].

Though pumilacidin has promising roles in the medical field, however, its high cost prohibits its applications requiring large quantities. For reducing the cost, surfactin was produced in solid-state fermentation (SSF), using agro-industrial residues as substrates. Slivinski et al. produced pumilacidin, from *Bacillus pumilus* (Brazilian strain UFPEDA 448), by the process of SSF, by using okara together with sugarcane bagasse [44].

12.6 Conclusion

The myriad biophysical traits and the dynamic structure of biosurfactants have gained attention in recent years. They have an increasing demand in different industries, from medicine and cosmetics to agriculture and petroleum. They exhibit antibacterial, antifungal, antiviral, antitumor, and potent immunomodulatory properties. They are arising as a suitable alternative to synthetic chemical surfactants. They can also be used as adjuvants in vaccines and gene transfection. Though their biomedical prospect is promising, the application of biosurfactants is still restricted; the probable reasons are: firstly, production of biosurfactants is expensive, and secondly, lack of sufficient information about its toxicity on humans. Nevertheless, the roles demonstrated by biosurfactants make them promising new candidates for the development of safe and effective therapeutic agents not only against gastric ulcers but also against other acid-related diseases.

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CHAPTER

Applications of biosurfactants as nonpyrogenic and nontoxic immunologic adjuvants

13

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13.1 Introduction

Biosurfactants belong to a diverse group of surface-active molecules that have unique chemical characteristics. They are of microbial origin and range in size from 500 to 1500 Da [1,2]. They are first discovered in the 1660s and from that time they were widely accepted as an effective alternative to their synthetic conventional counterparts [3]. Biosurfactants possess many advantages that mark their superiority over synthetic or chemical surfactants. Biosurfactants have higher environmental sustainability and are stable over a wide range of temperatures and pH. Moreover, they are highly biodegradable, less toxic, and are easy to produce [4]. All these characteristics make biosurfactants a potential candidate for use in various fields therefore from the last few decades' biosurfactants applications have been greatly extended throughout the world [5,6]. Biosurfactant molecules possess both hydrophobic and hydrophilic moieties and are amphipathic. They also play an important role in the survival of microorganisms from which they are derived by regulating various key cellular processes [7]. Being amphipathic molecules, they reduce the interfacial tension between two interfaces having varying degrees of polarity and hydrogen bonding. Due to diverse chemical characteristics, they are capable enough of reducing the interfacial tension between immiscible liquids and solid-liquid interfaces and act as bio-emulsifiers under certain conditions [8]. Due to such characteristics, they play a key role in various processes like emulsification, de-emulsification, wetting, forming, polymerization, and phase dispersion [9]. Moreover, from the biological point of view biosurfactants have a wide range of applications as they increase the bioavailability and solubility of poorly soluble hydrophobic substrates by increasing their surface area. They promote bacterial pathogenesis by facilitating heavy metal binding, biofilm formation, and quorum sensing [10,11].

13.2 Biological and therapeutic role of biosurfactants

Biosurfactants possess an immense therapeutic potential and their unique physical, chemical and biological characteristics make them enable to be used widely in pharmaceutical industries for

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various purposes. Biosurfactants exist in a great variety of chemical structures depending upon the species and strains of microorganisms from which they are derived. Broadly speaking biosurfactants are mostly glycolipid and lipopeptide in nature [12]. Studies proved the therapeutic role of biosurfactants as an antimicrobial agent as they disrupt the integrity of lipid bilayer by inducing pore formation in the plasma membrane. In this way, they not only toxify the microbial plasma membrane but also alter its permeability for various substances [13,14]. The antiadhesive property of biosurfactants enables them to restrict the growth of biofilm formation on catheters and other medical equipment used in hospitals. In this way, they significantly reduce the incidence of hospital-acquired infections without the use of antibiotics or any other chemical agents [15]. Biosurfactants because of their biological origin, possess less toxicity and are considered safe and suitable alternatives to their chemical counterparts and even antibiotics in some cases. Moreover, experimental data suggest that more potent and therapeutically effective biosurfactants can be produced by genetically altering the biosurfactant producing genes which can be used as a good alternate of antibiotics in an era where rapidly emerging antibiotic resistance is becoming a global crisis. Biosurfactants derived from Lactobacilli are used in probiotic preparations and play an important role in eradicating pathogenic colonization and combating various urogenital tract infections [10]. Lipopeptide-based biosurfactants are widely used as immunological adjuvants when mixed or covalently attached with conventional antigens which otherwise are not sufficient to produce a significant immune response. By acting as ligands, biosurfactants bind with immunoglobins and play an important role in stimulating immunomodulatory actions which overall boost the body's immune response. Glycolipid-based biosurfactants also modulate the signal transduction process by altering the properties of cAMP, PLA2 type molecules [16]. Biosurfactants such as MEL-A play a critical role in increasing the efficiency of liposome-mediated gene transfection and lipofection which is generally regarded as the most promising way of transferring a foreign gene to the target cells without showing any complications and adverse effects throughout the whole process [17,18].

13.3 Immunomodulatory role of biosurfactants

Glycolipid-based biosurfactants are among the most common types of surfactants that possess good immunomodulatory activity. They can be isolated from a variety of microorganisms particularly bacteria and are chemically composed of complexes of carbohydrates and long chains of fatty acids. Carbohydrate complexes and chains of fatty acids are linked with each other through ester bonds. So far, many glycolipids and lipopeptide-based biosurfactants like rhamnolipids (Table 13.1), trehalolipids, sophorolipids, mannosylerythritol lipids (MELs), WH1fungin, etc. have been identified and isolated from many different microorganisms. Studies showed that these biosurfactants are responsible for regulating key immunomodulatory functions and have a wide range of therapeutic applications [37].

Rhamnolipids are produced by various strains of pseudomonas and are basically bacterial endotoxins. Despite having good immunomodulatory activity, they also act as soldier molecule and plays a critical role in the survival of *Pseudomonas aeruginosa* in the host [38]. In addition to this, many studies demonstrated several other immunomodulatory roles of rhamnolipids like

Table 13.1 Applications of important biosurfactants.

Bio surfactant	Origin	Applications	References
Rhamnolipids	*Pseudomonas aerugenosa*	• Antimicrobial activity • Antiadhesive activity • Immunomodulatory activity • Immune restoration activity	[19–22]
Sphorolipids	*Candida bombicola* *C. bogoriensis* *C. apicola* *Yarrowia lipolytica*	• Potent Immunomodulatory agent • Anticancer activity • Antiviral, antibacterial, antimycoplasmic activity	[13,23–26]
Mannosylerythritol Lipid (MEL)	*Candida antartica*	• Antimicrobial activity • Neurological and immunological activity	[27–29]
Trehalose lipid	*Rhodococcus erythropolis*	• Antiviral activity • Immunomodulatory activity	[30–32]
WH1Fungin	*Bacillus amyloliquefaciens*	• Immunomodulatory activity • Adjuvant properties	[33–35]
Emulsan	*Acnetobacter calcoaceticus*	• Adjuvant properties	[36]

histamine release from the mast cells, increasing the oxidative responses, increasing the production of serotonin (5-HT), and 12-hydroxyeicosatetraenoic acid (12-HETE) from human platelets [39]. Furthermore, rhamnolipids negatively regulate macrophage activity by affecting the internalization process and inhibiting the fusion of phagosome and lysosomes within the macrophage [40]. Another biosurfactant sophorolipid obtained from different strains of yeast possess both immunomodulatory and emulsifying properties. Sophorolipid contains a dimeric carbohydrate called sophorose that is linked with long-chain fatty acid via glycosidic linkage [41,42]. Bluth and his colleagues showed that sophorolipids can act as a potent immunomodulatory agent because of their observed antiinflammatory activity in several animal models. Therefore, they proposed that sophorolipids can act as novel antiinflammatory agents for the cure of diseases like asthma and atopic eczema. Sophorolipids are capable of improving the survival rate of animals suffering from sepsis by significantly lowering the production of nitric oxide and proinflammatory cytokines [23].

Trehalolipids comprise a large group of biosurfactants derived from different genera of actinobacteria like *Rhodococcus*, *Nocardia*, *Godornia*, *Corynebacterium*, etc. They are generally composed of nonreducing sugars chemically linked with long-chain fatty acids [37]. Trehalolipids derived from *Rhodococcus ruber* are considered to be the most potent biosurfactants among all other glycolipid biosurfactants. Trehalolipids derived from Rhodococcus usually occur in the form of complex chemical mixtures. They can either be in the form of trehalose mono/di/tri-mycolate or acylated derivatives of trehalose. Due to their structural diversity, they possess less toxicity and good immunomodulatory activity [43]. These trehalose complexes induce the formation of IL-1 and TNF-α in monocytes without showing any stimulatory effect on IL-6. Whereas, in

mononuclear lecocytes, it activates the production of both IL-1, TNF-α as well as IL-6 [44]. Although it is not certain how trehalolipids show their activity because the actual mechanism of their biological activity is still not known. However, some studies showed that trehalolipids interact with different components of the mammalian cell membrane, such as phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine, and distort the overall structural integrity of the cell membrane. Consequently, such interactions lead to apoptosis and disruption in cell signaling [44].

J. Asselineau carried out the first detailed study of trehalose di-mycolate (TDM) in the 1930s and reported its presence in the cell wall of Mycobacterium tuberculosis [45,46]. But later researches showed that TDM is also present in other pathogenic microorganisms belonging to the genera *Nocardia* and *Corynebacterium* [47]. TDM isolated from mycobacterium seems to have a good immunostimulatory activity because it induces the production of nitric oxide, chemokines, and cytokines such as IL-4, IL-6, IL-10, IL-12, IFN-γ, TNF-α, promote granuloma formation, initiate angiogenesis, and help in annulling tumor [48]. Due to the relatively small molecular size of TDM, studies revealed that it shows granulomatogenic activity in the vital organs of the animal models used for experimentation [43]. TDM induces the pro-coagulating and chemotactic activity of macrophages by stimulating chemokines and pro-inflammatory cytokines [48].

MEL acts as a biosurfactant and shows a wide range of biological activity. It is also known as a yeast glycolipid biosurfactant and is extracted from vegetable oils produced by Candida strains. Studies showed that MEL possesses very good immunological, antimicrobial, and neurological activity. In addition to glycolipid-based biosurfactants, some other molecules of microbial origin are also discovered which possess good immunologic adjuvant activity. WH1fungin and Emulsan derived from *Bacillus amyloliquefaciens* and *Acinetobacter calcoaceticus*, respectively, act as good immunologic adjuvants and enhance the production of antigen-specific antibodies when combined with suitable antigen [49].

13.4 Biosurfactants and immunologic adjuvants

Immunologic adjuvants are substances that usually enhance the strength and duration of the antigen-specific immune response within an organism when given in combination with specific antigens. The immune response can either be humoral or cellular depending upon the type of antigen with which immunologic adjuvant is attached [50]. Immunologic adjuvants elicit antigen-specific immune response via several different mechanisms and utilizing different chemical pathways like activating chemokines and cytokines, utilizing depot effect which allows sustaining release of antigen at the injection site, increasing the uptake of antigen and its presentation on the antigen-presenting cells (APCs), increasing the expression of major histocompatibility complex (MHC), and activation of inflammasomes [51]. However, to elicit the immune response to the desired extent, immunologic adjuvants should possess good efficacy with minimum toxicity and adverse effects like induction of allergic and autoimmune reactions [52]. Conventional immunologic adjuvants like alum, monophosphoryl lipid A, CpG oligonucleotides, saponins, etc have many limitations and side effects. Therefore, the use of biosurfactants as immunologic adjuvants is becoming a popular idea because it overcomes most of the shortcomings of conventional adjuvants and is capable of stimulating both humoral and cellular immune responses effectively. Because of

their biological origin biosurfactant-based immunologic adjuvants show several advantages over their chemical counterparts like good stability, high biodegradability, low toxicity, chemical and biological diversity which make them suitable for use in various applications [39].

13.5 Applications of biosurfactants as immunologic adjuvants

Lipopeptide biosurfactants of bacterial origin possess good immunomodulatory activity and act as potent immunologic adjuvants. Haptens having low immunogenicity are usually mixed or combined with immunologic adjuvants to give high titers of antigen-specific antibodies. To elicit a good immune response, mostly low molecular weight antigens are coupled with suitable carriers like poly-L-Lysine (PLL), bovine albumin (BSA), keyhole limpet hemocyanin (KLH), etc before mixing and binding with immunologic adjuvants [53].

Lipopeptide *N*-palmitoyl-S-[2,3-bis(palmitoyloxy)-(2RS)-propyl]-(R)-cysteinyl-serine (P_3CS) act as good immunologic adjuvant when coupled with T_h-cell epitope. Studies proved that when P_3CS-T_h conjugates bind with low molecular weight antigens like Microcystin (MLR), Herbicolin A, and Iturin A, they significantly enhance the immune response either by activating T_h-lymphocytes which then produce lymphokines or by protecting antigens from proteolytic degradation and renal secretion. Toxic drug microcystin along with PLL which act as a carrier (MLR-PLL conjugate) significantly increases the production of antigen-specific antibody-secreting hybridomas in the presence of P_3CS-T_h. Similarly, low molecular weight cyclic peptides herbicolin A and iturin A also produce a good humoral immune response when combined with immunologic adjuvant P_3CS and T_h-cell epitope. P_3CS-T_h mediated immune response produces B-lymphocytes which are further used for preparing monoclonal antibodies [39,53].

Immunoprecipitating antibodies are also obtained when an oligopeptide segment of epithelial growth factor receptor (EGRF) which acts as nonimmunogenic hapten is coupled with nontoxin and nonpyrogenic immunologic adjuvant P_3CS. Peptide-specific antibodies thus produced are also recognized by native EGFR [54,55].

Similarly, another lipopeptide *N*-palmitoyl-S-[2,3-bis(palmitoyloxy)-(2RS)-propyl]-(R)-cysteinyl-seryl-serine (P_3CSS) seems to show good immunologic adjuvant activity when coupled with viral envelop protein gp160 of HIV-1 containing both B- and T_h-cell epitopes. Along gp160 peptide is nonimmunogenic and is unable to induce a humoral immune response. However, when covalently coupled with P_3CSS it elicits a marked humoral immune response without requiring binding of any additional carrier protein [56].

Another important lipopeptide analog *N*-palmitoyl-S-[2,3-*bis*(palmitoyloxy)-(2RS)-propyl]-(R)-cysteinyl-seryl-$(lysyl)_3$-lysine (P_3CSK_4) significantly enhance the immunogenicity of melittin which is the major pain-inducing component of bee venom. When synthetic melittin is covalently linked with P_3CSK_4 and T_h cell epitope it elicits the production of melittin-specific antibodies. The antisera thus obtained not only recognizes respective eliciting peptide but also native melittin. This technique is further extended in the preparation of vaccines against mushroom toxins like phalloidin and amanitin, influenza virus, and lethal *Salmonella* infections [57,58].

WH1fungin, a novel biosurfactant derived from gram-positive bacterium *B. amyloliquefaciens* reported having a good immunoadjuvant activity. When combined with antigens, WH1fungin

markedly enhance both Th1 and Th2 mediated immune response after subcutaneous or intramuscular immunization. WH1fungin exerts its immunoadjuvant activity mainly by inducing the accumulation of reactive oxygen species (ROS) which then initiates the immune response. ROS at low concentration usually acts as a secondary messenger for initiating the immune response, therefore, ROS accumulation at low concentration leads to the activation of APCs via the upregulation of specific markers like MHCI, MHCII, CD80, CD86, and cytokines. Characteristics like chemical stability towards the tissue, low toxicity, and high potency make WH1fungin a novel immunologic adjuvant in the field of vaccines [59].

Another biosurfactant emulsan, which is a complex extracellular polysaccharide derived from Gram-negative bacterium *A. calcoaceticus*, possesses good emulsifying and immunomodulatory activity. Studies showed that emulsan can induce the activation of macrophages in a dose-dependent manner. When combined with antigens, a significant increase in the production of antigen-specific antibodies is observed. This immunopotentiation property of emulsan makes it a novel candidate as an immunologic adjuvant [36].

13.6 General mechanism of immunologic adjuvant activity

The main purpose of vaccination is to elicit an antigen-specific innate immune response within an individual. However, most of the time the innate immune response thus produced is not sufficient to produce protective immunity. Therefore, specific immunologic adjuvants are extensively used which significantly enhance the antigen-specific innate immune response when administered in combination with antigen [60]. Despite the extensive use of immunologic adjuvants in vaccines, the exact mechanism by which they enhance protective immunity is still unknown. But recent discoveries in the field of immunology revealed several mechanisms by which immunologic adjuvant elicit an antigen-specific immune response. Studies showed that immunologic adjuvants can utilize either one or more mechanisms or their combination while performing their immunologic activity [61]. Immunologic adjuvants can either produce a depot effect by gradually releasing the antigen from the injection site or they can upregulate the release of chemical mediators like chemokines and cytokines (Fig. 13.1). Some immunologic adjuvants also play part in the recruitment of specific immune cells and uptake of antigens and their presentation to APCs while others show their activity by promoting the antigen transport via APCs to draining lymph nodes. [51,62]. Depending upon the type of immunologic adjuvants and the respective mechanisms utilized while performing an immunologic activity, they can alter the quantity and quality of immune response to a great extent [63]. A brief description of some of the mechanisms utilized by immunologic adjuvants are as follows:

13.6.1 Sustain release of antigen from injection site

Many adjuvants show their activity by forming a depot at the injection site thus allowing the release of antigen in a sustained manner. Once depot formation was recognized as the oldest and classical mechanism of adjuvant activity as adjuvants successfully produce high antibody titers using this mechanism of action [64,65]. This mechanism was first observed while studying the

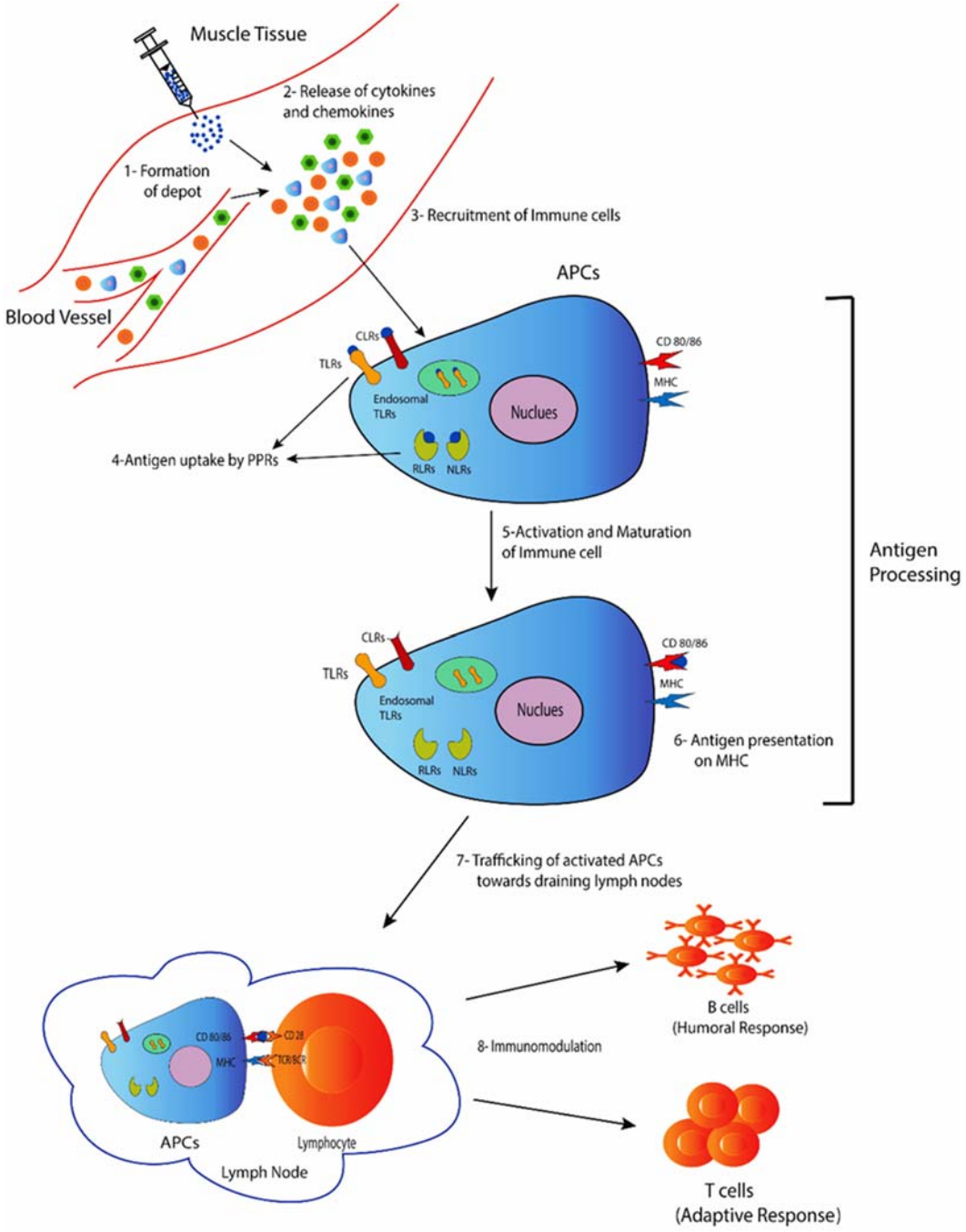

FIGURE 13.1 General mechanism of immunologic adjuvant activity.

Immunologic adjuvants can either produce a depot effect by gradually releasing the antigen from the injection site or they can upregulate the release of chemical mediators like chemokines and cytokines. Some immunologic adjuvants also play part in the recruitment of specific immune cells and uptake of antigen and their presentation to APCs while others show their activity by promoting the antigen transport via APCs to draining lymph nodes.

biological activity of alum as an adjuvant where the antigen attached with alum was found in a detectable concentration for about 2−3 weeks after the administration in the alumina-gel induced granulomas [66,67]. Studies showed that strong electrostatic interactions between alum and antigen play an important role in producing high antibody titers by enhancing the antigen uptake and its presentation via APCs [68]. Many other adjuvants like complete Freund;s adjuvant, biodegradable micro- and NANOparticles as well as water in oil emulsions shown to act by this mechanism. Alum in combination with monophosphoryl lipid A (MPL), when colocalized with antigen, produces an optimum immune response by utilizing this mechanism of depot formation [69]. Similarly, another cationic adjuvant formulation comprised of dimethyldioctadeclammonium (DDA) and trehalose-6,6-dibehenate (TDB) was also thought to show its activity by producing a long-lasting depot effect [70].

13.6.2 Upregulation of cytokines and chemokines and cellular recruitment of immune cells

Many immunologic adjuvants work by creating a local pro-inflammatory environment that facilitates the recruitment of immune cells. Mosca along with his colleagues showed that many immunologic adjuvants are capable of modulating the activity of cytokines, chemokines, interferon-induced genes, innate immune receptors, and genes encoding adhesion molecules [71]. All these factors are collectively responsible for recruiting various immune cells at the site of injection where some immune cells carry the antigen toward draining lymph nodes. At lymph nodes, antigen induces specific immune responses and stimulates the production of antigen-specific antibodies [72]. For example, when alum is used as an immunologic adjuvant it stimulates the production of chemical mediators like CCL2, CXCL1, and CCL11. These chemo-attractants then induce the infiltration of immune cells particularly neutrophils and eosinophils [73]. Studies showed that alum induces a Th2-type immune response via signaling either through interleukin-25 or interleukin-6. However, the complement cascade thus activated as a result of such signaling is responsible for creating a pro-inflammatory environment and recruitment of immune cells [74]. Likewise, Jordan and his colleagues showed that alum is responsible for the priming of B-cells via stimulation of interleukin-4 producing Gr1C cells. This alum-induced priming of B-cells is very important for the proliferation of antigen-specific B-cells and the consequent production of antigen-specific antibodies [75]. Intra-peritoneal injection of vaccine containing alum as an immunologic adjuvant induces the rapid recruitment of inflammatory monocytes. These monocytes then take up the antigen and undergo differentiation into CD11cC MHC class IIC DCs and migrate toward lymph nodes where they stimulate the production of T-cells specific to the antigen previously taken up by the monocytes [76]. Another immunologic adjuvant ASO4, upon administration, induces cytokine production and transient local NFkB activity. A component of ASO4 called MPL which is a TLR4 agonist stimulates the production of monocytes and DCs at draining lymph nodes [69]. Similarly, cationic liposomes such as DDA/MPL, when injected intraperitoneally induce the influx of monocytes, neutrophil macrophages, and natural killer cells into the peritoneal cavity. Another cationic liposome-based immunologic adjuvant CAF01 increases the recruitment of immune cells particularly monocytes at the injection site and facilitates their trafficking toward draining lymph nodes [70].

13.6.3 Increase antigen presentation on antigen-presenting cells

Many immunologic adjuvants work by increasing the extent of antigen presentation on APCs through the activation of MHCs. This plays an important role in the induction of a good adaptive immune response in the presence of antigen. Adjuvants like alum, biosurfactants, and oil emulsions mainly act by channeling antigens to the APCs and thus increase their uptake by DCs and ultimately alter the magnitude and duration of antigen presentation [77]. Adjuvants usually facilitate the process of internalization of antigen by interacting with membrane lipids. In the case of alum, the antigen is adsorbed on the alum surface and alum interacts with membrane lipids and causes activation of PI3 and recruitment of ITAM containing molecules. All these events ultimately lead to internalization of antigen, activation, and maturation of DC, and enhanced expression of MHC class II [78]. Ghimire and his colleagues showed that in addition to increasing the rate of internalization, alum also plays a critical role in decreasing the degradation rate of antigens within the human body [79]. Moreover, the size of the antigen is also very important in determining the overall efficacy of the vaccine, because the extent of antigen presentation directly depends on the size of the antigen. The extent of antigen presentation largely depends on the size of lipid vesicles containing the antigen. Large size lipid vesicles usually undergo early phagocytosis and cause increased antigen presentation while small size lipid vesicles localize more rapidly and undergo late phagocytosis that ultimately results in decreased antigen presentation [80].

13.6.4 Dendritic cells activation and maturation

Activation and maturation of DCs is a crucial step in the activation of the proper immune response. Enhanced expression of MHC class II and induction of CD83 and CD86 markers play a vital role in producing a significant immune response by enhancing the ability of APCs to stimulate the activation and differentiation of T lymphocytes [81]. Experimental studies on human specimens showed that both MF59 and alum can not stimulate DCs directly, rather than that they increase the expression of MHC class II mediators. Moreover, they also stimulate the activation marker (CD86) and maturation marker (CD83) on various immune cells which ultimately leads to increase proliferation of T cells [82]. Members of a new class of immunologic adjuvants that show their activity in a TLR-independent manner can directly activate DCs via FcgR-Syk-Card9-Bcl10-Malt1 pathways and concomitantly up-regulate the expression of stimulatory molecules like CD86 and CD83 [83]. However, some cationic-based liposomes (DOTAP) induce the activation and maturation of DCs via induction of MAPK while others (diC14-amidine) induce the activation of DCs by up-regulating the expression of CD80 and CD86 via TLR4/MD2 ligation [84].

13.6.5 Inflammasomes activation

Immune cells usually possess several types of pathogen-recognition receptors (PRRs) and their primary function is to recognize any foreign particle or infectious agent that enters the body. Extensive research on PRRs in recent years leads to the discovery of many new families of PRRs. TLRs, CLRs, NLRs, and RLRs are some of the important families of PRRs. Many immunologic adjuvants work by signaling through various PRRs to produce an antigen-specific immune response. However, some adjuvants still can induce an immune response without being recognized

by specific PRRs. In 1994 Matzinger proposed the "Danger Hypothesis" according to which danger signals produced as a result of tissue damage are responsible for activating the body's self-defense mechanism and induction of immune response [85]. Molecules associated with tissue damage serve the role of noninfectious damage signals and are commonly recognized as damage-associated molecular patterns (DAMPs). These are entirely different from pathogen-associated molecular patterns (PAMPs). While administration, adjuvants cause local tissue damage at the site of injection and the damage signals thus produced as a result of such tissue damage stimulate the nonspecific innate immune response which then produces adaptive immunity [86]. Inflammasomes mainly belong to the NLR family and include receptors such as NODs, NLRPs, MHC II trans-activator (CIITA), etc., and are extensively studied due to their role in stimulating immunologic adjuvant activity. Among these, the NLR family member, NLRP3 is the most studied receptor of this class which acts as an inflammasome receptor and is involved in the adjuvant activity. NLRP3 inflammasomes are usually activated via signals from DAMPs, PAMPs, environmental irritants, and metabolic stress. They also show their activity by inducing caspase-1 which later cleaves proforms of interleukin-1b, -18, and -33 to their active forms [87]. Studies showed that inflammasomes are involved in adjuvant-mediated cellular recruitment immune cells and ultimately in activating innate immunity. Experimental data revealed that the immunologic adjuvant activity of alum and MF59 is linked with inflammasomes but the actual mechanism of their activity is still unknown. Similarly, the role of inflammasomes in mediating adaptive immunity also remains elusive [88].

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CHAPTER

Antifungal activity of biosurfactant against profound mycosis

14

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14.1 Introduction

Mycoses are fungal infections that induce a wide range of diseases in humans. Two classifications are used for profound mycoses, namely subcutaneous infections and systemic infections [1–3]. The latter can be considered a type of endemic mycosis whose main agents are thermodimorphic fungi, that is, those that exist as molds at temperatures of from 25°C to 30°C, and as yeasts, at body temperatures. Regarding *Coccidioido mycosis*, the fungi as spherules-shaped of body temperatures [4]. Some of such diseases are endemic in the South and Latin Americas, mainly in Brazil, and recognized as important causes of morbidness and mortality, leading to serious complications in compromised patients [5]. Therefore, profound mycosis can be recognized as an opportunistic infection [2,6]. Costa et al. [5] claimed endemic mycoses occur in almost all Brazil, mainly due to the climate and the regions of high rainfall, high temperatures, and dense forests. Moreover, some economic and social distortions limit access to more effective health treatments for the poorest population.

In comparison to antibacterial agents, few therapeutically useful antifungal agents are found for treatments; both humans and fungi are organisms formed by eukaryotic cells, consequently, most substances that inhibit or kill fungal pathogens are also toxic to humans, thus hampering the development of a drug selectively toxic to fungal cells, rather than to the host [3,7]. Conventional treatments employ mostly, antifungal drugs such as azoles and polyenes [8], which act in the synthesis of ergosterol, a substance present in large quantities only in fungi cells, or forming pores in the cell membrane; however, the development of resistance to the agents is commonly observed [7,9]. In recent years, new substances have been investigated toward a better therapeutic response [7,10,11].

Among such substances are biosurfactants [12–15], a group of compounds that display a wide range of properties and functions, and, due to their origin from different microorganisms (bacteria, fungi, and yeasts), they are more environmentally friendly than surfactants obtained in a chemically

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synthetic way [16,17]. They can be classified according to their chemical composition into several classes, such as glycolipids, lipopeptides, polysaccharide−protein complexes, phospholipids, and fatty acids [14,16,18]. Due to their characteristics (e.g., low toxicity, diverse sources of obtaining, biodegradability, among others), their use has been studied toward different applications, including biotechnology and environmental aspects [19,20], and, more recently, in the medical field [13−15,21,22].

This chapter is devoted to the use of biosurfactants for treatments of profound mycoses. Some basic concepts related to mycoses and usual treatments are provided toward discussions on the state-of-the-art of this study area. Aspects related to the extraction and production of biosurfactants for medical applications, techniques for their characterization, and the important properties for their use as antifungal agents, as well as their application in systemic mycoses are also addressed.

14.2 Production of biosurfactants

Given the need for using surfactants in diverse industrial applications and their potential as medical-pharmaceutical, the study of their production and purification techniques must be addressed—the biosurfactants market is projected to reach US$39.86 billion in 2021 [23].

The production of biosurfactants can follow the route of synthesis from simpler components and the use of microorganisms—the latter are the most common and widely studied [24]. Some challenges in bioengineering related to the development of technologies and strategies for the production of biosurfactants include high costs and difficult purification—downstream process [25], common in alternative technologies that use microorganisms. They must be soon overcome, due to the sustainable need for those materials, and the physical and technological depletion of oil reserves.

The methodologies and strategies for the production of biosurfactants widely vary, depending on the type of product to be designed. Therefore, different microorganisms (e.g., bacteria and yeasts) [26], as well as genetic engineering technologies [27], such as the use of heterologous hosts and selected strains, experimental designs [28], and types of processes, can be implemented. However, industrial batch fermenters are commonly used, through the control of pH, temperature, oxygenation, and concentration of carbon and nitrogen sources. Cheaper substrates, such as agricultural and industrial residues, and by-products that decrease production costs can also be employed [24].

The use of those materials as substrates offers advantages and disadvantages. Among the disadvantages are the need for the preprocessing of both substrates and the product's impurities, given their by-product nature [29]. Studies of costs and processes are, therefore, required so that the most economical and simplified path can be found.

The following topics elucidate such points since they address advances in technologies and what has been consolidated regarding the production of biosurfactants, focusing on their three most prominent classes, namely lipopeptides, nucleolipids, and glycolipids (Figure 27.1).

Because of the focus of the study presented in this chapter, some main points on the production of biosurfactants must be highlighted, regarding the antimicrobial nature of these compounds. Biosurfactants that have excelled regarding antifungal and antibacterial actions are viscosinamide, surfactin, iturin, and rhamnolipids [30] (see Fig. 14.1 for some molecular structures). Some of such compounds are already produced and commercialized (e.g., biofungicidal rhamnolipids ZONIX™, from Jeneil Biosurfactant Company, USA [33]).

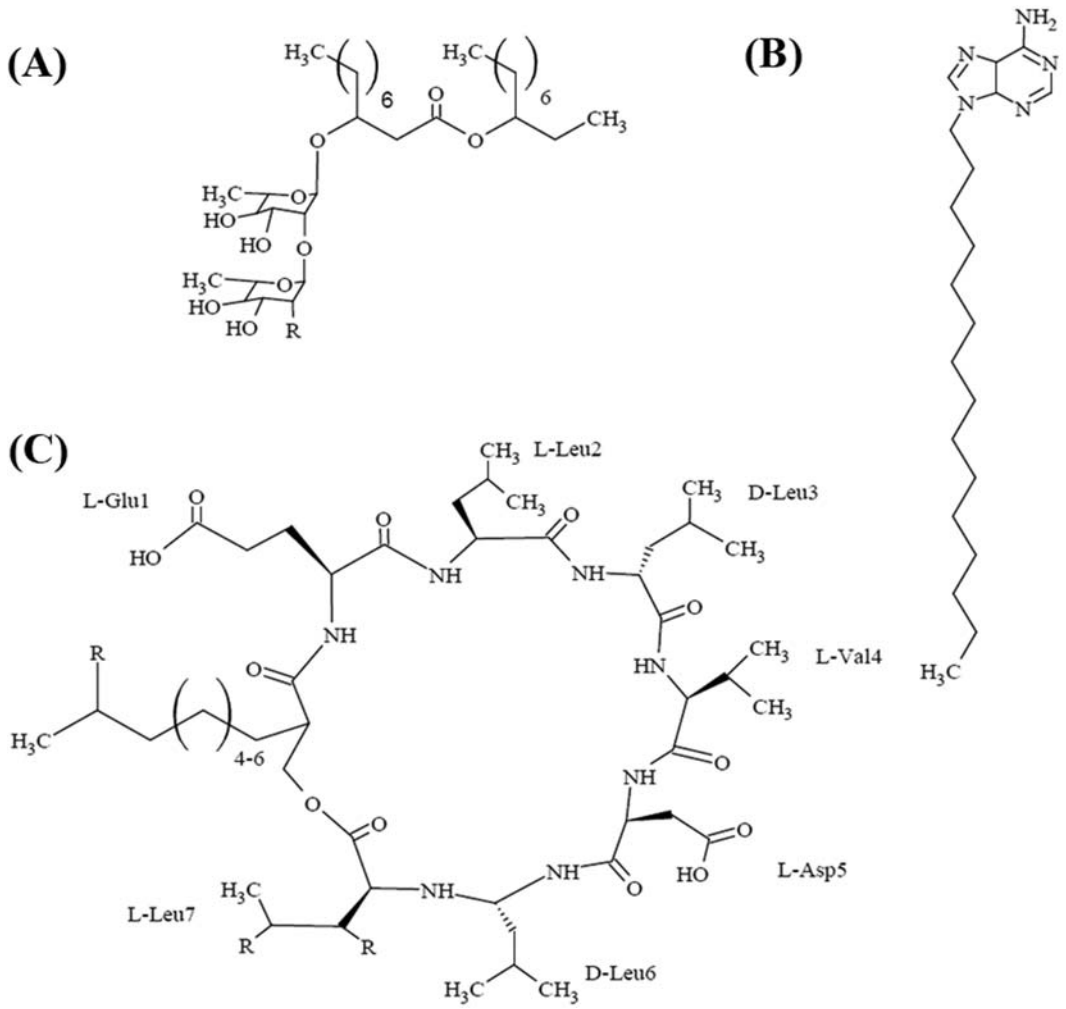

FIGURE 14.1

Molecular structures of (A) rhamnolipid, (B) nucleolipid, and (C) surfactin, biosurfactants with antifungal potential.

Adapted from I.M. Banat, A. Franzetti, I. Gandolfi, G. Bestetti, M.G. Martinotti, L. Fracchia, et al., Microbial biosurfactants production, applications and future potential, Appl. Microbiol. Biotechnol. 87 (2010) 427–444. https://doi.org/10.1007/s00253-010-2589-0 and B.N. Paulino, M.G. Pessôa, M.C.R. Mano, G. Molina, I.A. Neri-Numa, G.M. Pastore, Current status in biotechnological production and applications of glycolipid biosurfactants, Appl. Microbiol. Biotechnol. 100 (2016) 10265–10293. https://doi.org/10.1007/s00253-016-7980-z.

14.2.1 Metabolic pathways/biosynthesis and optimization strategies

Several strategies have achieved good production of biosurfactants. Kosaric et al. [34] established four factors for reducing production costs, namely selection of microorganisms, processes, the adaption of cheap substrates, and cheap processing of products. Decrease in water's surface tension and critical micellar concentration have gained prominence for the characterization of a given biosurfactant and can be used as parameters for a given application [24,29]. A tensiometer and a goniometer (contact angle) measure changes in surfaces and interfacial tensions—surfactin, for example, can decrease the water's surface tension from 72 to 27 mN/m [25,29].

Critical micellar concentration enables a biosurfactant to interact with an aqueous phase. In addition to more robust characterization techniques (described in Section 27.3), a critical micellar concentration and a decrease in water's surface serve as quality standards for the evaluation of the processing products [35].

Techniques that optimize the production of microbial biosurfactants based on the metabolic characteristics of the microorganisms involved (e.g., careful selection of strains [27] and use of genetic engineering) can be implemented. Carefully selected cell lines are cataloged and tested for the production of a certain type of biosurfactant. However, it requires the knowledge of biosynthetic routes and gene expressions for each different type of biosurfactant in each microorganism [36].

Production by genetic engineering techniques involves the implementation of mutations through gamma radiation and ultraviolet radiation, which provides mutant strains, and the development of multiomic analyzes toward more productive strains and genes responsible for this production [27,37]. A heterologous expression of rhamnolipids with the use of *Escherichia coli* and *Pseudomonas putida* was made in an attempt to improve the yield of the biosurfactant production [36]. This technique can be used due to pathogenic issues of *P. aeruginosa* species [38]. Despite the cost relationships, aimed at the need for sophisticated techniques and apparatus, these approaches are essential for the application of processes in the industrial production of biosurfactants, making them more viable and the safety production [37].

14.2.2 Industrial production of biosurfactants

Bioreactors fed in batch and continuous are generally used for the production of biosurfactants, and some cost issues can be pointed out. Continuous flow reactors are more economical and profitable [37]. Special reactors, such as BMBR membrane (bubble less membrane bioreactor), can also be used [37,38], and aerobic conditions are usually employed, although some studies present results contraries [39].

The use of this reactor in a continuous process for the production of lipopeptides from *Bacillus subtilis* resulted in up to 95% purity [40]. Dolman et al. [41] investigated the use of continuous systems and showed the main problems related to those processes were foaming, accumulation of viscous products, thus limiting the transfer of oxygen, and need for high inflows into the bioreactor (the economically limiting factors). Bator et al. [42] used ethanol as a substrate, pointing as exceptional in reducing foam, with mutant strains of the nonpathogenic bacterium *P. putida* KT2440, obtaining after 23 hours productivity of 5 g/L. Genomic sequencing was performed for detecting phenotypic changes in mutations in the adaptive evolution laboratory, hence, the optimal strain [42].

The growth of microorganisms for the production of the compounds in question can be mathematically described by the modified Gompertz equation, which is a nonlinear mathematical model of growth that describes the production of metabolites of microorganisms [43], and supplies parameters of specific growth rate and specific component productivity [36]. Like other metabolic reactions, the production of biosurfactants is affected by several factors that can either increase or inhibit productivity. Rahman and Gakpe [29] extensively analyzed salinity, pH, agitation, oxygenation, and temperature.

The pilot-scale/large-scale production of biosurfactants establishes a disadvantageous cost relationship with synthetic surfactants. Different strategies have aimed to overcome it at a biotechnological level (e.g., more efficient processes that employ optimization of fermentation conditions and parameters of purification, use of cheaper substrates, and high-performance microbial strains) [24].

The production of probiotics, such as bacteria of the *Lactobacillus* genus, can be interesting due to their low toxicity and by-products for human health mainly for therapeutic applications [30]. *Pseudomonas*, *Bacillus*, and *Candida* are the most studied genera for large-scale economic productions [38].

Solid-state fermentation and experimental designs that result in response surfaces have been employed for optimizing the production of biosurfactants [28,36,44]. A complete factorial (24) and Central Composite Rotational Design (CCRD) using concentrations of soy oil, nitrate, phosphate, and iron as critical components were applied for the production of rhamnolipids, obtaining response surfaces [36]. The maximum production of biosurfactants was determined by specific concentrations of those components.

A biosurfactant of antimicrobial capacity was identified in the ZDY2 strain of *B. aryabhattai* microorganism, and its production was 8% optimized by the response surface methodology in Yaraguppi and collaborators [44]. This biomolecule showed antimicrobial activity against *Salmonella typhimurium*, *E. coli*, *Micrococcus luteus*, *Sthaphylococcus aureus*, and *Candida tropicalis*. An infrared spectroscopy analysis revealed a lipopeptide of potential for pharmaceutical applications [44].

The pH control can be used in some cases—mainly in lipopeptides—for the control of micelization in both the production and application of such types of biosurfactants [25]. The elaboration of nucleic acids with lipopeptides can favor the stability of acids controlling pH. Therefore, different approaches can employ nucleic acids [25] as, for example, genetic vectors and drug carriers, and in gene therapies. Hydrogels have also been developed from nucleolipids to be potentially used in tissue engineering [45].

During the pilot/large-scale production of biosurfactants, the product of the fermentation media showed a mixture of molecules of close similarity to molecular structure and physicochemical properties [32]. Because of such an obstacle, some of those molecules were elaborated by synthetic routes for the production of biosurfactants to be implemented on a large scale [25].

Makovitzki et al. [46] produced a lipopeptide composed of only four amino acids linked to a fatty acid, and observed its good bactericidal and fungicidal characteristics through the cell lysis mechanism. In vitro and in vivo tests were conducted with *Botrystis cinerea* and *Chochliobolus heterostrophus* pathogens obtained potent fungicidal power with almost no damage to plant tissue. A synthetic route was used in the present study, given the simplicity of the biosurfactant molecule, which offers a range of possibilities for the production of such substances. Peptides were linked to N-terminations using a technique based on the flueronylmethocycarbonyl protecting group (FMOC), a type of mechanism that uses the 9-fluoerenylmethoxicarbonyl group subsequently purified by high-performance liquid chromatography (HPLC). Sha et al. [47] used a rhamnolipid from

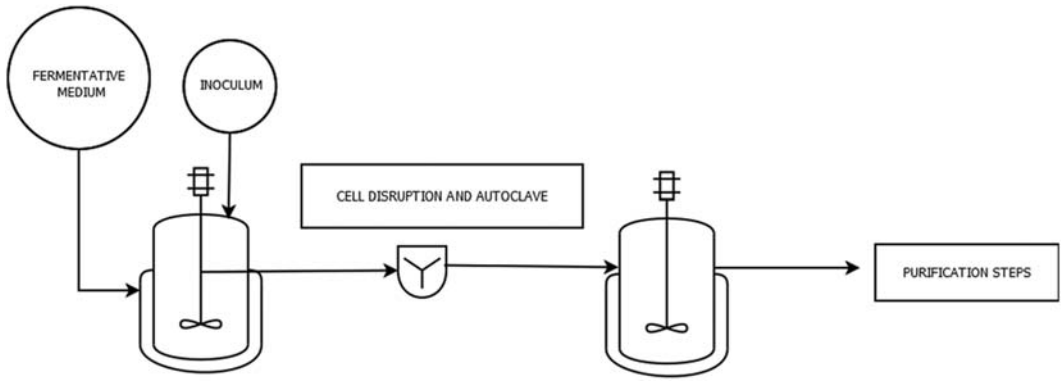

FIGURE 14.2

Experimental scheme of the two fermentation stages usually implemented in the production of biosurfactant.
Adapted from I.M. Banat, S.K. Satpute, S.S. Cameotra, R. Patil, N.V. Nyayanit, Cost effective technologies and renewable substrates for biosurfactants' production, Front. Microbiol. 5 (2014) 1–18. https://doi.org/10.3389/fmicb.2014.00697.

cultures of *P. aeruginosa* with free-culture cells in vitro and in vivo tests, which showed high fungicidal power mainly against the botanical pathogen of *Oomycetes* genus.

The production strategy that uses two fermentative stages with different organisms (or not) in each stage was adopted, as shown in Fig. 14.2. In the first fermenter, a first microorganism consumes the carbon source, thus resulting in biomass and other compounds that, when moving to the second fermenter, followed by cell disruption and autoclaving, serve as a growth substrate [24]. This substrate that contains the cell extract is rich in single-cell oil.

Helmy et al. [37] reviewed the production of some biosurfactants using the aforementioned technique and found high yields of up to 60% in productivity compared to those of simple fermentation. In this study, enzymatic syntheses are also cited, however, with more specific control of parameters, which results in technologically complex processing. Such syntheses can be used in the production of special compounds, such as cosmetics and drugs, since, in these cases, the purities of the products must be higher [37]. Singh et al. [23] highlighted the most promising strategies for a lower-cost and improved production of biosurfactants are the use of nanoparticles, an industrial coproduction with another bioprocess, and the use of bioreactors with films and rotating disks for product recovery, among other solid-state fermentation approaches.

Despite recent implementations and advances, few large-scale productions of biosurfactants have been observed - only has Jeneil Biosurfactant Co. (Saukville, Wisconsin) developed a line based on rhamnolipids with efficient strains of *P. aeruginosa*; the purification processes implemented can obtain rhamnolipid purity of up to 99%, with a 20,000-gallon capacity. It has been considered the most successful large-scale production of biosurfactants [37].

14.2.3 Low-cost substrates in the production of biosurfactants

Apart from the traditional substrates used (e.g., crude oil, diesel, vegetable oils, and glycerol [29]—the latter widely used [24,38]), some applications with agricultural and food residues [25,33] have

been studied, and even bolder approaches, such as those that employ engine oil, require costly downstream processes, despite their good conversions [31]. For example, some pressing cakes residues evaluated showed a lower amount of oleic acid [24].

Among the different types of substrates available for the production of amphiphilic molecules, some properties can be mentioned, since they are relevant for obtaining different biosurfactants. Within agro-industrial residues, a trend toward the use of agricultural residues from molasses of different sources has been observed mainly in countries with a strong agricultural economy, such as India and Brazil [24,48]. Animal fat has been predominantly applied for the production of sophorolipids [24], and combinations of carbon and nitrogen sources, such as protein concentrates, have been analyzed. In the dairy industry, whey and cheese are low-cost and relatively high-purity substrate options. Aqueous effluents, such as cereal washing water and aqueous dairy waste have been used and proven promising alternatives, despite the difficult removal of nonesthetic properties from the products [24].

14.2.4 Downstream processes in the production of biosurfactants

As addressed elsewhere, the values of conversion of substrates into products are generally low, even those that use more advanced optimization techniques, advanced extraction, and purification processes must be carried out for the obtaining of microbial biosurfactants [38]. Therefore, approximately 60%−80% of the processing costs of biotechnological products are estimated to be related to downstream processes [24].

Several purification and extraction processes can be implemented for the products of fermentation media and applied for the extraction of biosurfactants (e.g., solvent extraction, precipitation, crystallization, centrifugation, and foam fractionation) [38]. Advanced extraction and purification processes are required when industrial residues are used as fermentation media, due to the impurities they may contain. Among such techniques, simple gravitational separation has shown effective for product separation and recovery, as well as for increasing the bioprocess productivity [41].

Extraction with the use of organic solvents has been mostly implemented for the purification of biosurfactants [37], and among the solvents highlighted are mixtures of chloroform-methanol and dichloromethane-methanol. Despite resulting in good purity rates, such mixtures are toxic to human health and the environment [24]; therefore, solvents are more eco-friendly solvents, such as acetic acid, ethyl acetate, and methyl tertiary-butyl ether (MTBE) should be implemented.

Few studies apply continuous flow strategies for the purification of biosurfactants, and generally obtain products of inferior quality [37]. Dubey et al. [49] used several types of absorbents (silica gel, activated alumina, and zeolite) for the continuous removal of biosurfactants using activated carbon. For this specific case, a critical micellar concentration twice lower than that of the biosurfactants produced by the traditional downstream technique was observed.

Ferreira et al. [50] simultaneously extracted a biosurfactant and a bioemulsifier from *Mucorhiemalis*, isolated from the soil of the Brazilian *caatinga* biome, which was obtained under different cultivation conditions. A mixed fermentation was conducted under the first cultivation condition, thus obtaining a product of reduced surface tension of water (from 72 to 32 mN/m), and 96% emulsification index. A static cultivation condition was performed under the second cultivation condition with a biomolecule of 40 mN/m surface tension. The compounds found showed to be of glycolipids' nature [50].

Ekpenyong et al. [51] used artificial neural networks (ANN) and response surface analysis to optimize the production of glycolipopeptides: *P. aeruginosa*, controlled the conditions of temperature, pH, agitation, and duration of the process. The authors found 4.34 (Y_p/x) productivity under optimum conditions of 32°C, pH 7.6, agitation of 130 rpm, and 66 hours of fermentation. Glycolipopeptide showed antimicrobial, antibiofilm, and anticancer activity especially against MCF-7 and HeLa strains [51].

Conceição et al. [52] used *P. aeruginosa* for the production of rhamnolipids using bacterial cellulose membranes and residual corn bran oil, glycerol, and nutrient salts magnesium-based, sodium, and calcium; urea was employed as a nitrogen source. A two-level CCRD optimized the values of the amount of glycerol and aqueous extract of the corn bran residue and produced 15.8 g/L using such a solid fermentation apparatus. Extraction and quantification were performed with the aforementioned chloroform-methanol mixture, along with nuclear magnetic resonance spectroscopy [52].

A biosurfactant of antimicrobial capacity was identified in the ZDY2 strain of *B. aryabhattai* microorganism, and its production was 8%optimized by Yaraguppi et al. [44] through the response surface methodology. The biomolecule showed antimicrobial activity against *S. typhimurium*, *E. coli*, *M. luteus*, *S. aureus*, and *C. tropicalis*. An infrared spectroscopy analysis revealed a lipopeptide of potential for pharmaceutical application. Shatila et al. [53] studied the effect of carbon sources (glucose and glycerol), nitrogen ($NaNO_3$ and NH_4NO_3), and iron ions (Fe^{+2} and Fe^{+3}) on gene expression during the production of rhamnolipids from *P. aeruginosa* ATCC 15442. The rhlA, rhlB, rhlC genes were analyzed by PCR (polymerase chain reaction) technique. As a result of maximum expression of rhamnolipid synthesis genes, the preference for divalent iron ions was observed, in the generally low concentrations of glucose and $NaNO_3$ of 0.05 g/L. This is a study in the synthesis of biosurfactants, due to using the technique of gene expression as a factor in its production.

Studies on the optimization of microorganism processes and strains for the production of biosurfactants have been conducted. However, some properties necessary for sustainable and lower-cost production of biosurfactants from substances of high industrial and therapeutic potential are obtained with a set of data and tests.

14.3 Properties characterization of the biosurfactants

Many living microorganisms are known to generate an infinity of active compounds frequently found on cell walls or released in an intra- and extracellular way [54]. Surfactants or biosurfactants are produced from microbial or living microorganisms that display different morphological structures, physicochemical properties, thermal behavior, and antifungal activity [14]. Such biosurfactants can be classified into several groups, according to their physicochemical composition and mainly microbial origin, including glycolipids, lipopeptides, phospholipids, neutral lipids, fatty acids, and polymeric and particulate biosurfactants [55]. Korenblum et al. [56] claimed the main characteristics of biosurfactants directly contribute to their applications (e.g., biomedical, industrial, food, among others), and also highlighted lower quantities of biosurfactants are necessary for each application.

This section discusses studies developed toward a better understanding of the application of biosurfactants for treatments of systemic or profound mycoses, predominant in underdeveloped or

developing countries. Such understanding is based on the complete characterization of biosurfactants' physicochemical and structural properties, thermal behavior, microbiological or antifungal activities, and mechanical properties [17]. Some widely applied analytical techniques, such as FTIR, CA, TG/DTG, curves, SEM-TEM, LTC, GC-Ms are discussed in what follows.

14.3.1 Physicochemical and structural characterization

According to Sen et al. [57], several biosurfactants isolated from living microorganisms display distinct components and physicochemical structures (e.g., glycolipids, lipopeptides, polysaccharides, protein complexes, phospholipids, fatty acids, and neutral lipids); therefore, more in-depth knowledge of such specific characteristics is required.

Kaur et al. [58] evaluated the production of biosurfactants from living organisms isolated from the lateral vaginal wall and their activity against *C. albicans* biofilm formation. FTIR (Fourier transform infrared) analysis revealed the biosurfactant extracted and separated by thin-layer chromatography (TLC) plates showed predominantly a lipopeptide. The percentage biofilm inhibition activity of the produced biosurfactant was analyzed by colony-forming unit (CFU), adhesion force, and precoating tests. The lipopeptide extracted from isolated B1 showed a high capacity of penetration in the biofilm, eliminating 91% *C. albicans*, and highest inhibitions at 2500 μg/mL and 1250 μg/mL concentrations, respectively, were observed in precoating experiments. According to Kaur et al. [58], biosurfactants produced from living organisms can be efficient against colonizing opportunistic *C. albicans* and applied to biomedical apparatus for inhibiting biofilms formation.

Applying FTIR analysis, ESI-Ms, and Emulsification Index (EI_{24}), De Gregorio et al. [13] assessed a biosurfactant isolated from vaginal *Lactobacillus crispatus* (BC1) as nonhomogeneous lipopeptide molecules. The FTIR results showed different functional groups in the biosurfactant structure and the presence of mainly aliphatic chains and peptidic groups in the molecules. However, ESI-Ms tests indicated a lack of acid functions, characteristics of glycolipids bonds (mainly rhamnolipids), which generally increase signals attributed to negatively charged ions.

Sen et al. [57] characterized the sophorolipid biosurfactant produced by a locally isolated novel yeast *Rhodotorula abjevae* (YS3) collected from the soil of Assam (India) was evaluated its antifungal activity. The highest biosurfactant yield was achieved after 192-h growth with a 16.61 g/L cell biomass production at 19°C. TLC confirmed the *R. abjevae* as glycolipid from the positive reaction with sugars and lipids using anthrone reagents and iodine vapors, respectively, and also predicted the biosurfactant produced as a sophorolipid, which was detected by the presence of spots of standard and acidic lactonics. The FTIR spectra revealed functional groups related to the presence of lactone and a mixture of standard and acidic lactonics, also detected by TLC. The Liquid Chromatography coupled with Mass Spectrometry (LC-Ms) confirmed the presence of five standard and acidic lactonics [57].

Jemil et al. [59] investigated biosurfactants produced from lipopeptides molecules using TLC and FTIR techniques. FTIR analysis revealed the main functional groups and the chemical linkages present in the biosurfactants and their chemical nature. However, TLC showed the separation of some compounds in the mixture, which can be used for determining a large number of active components in solutions. Interestingly, the *in natura* biosurfactants samples revealed yellow spots (iodine vapor), indicating an incidence of polar lipids.

Jadhav et al. [60] studied microorganisms producing biosurfactant from a diesel-contaminated soil isolated and with an *Enterobacter* sp.-enriched culture. The maximum biosurfactant production was 0.48 g/L day, achieved with sunflower oil cake as substrate; the same substrate reached a maximum concentration of 1.5 g/L. TLC plate tested with iodine revealed some spots presenting a value of 0.75. FTIR spectra showed groups related to aliphatic chains, carboxyl groups, acetyl ester bonds, alkene, anomers in polysaccharides, and carbohydrate molecules. The acid biosurfactant was hydrolyzed and studied for the determination of the amount of FAMEs. GC-Ms analysis revealed a hydrophobic portion, predicted as C_{16} and C_{18} methyl esters, and a carbohydrate moiety found as sugars from plant polymers (e.g., glucose, galactose, and arabinose). Since the biosurfactant produced showed antifungal activity against *Aspergillus niger* and *Penicillium chrysogenum*, with complete inhibition at 12.5 mg/mL concentration, it can be used as an antifungal agent.

Abruzzo et al. [61] characterized an innovative formulation for vaginal delivery of econazole nitrate, frequently used in treatments of *Candida* infections. The biosurfactant isolated from vaginal *Lactobacillus* was employed in the preparation of phosphatidylcholine-based mixed vesicles. The biosurfactant produced by *L. gasseri* (BC9) and isolated from the vagina of a healthy premenopausal woman was characterized by FTIR and ESI-Ms analysis. FTIR revealed the chemical composition of such biosurfactant was nonhomogeneous and characterized by peptide-type molecules containing hydrocarbon chains. However, ESI-Ms showed no significant peaks in the parameters evaluated, thus suggesting the absence of chemical activities, but the presence of organic acid functions, for example, glycolipids (mainly rhamnolipids), which showed intense negative signals attributed to pseudomolecular ions [61].

Sarwar et al. [62] studied six types of lipopeptide-producing *Bacillus* species of considerable potential to be applied in pharmaceutical industries due to their dynamic surface properties, and antimicrobial, antifungal, immunosuppressant activities. Their bath ability (capability for adhesion in crude oil) ranged from 77% to 90%, whereas no activity was observed in *Paenibacillus polymyxa* and *B. subtilis*. Maximum aggregation was obtained by *B. amyloliquefaciens* (40.13%), and the minimum was obtained by *B. subtilis* (11.08%). However, the antifungal activity achieved an approximately 87% inhibition value for *B. amyloliquefaciens* and a lower value for *B. subtilis* (3%−5%).

Ma and Hu [63] produced *B. mojavensis* (B0621A), a biosurfactant isolated from a *Pinctada martensii* pearl oyster and originated from South China Sea. Three cyclic lipopeptides compounds potentially useful for biological control against fungal pathogens were isolated, purified, and evaluated by vacuum flash chromatography (VFC) coupled with reversed-phase high-performance liquid chromatography (RP-HPLC). However, their structural properties and identification were performed by gas chromatography coupled with mass spectrometry (GC-Ms). The spectroscopic analyses identified the lipopeptides elements analogous to *B. mojavensins*, which contain amino acids of asparagine, tyrosine, glutamine, proline, but are different from each other due to their saturated β-amino fatty acid chain residues. All results confirmed the lipopeptides produced by *B. mojavensis* can be useful as biological control agents for combatting pathogenic fungi.

Garg et al. [64] investigated and characterized the ability of *C. parapsilosis* to produce a biosurfactant for biomedical applications. The FTIR spectra revealed the presence of functional groups, for example, phenol or alcohol, and amide in the sample, which validated the biosurfactant type. GC-Ms showed the presence of 13-docosenamide due to its molecular weight (337.5 g/mol). *C. parapsilosis* showed antimicrobial activity against *S. aureus* and *E. coli* due to the inhibition

zone. The higher concentrations of the biosurfactant increased the diameter of inhibition, and the best inhibition yields were achieved at 1.5 mg/mL concentration, with 2.87 and 2.66 cm diameters for *E. coli* and *S. aureus*, respectively.

Kumar et al. [65] characterized *Rhizosphere* bacteria for a high biosurfactant production of biosurfactants. 100 bacteria were isolated from different soil samples of *Rhizosphere* by the method of culture by soil enrichment and selected for activity as a biosurfactant. Only 20 were selected and analyzed, and RHNK22 showed greater prominence as a biosurfactant. The antifungal activity of isolated RHNK22 against *M. phaseolina* and *S. rolfsii* phytopathogens showed inhibition in potato dextrose agar (PDA) environment of 76.9% and 73.3%, respectively. However, on glucose yeast extract (GCY) as amino acids were 80% for both pathogens, and the average value on Kings B (KB) were 72.5% and 75.5%, respectively. Iturin A biosurfactant produced by isolate RHNK22 displayed a 77.7% inhibition of the pathogens tested at 5 mg/mL concentration. FTIR spectra of the extracted surfactant indicated the presence of peptide linkages, lactone ring, aliphatic chain, and amide II, thus confirming the production of iturin A by RHNK22.

Scanning electron microscopy (SEM) images or transmission electron microscopy (TEM) images, is another important experimental analytical technique for the characterization of biosurfactants for biomedical applications through their morphological characteristics.

Jiang et al. [66] evaluated the morphological structures (SEM and TEM images) of biosurfactants produced from *Fusarium moniliforme* hyphal with treatment (50 μg/mL [ΔLeu6]-surfactin) and without it and observed a normal growth of the structures of biosurfactants without pretreatment, that is, they showed flat direction and smooth appearance; however, the samples after pretreatment showed twisting and rough structures. The TEM images of the untreated samples revealed growth, healthy hyphae, typical structures, and smooth surfaces. Moreover, all cellular organelles were visible and showed regular arrangements. On the other hand, although the hyphal structures remained unchanged during treatment, the organelles were grouped into clusters, and a few huge vacuoles were observed in central regions. The SEM and TEM images evidenced the pretreatment considerably affected *F. moniliforme* growth, causing morphological alterations in hyphae, hence, inhibition fungal growth.

Santos et al. [67] evaluated and characterized *Streptomyces* sp. (DPUA1559) isolated from lichens of the Amazon region. The kinetic method used for biosurfactant production was studied in a mineral environment containing 1% residual soybean oil and 1% peptone during 120 hours of cultivation. The results reached values of 1.26, 4.81, and 5.02 g/L at 24, 96, and 108 hours of culture, respectively. During the fermentation, pH ranged from 6.8 to 8.7, thus demonstrating the microorganism adaptation to the environment. The biosurfactant produced showed a chemical composition of 20% proteins, 38% carbohydrates, and 12% lipids, indicating a preliminary presence of glycoproteins. The ATR-FTIR spectra of the solubilized biosurfactant in methanol showed intense bands, hence, the presence of hydroxyalkyl, amides, aromatic rings, and other compounds, representing a chemical structure of peptides. However, more experimental details are necessary for the determination of their specific chemical structures.

Using SEM images, Ceresa et al. [68] investigated the capability of a biosurfactant produced by *L. brevis* isolated (CV8LAC) for inhibition adhesion and biofilm formation of *C. albicans* on medical-grade silicone elastomeric disks (SEDs). A significant reduction in the biofilm's covered surface was observed in precoated disks, however, with no differences in the production of hyphae or blastospores, except at 1.5 hours incubation time. The authors also demonstrated the capability

of the biosurfactant produced (CV8LAC) the significantly counteract the initial deposition of *C. albicans* onto silicone surfaces and slow the biofilm growth.

14.3.2 Thermal behavior

Thermal analysis is commonly used for assessments of the thermal stability and decomposition of several biosurfactants, which are significant properties for some commercial applications at high and/or moderate temperature ranges, for example, microbial enhanced oil recovery, and biomedical and food industries [69]. Interestingly, the heat generated in such systems during the use or production of biosurfactants must be controlled, since this temperature variation can directly affect the biosurfactants produced [23].

A recent study on biosurfactants for industrial applications verified approximately 1% of the mass loss occurred after initial temperature increase, that is, from 50°C to 200°C (TG/DTG curves), possibly due to the loss of organic solvents, moisture molecules, other unidentified components, and complete loss of biosurfactant mass after 270°C [69]. Interestingly, the high moisture loss during the biomaterial heating suggested the biopolymer was not totally anhydrous. However, Jain et al. [70] showed the biosurfactant produced from an alkalophilic strain of *Klebsiella spp*. reached its maximum decomposition step between 350°C and 400°C (TG/DTG curves). Similar results were described by Abbasi et al. [71], who used rhamnolipid produced from *P. aeruginosa* (MA01). The authors stated the biopolymer mass was completely lost around 290°C, showing a continuous decrease. The biosurfactant isolated from *Enterococcus faecium* strain exhibited a thermal behavior similar to that of glycolipids and characteristics of thermostable biomaterials. The molecular mass was also determined by gaseous chromatography coupled with mass spectrometer (GC-Ms), confirming the molecular mass of the biosurfactant isolated in this study was similar to that of glycolipid biosurfactant, and, consequently, their thermal decompositions were the same [69].

Sharma et al. [72] evaluated the thermal degradation of *L. helveticus*-derived biosurfactant. TG/DTG curves determined the maximum degradation temperature at approximately 250°C. The total mass loss of the polymer was reached above 290°C, and a gradual decrease was clearly observed up to the residual mass. The authors verified the biosurfactant isolated from MRTL 91 strain displayed similar thermal behavior, that is, close to that of rhamnolipids. Regarding thermal stability at different temperatures, the biosurfactant remained stable after incubation for 120 hours to at 25°C−60°C temperatures, showing no apparent loss of activity. The molecular mass, determined by mass spectroscopy (Ms), confirmed the isolated biosurfactant showed molecular weight similar to that of glycolipid biosurfactant and the same thermal behavior.

Kaskatape and Yildiz [73] studied the optimal temperature ranges for the thermal decomposition of Rhamnolipid biosurfactants produced via *Pseudomonas* sp. The authors evidenced the ranges for different *Pseudomonas* sp. varied between 30 and 37°C, and reported the optimal temperature found was 37°C for *P. aeruginosa* based on the experimental observations of temperatures between 30°C and 42°C.

14.3.3 Antimicrobial or antifungal activity

According to Goswami et al. [12], the highest microbiological or antifungal activities were observed with the use of glucose as the main carbon source and attributed to the maximum

biosurfactant production and differences in the constituent's structure, which are analogous to those of rhamnolipid biosurfactants for each carbon source used.

The biosurfactant isolated with maximum yield was identified as *L. fermentum* by the compact system (e.g., VITEK II) for microbial identification, which showed an 85%−11% decrease in the microbial adhesion, with 78.125 to 2500 μg/mL biosurfactant [58]. The study developed by Sharma et al. [69] considered the antiadhesive properties of the produced biosurfactant (*Lactobacilli fermentum*), which offered new perspectives and challenges on the use of those biosurfactants for combatting microbial colonization, making them a suitable alternative for conventional antimicrobials.

Jemil et al. [59] evaluated the antioxidant activity of lipopeptides (DCS1), and their in vitro antimicrobial function showed considerable antibacterial and antifungal activities. The authors also assessed their antiadhesive action for combatting several pathogenic microorganisms, which proved excellent at low concentrations (≈ 1 mg/mL) when a polystyrene surface pretreatment. The present study also demonstrated the high potential of these lipopeptides as natural antioxidants and antimicrobial and/or antiadhesive agents for several biomedical applications.

De Gregorio et al. [13] studied the mucoadhesive properties of biosurfactants isolated from vaginal *L. crispatus* (BC1) and verified they were not cytotoxic and decreased *Candida* strains' capacity to adhere to the human cervical epithelial cells, mainly by exclusion mechanism. Moreover, their intravaginal inoculation in a murine experimental model was secure and did not alter vaginal cytology, histology, and cultivable microbiota. The biosurfactants reduced the leukocyte influx in intravaginal experiments of mice with *C. albicans*. The insights demonstrated the biosurfactants from vaginal *L. crispatus* were capable of intervening with *Candida* adherence in in vitro and in vivo tests, and suggested their probable application as a prevention factor for reducing mucosal harm caused by *Candida* in vulvovaginal candidiasis.

Mnif and Ghribi [74] revised several lipopeptide biosurfactants for biomedical applications and observed their capacity to form pores and disestablish biological membrane enabled their use as antimicrobial, antifungal, hemolytic, antiviral, antitumor, and insecticide agents. Moreover, lipopeptides can act at the surface and modulate enzymes action, increasing both the activity of some enzymes and the microbial process or inhibition of certain enzymes that can be used as antifungal agents.

Abruzzo et al. [61] investigated the antimicrobial action of the mixed vesicles for planktonic cultures and biofilms of *C. albicans* and observed biosurfactants fabricated by *L. gasseri* (BC9) contained peptide molecules, showing hydrocarbon chains, high surface activity, and low critical micelle concentration. According to Abruzzo et al. [61], biosurfactants containing econazole-loaded mixed vesicles showed higher encapsulation efficiency and mucoadhesion capacity, regarding vesicles with Tween 80. They also promoted an adequate release of econazole nitrate, keeping the antifungal function against *C. albicans* planktonic culture. Finally, the authors highlighted biosurfactants based on vesicles were more efficient than free econazole in the eradication of *Candida* biofilm mixed vesicles and promising new vaginal delivery systems for the treatment of persistent infections [61].

Ishaq et al. [75] produced, purified, and characterized a novel glycolipid biosurfactant (called Uzmaq) from *Aspergillus flavus* (AF612), which was isolated from citrus fruits. The authors assessed its microbiological properties or antifungal activity and observed Uzmaq was composed of methoxy phenyl oxime glycosides (first time from a filamentous fungus). They also reported its

surface activity was more efficient in comparison to the values found for synthetic surfactants, and the biosurfactant evidenced its antifungal function and self-assembling characteristics. *A. flavus* can be used for the commercial fabrication of biosurfactants to be applied for the controlled delivery of drugs and bioremediation.

14.3.4 Functional properties

As addressed elsewhere, biosurfactants show hydrophilic and hydrophobic fractions in some properties. The location and size of such functional groups (hydrophilic and hydrophobic) can predict the characteristics of the biosurfactants, which are related to functional properties, and determine their application (food, cosmetics, pharmaceutics, agricultural, and others) [76].

The main surface activity of biosurfactants, called surface tension, is determined by the functional property, measured by the force per unit length applied by a liquid in contact with a solid surface or another liquid (e.g., highest surface tension value of water, that is, 72 mN/m (or dyne/cm), can be reduced with the addition of a biosurfactant) [76].

The purification step of the biosurfactant production contributed up to 60% of the total production cost since most biosurfactants require whole-cell culture broths or crude preparations [67].

The biosurfactant developed by Santos et al. [67] from *Streptomyces* sp. (DPUA1559) in its crude form and with no previous extraction steps, which are costly, was investigated, and the main results showed slight changes in the surface tension at different pH values (4, 6, 8, and 10), with the surface tension value around 30 mN/m. The biomolecules also showed thermal stability at different temperatures and surface tension stability between 4°C and 8°C—surface tension also exhibited a small change in different NaCl concentrations (0%−12%) with the highest value (33.38 mN/m) at 12% of NaCl.

From the 20 samples of biosurfactants investigated by Kumar et al. [65], the highest surface tension reduction was observed in RHNK22—from 60.50 to 26.12 N/m in 24 hours of experiment, and to 29.04 mN/m after 48 hours. The surface tension value ranged from 60.39 to 27.96 mN/m and 50.12−29.86 mN/ m, for 24 and 48 hours, respectively, for the other isolated materials. The surface tension of a biomolecule studied by Sarwar et al. [62] was performed to a mixture of biosurfactant with water and revealed that after 24 hours of incubation, the surface tension was reduced from 72 to 33.4−70 mN/m.

Toward reducing production costs, Jadhav et al. [60] used carbon-based substrates supplemented with the biosurfactant a mineral salt environment. They evaluated surface tension reduction, the time course of production, and the growth of *Enterobacter* sp. The surface tension showed a 34 mN/m average reduction.

The property known as wettability is associated with surface tension since surfactants act as wetting agents. It is defined as the spread and penetration capability of a substance that decreases the surface tension when a liquid is added [76].

Another important functional property of biosurfactants is Emulsification Index (EI_{24}), which represents the dispersion of a liquid into another, resulting in a mixing of two immiscible liquids, meaning that molecular solubilization produces large solubilized particles [76].

Kaur et al. [58] found a 20.83% Emulsification Index (EI_{24}) for cell-free extract (growth medium of isolate B1), and the average yield of B1 was 4.55 g/L. In a study of a biosurfactant isolated from vaginal *L. crispatus* (BC1) conducted by De Gregorio et al. [13], EI_{24} was around 50%, confirming the results of FTIR and ESI-Ms analyses.

The sophorolipid biosurfactant produced by *R. abjevae* (YS3) collected by Sen et al. [57] showed an expressive emulsification activity and stability behavior after 168 hours for the hydrophobic substrates of crude oil, n-hexadecane, and diesel, which achieved Emulsification Indexez (EI_{24}) of 98.0%, 88.0%, and 20.0%, respectively.

Enterobacter sp. was efficiently emulsified with various hydrocarbons and oils, achieving Emulsification Indexes (EI_{24}) of 70.5%, 60.1%, 55.52%, and 60.5% for diesel, kerosene, groundnut oil, and olive oil, respectively [60]. EI_{24} for the four types of *Bacillus* (*B. amyliliquefaciens* FZB42, *B. subtilis* NH-100, *Bacillus* sp. NH-217, and *B. atrophaeus* 176 seconds) was studied by Sarwar et al. [62] and displayed 2.7% average values.

In the research performed by Kumar et al. [65], the Emulsification Index (EI_{24}) was carried out with RHNK22 (isolated) and some hydrocarbons types: kerosene, diesel, coconut oil, and sunflower, which showed the highest percentage (EI_{24}): 78.1%, 72%, 53.1%, and 62.5%, respectively. *Bacillus* sp. isolated from RHNK22 also showed the highest emulsification activity [Eu mL^{-1}], that is, 214.0 in kerosene, 253.5 in diesel, 226.7 in coconut oil, and 291.4 in sunflower oil.

EI_{24} for *Streptomyces* sp. (DPUA1559) from Amazon, studied by Santos et al. [67], was 38% and 40%, respectively, for the subtractive diesel and kerosene, 89% and 95%, for engine oil and residual engine oil, respectively. The results for corn and sunflower oils were 47% and 30%, which showed an affinity with hydrophobic compounds, that is, action as surfactant and emulsifier.

Finally, in a study with a biosurfactant isolated from liquens from the Amazon region, Santos et al. [67] claimed temperature, pH, and ionic strength tolerance of the biosurfactants are related to their stability.

14.4 Etiological agents of profound mycoses and application of biosurfactants against them

The classification of mycoses is based on the infection site (superficial, cutaneous, subcutaneous, and deep), route of pathogen acquisition (exogenously by airborne, cutaneous or percutaneous, or endogenously by abnormal mycobiota proliferation or reactivation of latent pathogen infection), and pathogen virulence (by either primary etiologic agents of diseases, or opportunistic agents) [77].

The main fungi that cause systemic infections are included in genera *Paracoccidioides*, *Coccidioides*, *Histoplasma*, and *Blastomyces*, which are thermodimorphic. However, species belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Candida*, *Cryptococcus*, *Pneumocystis*, *Mucor*, *Pythium*, as well as etiological agents of subcutaneous mycoses as *Lacazia loboi, Sporothrix*, and dematiaceous fungi (*Fonsecaea*, *Cladosporium*, and *Phialophora*); and many other have also led individuals with immune weakness and/or comorbidities to systemic clinical conditions. The primary etiologic agents of deep mycotic infections have a restricted worldwide distribution, although some sporadic cases have been reported in nonendemic regions [78].

14.4.1 Etiological agent of profound mycoses

The main profound mycoses of interest to developing countries in South America, such as Brazil, are systemic candidiasis, pulmonary aspergillosis, paracocciodoidomycosis, cocciodoidomycosis,

cryptococcosis, histoplasmosis, and pneumocystosis. The main routes of infection and dissemination of agents of profound mycoses were illustrated in Fig. 14.3.

14.4.1.1 Systemic candidiasis

Fungi of *Candida* genus belong to Blastomycetes class and Cryptococcacea family [79], and the most common species are *C. albicans, C. tropicalis, C. glabrata, C. dubliniensis, C. parapsilosis, C. ortopsilosis, C. metapsilosis, C. krusei, C. famata, C. guilliermondii and C. lusitaniae* [80]. *Candida* sp. live as diners and are part of the normal microbiota [81–83] colonizing the intestine and oral and vaginal cavities of healthy individuals [84].

Candida sp. are opportunistic pathogens and, in immunocompromised hosts, can proliferate and cause infections [85]. Candidiasis can manifest as cutaneous (in the skin and its attachments), mucous (oropharyngeal, esophageal, and vulvovaginal) and systemic (as bloodstream infections),

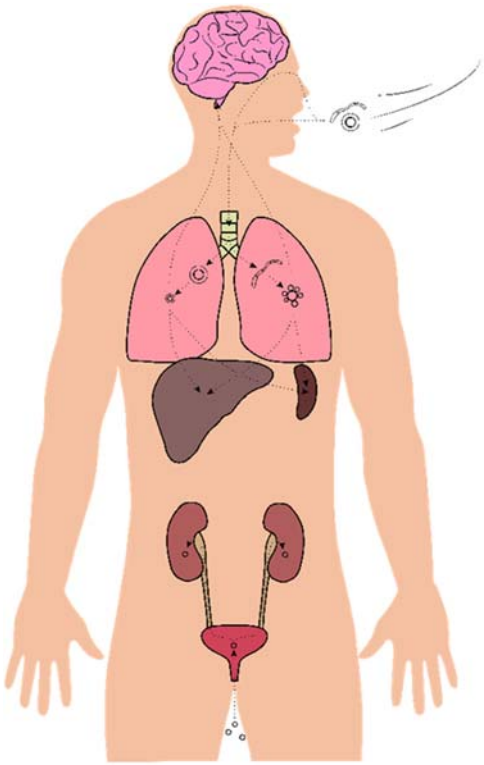

FIGURE 14.3

Routes of infection and dissemination of agents of profound mycoses. The main route of infection is respiratory (i.e., *Cryptococcus, Paracoccidioides, Coccidioides, Aspergillus, Pneumocystis*, etc.) which, from the lungs, can spread to other tissues via the lymphohematogenic route. Systemic candidiasis is mainly due to cystitis that progresses to pyelonephritis.

that is, candidemia and other forms of invasive candidiasis [86], such as deep candidiasis (most often intra-abdominal candidiasis) [83].

The infection caused by *Candida* sp. is one of the most prevalent and opportunistic worldwide; *Candida* sp. are responsible for 80% of all systemic fungal infections and is associated with high mortality rates [82]. Most studies have reported 30% to 40% crude mortality rates of infections by *C. albicans*, and some have estimated 46% to 75% [87].

Candida sp. are responsible for more than 400,000 annual cases, with an incidence of 0.24 to 34.3 patients/1000 in intensive care unit (ICU) admissions and mortality close to 40%. They are the third or fourth most common causes of health-related infections worldwide [88].

Although most cases of invasive fungal infection are attributed to *C. albicans*, the rate of infection by both nonspecies and closely related *C. tropicalis* and *C. parapsilosis* species has increased in recent years in various parts of the world. Infections caused by *C. parapsilosis*, for example, are commonly associated with newborns in hospitals, and *C. tropicalis* has become an emerging pathogen in certain regions, such as the Indian subcontinent and Latin America [89].

14.4.1.2 Pulmonary aspergillosis

Aspergillus species are widely distributed in the environment, and can be found in plants, decomposing organic matter, soils, air, bioaerosols, animal systems and freshwater and marine habitats, as well as in indoor environments (building surfaces, air, appliances, etc.) and drinking water and dust [90].

Aspergillus spp. are filamentous environmental fungi that cause a wide spectrum of infections in humans, including hypersensitivity reactions, and chronic lung and acute life-threatening infections—the latter occurring mainly in immunocompromised individuals [91].

Aspergillus genus covers more than 250 species and is one of the largest genera of filamentous fungi that cause human diseases. Among the several hundred species of *Aspergillus* genus, only a few exert considerable impacts on human and animal health. Infections are usually caused by *A. flavus, Aspergillus fumigatus, Aspergillus nidulan, A. niger*, and *A. terreus*, among other species [90,92].

In immunocompetent individuals, conidia (spores) released into the atmosphere can be inhaled and are usually eliminated by mucociliary and innate immune systems, with no underlying lung disease. Histological, clinical, and radiological manifestations of pulmonary aspergillosis are related to the balance between fungal virulence and the host's immune integrity. Pulmonary parenchyma disease caused by *Aspergillus* is classified into five categories, namely saprophytic aspergillosis (commonly known as aspergilloma, mycetoma, or fungal ball), allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis, and semiinvasive and invasive aspergillosis [93].

A. fumigatus, a saprophytic fungus of the air, is the most common species responsible for 80%−90% of cases, followed by *A. flavus*, *A. nidulans, A. niger, A. terreus*, and *A. versicolor* [93].

14.4.1.3 Paracoccidioido mycosis

Paracoccidioides sp. cause deep mycosis predominantly in individuals who manage the soil in endemic areas such as Brazil, Colombia, Venezuela, and Argentina, which concentrate most cases in the continent [94,95]. Its endemicity in South America and clinical similarity to North American *Coccidioido mycosis* (caused by *Coccidioides* sp.), with high incidence in rural workers, led to the name of *Pseudococcid mycosis* [96] and, later, *Paracoccidioido mycosis* (PCM), when referring to the morphological aspects to the microorganism [97].

PCM affects mainly the lungs, but it can be disseminated to other systems. Its main contagion route is respiratory, through the inhalation of fungal propagules from soil, vegetables or water, during the saprobiosis stage. The thermoconversion from mycelia (infective) to yeast (parasitic) occurs in the lungs [98], and the disease can develop in both acute and chronic forms. In the acute form, rarer that affects children and adolescents, cellular immunity is compromised with the formation of loose granulomas that facilitate the spread of the microorganism to other anatomical sites. The chronic form, more common that affects adults, is characterized by an exacerbated cellular immune response and formation of compact granulomas that confine yeast and prevent their spread, thus inducting fibrosis [99–102].

The disease affects more men than women (13:1) in the childbearing age since estrogen prevents thermoconversion from mycelium to yeast, and men are more engaged in activities in soil. In addition to pulmonary infection, there may be involvement of the skin, mouth, and mucous membranes in multifocal form and other anatomical sites after lympho-hematogenous dissemination such as the central nervous system and the adrenal gland [103,104]. Ulcerative lesions in the head, nose and neck have also been reported [105,106].

14.4.1.4 Coccidioido mycosis

Coccidioides genus comprises two species, namely *Coccidioides immitis* and *C. posadasii*, which cause severe pulmonary manifestations or disseminated disease [107]. Although it is an endemic disease in the North American continent, with higher incidence in southern-central California valley and southern Arizona deserts, [107], some cases have also been reported in northeastern Brazil [108]. The infection mostly occurs through the inhalation of arthroconidia during soil management. The thermoconversion of arthroconidia to spherule occurs in the lungs, thus inducing granulomatous reactions. The incidence of the disease is slightly higher in men than in women and in individuals with HIV/AIDS, diabetes mellitus, lung diseases, and those pregant. Mortality is high in HIV-infected people with diffuse lung disease, who are predisposed to the disseminated form of the disease [107].

14.4.1.5 Cryptococcosis

Cryptococcosis is caused by fungi of *Cryptococcus* genus, especially *C. neoformans*, *C. gattii*, *C. laurentii* and *C. albidus*. Present in the environment, *Cryptococcus* has the soil, bird excreta, hollows, leaves, bark, fruits of trees and decomposing wood, especially eucalyptus (*Eucalyptus* or. exserta Blakely, *Eucalyptus camaldulensis*, *E. tereticornis*, and *E. gomphocephala*), as its main habitats [109–111]. The export of *Eucalyptus* seeds and trees from Australia is believed to contribute to the worldwide spread of *C. gattii*. In the Amazon rainforest, *C. gattii* was are found in hollow trees of *Guettarda acreana*, thus reinforcing other ecological niches for this pathogen [109–112].

The fungus shows neurotropism and can migrate to the CNS through hematogenous dissemination. When crossing the blood-brain barrier, it can cause meningoencephalitis with neurological abnormalities, intracranial hypertension, papilledema, optic atrophy commonly secondary to lung injuries, as well as episodes of mental confusion and behavioral changes [110,113–116].

Annually, more than 1 million new cases of cryptococcosis are worldwide estimated, with approximately 600,000 deaths. In addition, 220,000 annual cases of cryptococcal meningitis are estimated to develop among people with HIV/AIDS, resulting in almost 181,000 deaths, with the highest number of cases in SubSaharan Africa [117].

14.4.1.6 Histoplasmosis

Histoplasmosis is caused by *Histoplasma capsulatum*, a dimorphic fungus found in soil contaminated with bird and bat feces [118]. It is widely distributed in the American continent, in countries such as Guatemala, Mexico, Nicaragua, Panama, Argentina, Brazil, Colombia, Peru, Uruguay, and Venezuela, as well as in several Caribbean islands, Africa (in both varieties, that is, *H. capsulatum* and *H. duboisii*), and Southeastern Asia [118–121].

In Brazil, outbreaks have been reported in the states of São Paulo, Rio Grande do Sul, Rio de Janeiro, Espírito Santo, Mato Grosso, and Minas Gerais. However, despite some outbreaks or deaths resulting from complications, histoplasmosis is not found in the compulsory notification system, thus preventing information on the current distribution of the infection [118,119].

14.4.1.7 Pneumocystosis

Pneumocystis pneumonia (PCP) is an invasive respiratory infection caused by *Pneumocystis jirovecii*, a ubiquitous fungus and unusual opportunist [122]. When inhaled, *Pneumocystis* has tropism for alveolar epithelial cells in the lung and rarely spreads to other organs, although cases of extrapulmonary dissemination have been reported in patients with profound immunosuppression, such as HIV-infected patients [123].

Pneumocystosis has nonspecific symptoms such as progressive dyspnea, nonproductive cough, low fever, partial arterial oxygen pressure below 65 mmHg, and chest X-rays that demonstrate bilateral interstitial shadow [124].

Initially, pneumocystosis was responsible for most cases of morbidity and mortality in HIV-infected patients, which were subsequently reduced with the introduction of antiretroviral therapy, as well as antipneumocystic prophylaxis in these patients, however, it is still considered a significant cause of mortality among HIV patients. negatives who are under immunosuppression caused by several factors, such as being transplanted or being treated for cancer [124].

Pneumocystis infection has a global distribution among humans and most individuals show serological evidence of infection at 2 years of age. The incidence of PCP is related to the extent of immunosuppression, mainly to the impairment of cell-mediated immunity, evidenced by the frequent occurrence of PCP in AIDS patients [123]. With the change in epidemiology, changes in prognosis also become apparent: the mortality rate in patients without HIV infection is over 20% and increases to over 50% if patients undergo intensive care [125].

14.4.2 Antifungals

The discovery of molecules with antifungal activity has raised high expectations regarding their possible clinical use due to the emergence of microbe-resistant strains and the small arsenal of antifungal drugs licensed for human use [126].

Studies of antifungal compounds range from evaluation of action mechanisms to selective toxicity, safety, efficacy, bioavailability, and biotransformation when administered in successively more complex organisms (from preclinical to clinical trials), and costs for large-scale procurement [127].

Until the 1980s, therapeutic options for the treatment of invasive fungal infections had been restricted, mainly due to the high toxicity of antifungals [128]. Currently, four classes of antifungals, namely azoles, polyenes, allylamines, and echinocandins, are available for clinical use—only

the former two are effective in the treatment of all the profound mycoses; allylamines are ineffective and echinocandins have limited action against dimorphic fungi and *Cryptococcus* sp. Effective treatment of systemic fungal infections depends on the understanding of the disease epidemiology and the profiles of antimicrobial susceptibility. Despite the availability of several in vitro tests, the treatment of invasive mycoses is challenging, due to the scarcity of studies that correlate in vitro−in vivo data, thus hampering the transposition of results from experimental models for clinical applications [129,130].

The main effective drugs for the treatment of deep mycoses are echinocandins, such as caspofungin, azoles, fluconazole, itraconazole, voriconazole and posaconazole, and polyene amphotericin B with or without a combination with 5-flucytosine, which is not available for use in developing countries, such as Brazil [131,132]. Besides the restricted antifungal arsenal, cases of resistance mainly to azoles limit the antifungal therapy of profound mycoses, highlighting the need for new therapeutic options, such as biosurfactants, which are more environmentally friendly than surfactants obtained in a chemically synthetic way.

14.4.3 Biosurfactants

Although most biosurfactants have been used as natural pesticides, emulsifiers in the food industry, oil spill bioremediation, detergent, cosmetic formulations, and as antiadhesives for preventing and/or combating biofilm on surfaces, a few of them have been sufficiently evaluated to be licensed for use in antimicrobial therapies in humans. Currently, most studies have focused on microbial growth inhibition (broth microdilution and agar diffusion assays, for example), while a few assays have advanced to the preclinical phase [133] and there is no clinical trial currently in progress.

Lipopeptide produced by *Acinetobacter junii* B6 could inhibit the growth of two species of *Candida*, namely *C. albicans* and *C. utilis* at lower concentrations (5.0 μg/mL). The MIC values were lower than those of MIC for fluconazole and showed almost 100% inhibition. Its mechanism of action consists of the formation of pores in the cytoplasmic membrane due to its ability to interact with membrane phospholipids [18].

Ferreira et al. [134] used a polymeric biosurfactant (CLOA72) produced by *Trichosporon montevideense*, and suggested its possible application for inhibiting the adhesion and formation of biofilms on biological surfaces by *Candida* sp. The inhibition of biofilm by the biosurfactant (25 mg/L) in *C. krusei* and *C. albicans* in polystyrene was reduced to 79.5% and 85%, respectively. Preventing biofilm formation on the catheter surfaces is useful to minimize the risks of cystitis that may predispose to pyelonephritis, in the context of invasive candidiasis. Changes in the characteristics of the cell surface and the interface can inhibit the initial adhesion of yeast cells to the surface.

Sophorolipid (SL), a glycolipid biosurfactant, has shown antifungal properties against the formation of *C. albicans* biofilm and reductions in the viability of preformed biofilms. When combined with amphotericin B or fluconazole, it has shown synergistic activity against the formation of preformed biofilms and biofilms. Moreover, it negatively regulates the expression of specific hyphae genes HWP1, ALS1, ALS3, ECE1, and SAP4, which possibly explains its inhibitory effect on the formation of hyphae and biofilm [135].

Lipopeptides, mainly surfactin, a lower percentage of fengicin, AC7 BS significantly reduced the adhesion and biofilm formation of three strains of *C. albicans*. AC7 BS did not inhibit the viability of *C. albicans* in planktonic and sessile forms; it expressively prohibits the initial deposition

of *C. albicans* and slows down the growth of biofilm, suggesting a potential role for biosurfactant coatings in preventing fungal infections associated with medical silicone devices [136].

Liu et al. [137] demonstrated the synergistic antifungal activities of C-surfactin against *C. albicans* at 12.5 and 6.25 μg/mL, respectively. Based on such MIC values, C-surfactin and C-FICIs-surfactin were lower than 0.4 and 0.3 (all of them were lower than 0.5), respectively, only the combination of surfactins and KTC were synergistic.

WF11899A, B, and C produced by strains of *Coleomycetes* and *Hyphomycetes* groups, strongly inhibited 1,3-glucan synthase and were active against *C. albicans* and *A. fumigatus*. The inhibitory activity of the WF11899A enzyme was four times more potent than that of echinocandin B. Semisynthetic FK463 derived from WF11899A, called micafungin, was one of the last echinocandins licensed for the treatment of invasive aspergillosis [138,139].

L. gasseri 1. *L. rhamnosus* ATCC 9595, *L. acidophilus* ATCC 4356 and *L. paracasei* 11 produced biosurfactants that decreased the adhesion of *C.* albicans and interrupted the formation of biofilms. Overall, *Lactobacillus* strains showed significant anti-*Candida* activity, and their biosurfactants exhibited considerable antiadhesion and antibiofilm activity against *C. albicans* [140]. In the study of Gregorio et al. [13], *L. crispatus* BC1 vaginal biosurfactant reduced the ability of *Candida* strains to adhere to human cervical epithelial cells, mainly by exclusion mechanism.

Lactobacillus BS antimicrobial tests showed an improvement in antifungal activity. In particular, EN-BSMV almost completely eradicated *C. albicans* biofilm [61].

Zoysa, Glossop, and Sarojini [141] reported an anti-*Candida* activity of battacinlipopeptides. Trimeric lipopeptide 13 conjugated to 4-methyl hexanoyl emerged as the main candidate with a 6.25 μM MIC, and the antifungal activity was further increased with amphotericin B. The biosurfactant prevented the biofilm colonization and inhibited preformed biofilms of *C. albicans*. DCS1 lipopeptides, on the other hand, exerted a stronger antiadhesive effect against *C. albicans* with an approximately 89.3% inhibition percentage [59].

The antifungal activity of purified biosurfactants was verified against *A. niger*. Such biosurfactants showed remarkable antifungal activity against *A. niger* [75]. All 40 *Bacillus* sp. were selected for the production of biosurfactants and showed inhibitory activity against *A. flavus*, *A. niger*, *A. versicolor* [142]. Liang et al. [143] reported the tested biosurfactant exhibited significant inhibitory effects against *A. fumigatus* BCRC30099.

B. subtilis surfactin showed high inhibitory effects against *A. flavus* (100%), and the effect of purified surfactin on the growth of *A. flavus* was evaluated. The growth of mycelia was considerably reduced with increasing surfactin concentration [144].

Few studies have reported the antifungal activity of rhamnolipids. Abalos et al. [145] observed rhamnolipids produced by strains of *P. aeruginosa* exhibited excellent antifungal properties against *A. niger* (16 μg/mL).

Residual cooking oil (KWO) was analyzed as a substrate for the production of biosurfactant by *Wickerhamomyces anomalus* CCMA 0358, and its antifungal activity was evaluated against *Aspergillus*, obtaining results of up to 95% inhibition [146].

Regarding antifungal activity, DCS1 lipopeptides showed significant activity against several strains of fungi. More intense activity was observed against *A. niger* and *A. flavus* antimicrobial and antiadhesive activities of DCS1 lipopeptides from *B. methylotrophicus* DCS1 [59].

López-Prieto and collaborators [147] demonstrated the fungicidal (complete inhibition) and fungistatic (50% inhibition) capacity of the biosurfactant extract obtained from corn maceration water

on *A. brasiliensis*. *C. albicans* were observed more resistant than *A. brasiliensis*, although it was possible to achieve large growth inhibitions.

Other members of the iturin group, including *Bacillomycin* D and *Bacillomycin* L, also showed antimicrobial activity against *A. flavus*; however, the different lengths of the lipid chain apparently affected the lipopeptide activity against other fungi. Therefore, members of the iturin-like group of biosurfactants can be used as potent alternative antifungal agents [148].

14.5 Final considerations

Due to properties such as low toxicity, biodegradability, among others, biosurfactants have shown an alternative for different applications, including their use as antifungals. This chapter reported some studies on the feasibility of treating profound mycoses with biosurfactants. Research has shown characterization is an important way for the understanding and correct application of such substances since they display diverse structures and enable the production of biosurfactants from a variety of substrates.

Although different routes can be used in the production processes, costs are still a major factor for the application of viable biosurfactants for the treatment of mycoses, especially regarding their industrial production.

Despite the necessity of further studies, the results achieved so far have proven such substances are promising for antifungal therapies.

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CHAPTER

Hemolysis and formation of ion channels in lipid membrane

15

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15.1 Introduction

The purpose of the chapter is to shed light on understanding the mechanism of pore formation in the lipid membrane, hemolysis, and factors influencing the pore size. It is crucial to understand the mechanism hemolysis and formation of ion channels in lipid membrane as various progressions such as the antimicrobial activity of peptide; membrane fusion and permeation require the transmembrane pores The various biological applications encompasses important phenomena such as pore formation, solubilization, permeabilization, and lysis of membrane, the biogenic surfactant and detergents are playing a key role in these. These phenomena include the study of interactions of surfactants with membranes of lipids, membrane proteins, leakage and lysis of cell membranes by lipopeptides, antimicrobial activities, thermodynamic and structural facets of membrane−water partitioning, membrane solubilization to micelles and other lipid−surfactant systems, and alterations in properties of membrane brought about by surfactants [1−7].

15.2 Role of biosurfactants

The biosurfactants are rendering a significant role in hemolysis and pore or channel formation. The biosurfactant molecules interact with lipid bilayer which is an essential step to various processes. Biogenic surfactants derived from natural sources are also largely being used in biotechnological as well as in healthcare fields for their antimicrobial properties, permeabilizing effects for transport of drugs or DNA across cell membranes, and isolation of membrane proteins. Notably, the surfactants can introduce themselves within the lipid bilayer and can modify the surface area of the membrane through lipid solubilization. This forms the basis for surfactant-membrane interactions [2,5,8−10].

To understand the deformation in the cell membrane of erythrocytes, it is essential to know the characteristics of the erythrocyte membrane. The surfactants are known to contribute greatly to the cell membrane action mechanisms of erythrocytes and in the lysis of the erythrocytes [5].

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15.3 Classification of surfactants

Nazari et al. [1] categorized the effects of surfactants on membrane order based on fluorescence anisotropy as follows:

- Biosurfactants with membrane protein isolation and heterogeneously perturbing effects, for example, lipopeptides, and saponins.
- Biosurfactants induce the solubilization of cargo molecules in the micelles to bring about homogeneous disordering.
- Biosurfactants induce curvature stress in membranes, for example, Micelle-forming surfactants.
- Biosurfactants cause lysis of membrane once the disordering reaches critical extent, for example, lauryl maltoside, $C_{12}EO_8$, and octyl glucoside.
- Biosurfactants cause spontaneous segregation from the lipid or cause packing defects to disrupt the membrane, for example, lysophosphatidyl choline, fengycin, surfactin, and digitonin.

15.4 Mechanism of hemolysis caused by surfactants

The cell hemolytic alterations brought about by biogenic surfactants induce changes raging from minor shape modification to lysis of cell membrane [2,3,7,11,12].

The various mechanisms proposed are as follows:

- osmotic lysis;
- lysis by solubilization;
- lysis by the formation of pores in the membrane.

The ability to modify the curvature of the lipid membrane and surfactants affinity of the cell membrane determines its pore formation and lytic potency. The hydrophobic and hydrophilic moieties of the surfactants determine their shape. Saponins are being processed in regards to the formation of membrane pores.

15.5 Role of lipid layer in pore formation and membrane lysis

There are various ways through which the lipid bilayer causes pore formation using polypeptides. It alters the molecular configuration of membrane complex, conditions or maintains the active structures of peptides and protein, importantly, provide the binding sites to peptides and protein and thus contributes to all phases of pore formation. In the absence of proteins or peptides, spontaneous lipid flip-flop and nonassisted ion permeation are rare short-living events with properties dependent on the lipid composition of the membrane. The internal membrane disturbance of the lipid packing and coexistence of membrane-bound proteins or peptides raises the frequency of such events. Assembling of proteins, lipids, and polypeptides together forms the dynamic supramolecular complex to give rise to transmembrane pores. The assembly of lipids, proteins, and peptides act to induce pore formation by causing thermodynamic membrane balance which enhances

the possibility of the formation of pores. It involves various processes such as lowering the activation energy for pore formation, destabilizing the membrane lamellae, and pore structure stabilization [6,13,14].

The membrane rupture limiting kinetics are driven by the nucleation and growth of pores, the model dealt with the function of peptides in solution where they incorporate themselves into the bilayer. At concentrations below those required to spontaneously rupture the membrane, the effect of the peptides is to lower the rupture tensions systematically for all tension rates. The rupture of fluid membrane vesicles produces a distribution of breakage tensions, the tension rate and mean are known to rise with each other [15].

The micelle-forming amphiphile is incorporated in the lipid bilayer with a particular purpose knowing its affinity for a curvature or locally curved interface. This is in contradiction to the seemingly planar topology of lipid bilayer. This mismatch induces curvature stress which pops up many issues such as hydrophobic membrane core thinning, disordered strain, enthalpy, and free energy [1,16,17]. The strengths of the detergents are based on their chemical configuration of polar and apolar parts with their size and shape, accordingly its ability to induce the curvature stress can be determined. The detergent-like or carpet-like action of antimicrobial peptides will also influence their membrane-permeabilization ability [1,18–22].

15.6 Mechanism of pore formation and membrane lysis

It is essential to understand the mechanism of pore formation as various progressions such as the antimicrobial activity of peptides; membrane fusion and permeation require the transmembrane pores. The pore formation in lipid membranes is based on its atomic and molecular configurations and their dynamics [23].

15.6.1 The three-stage model by helenius and simons

Helenius and Simons configured the three-stage model to describe the role of the relative concentration of surfactant with lipid–surfactant system [5]. It encompasses the phenomenon at various concentrations of surfactants.

- The first stage occurs at a low concentration of surfactant in which the surfactant molecules partition into the membrane at low surfactant concentration.
- The second stage occurs at a concentration higher than critical concentration and includes two events, the coexistence of micelle with lipid membrane and solubilization of surfactant membrane.
- The third stage occurs at a concentration higher than the second critical concentration in which only micelles stay behind.

Notably, the above-mentioned description is of state at equilibrium, and the researchers observed the mismatch between the stages and the phase of surfactant concentration to bring about micelle formation in advanced proposing the call for researches on solubilization kinetics of membrane that are based on out-of-equilibrium setup [24].

15.6.2 Modes of membrane disordering

The surfactants bring about the solubilization and permeabilization of lipid membranes by two modes as a homogeneous or heterogeneous disordering of the membrane. Thus, the activity of surfactants can be two ways as follows [1]:

- Homogeneously disordering,
- Heterogeneously disordering.

In homogeneously disordering mechanisms, the disruption of the membrane occurs at the reach of curvature stress at a critical level. This is influenced by factors such as threshold concentration needed to lyse membranes, head group size, hydrophobic group volume, and partition coefficient. Based on the observations it is revealed that homogeneous disordering is usually caused by detergents or synthetic origin.

In heterogeneously disordering mechanisms, the disruption of the membrane occurs locally due to defects in structures. Saponins and lipopeptides act through heterogeneous disordering to result in selectivity, synergisms and greater activity of biosurfactants. Heterogeneous disordering shuns the tapering of the protein's environment and strong disordering. Heterogeneous disordering favors various processes such as micelle loading, destruction of target cells that occurs through membrane permeabilization effects, and membrane protein isolation. Time-resolved fluorescence anisotropy of diphenylhexatriene membrane probes makes a distinction between homogeneously and heterogeneously disordering mechanisms [1].

15.7 Applications of biosurfactants

Surfactants play major roles in many biological processes including hemolysis and channel formation in lipid membranes [1,2,5,25]. The biogenic surfactants derived from plant resources like saponins can permeabilize lipid membranes as well as complex cholesterol and thus play their role in defense mechanisms. In addition, the actions of antimicrobial active peptides are often compared to surfactants owing to their tendency to incorporation into the lipid bilayer and thus to permeabilize the lipid bilayers [5,25]. There are other biomedical applications of biogenic surfactants such as surfactants acting on lung alter alveoli surfaces to facilitate breathing. Surfactant acting on the gastrointestinal tract, such as bile salts interact with the bacteria flora in the colon as well as in the small intestine to assist in fat absorption. Additionally, biosurfactants of microbial origin act on their surfaces to change the adhesion properties or regulate the water-insoluble molecule availability to surfaces and thus to control the properties of microbial surfaces [1,26–28].

15.8 Structural aspects of biosurfactants playing role in hemolysis and membrane lysis

- Chemical structure of biosurfactants is more complex than synthetic [29–35];
- Spherical, circular, or branched structural components in varying sizes;

- At the interface, the closer packing of surfactants is possible due to spherical structural components in them to act efficiently;
- presence of proteases;
- low critical micelle concentrations;
- Lack of clear demarcation between hydrophobic and hydrophilic zones;
- hydrophobic zones showing variations in saturation;
- variable acetylation levels and sugar moieties causing alterations in hydrophilic zone;
- amphiphilicity of surfactants facilitate insertion and modulation of membrane structure;
- polarity in a mosaic pattern of distribution;
- mosaicity is responsible for the weaker binding of proteins to facilitate refolding of membrane protein.

15.9 Factors influencing pore formation

- Lipid composition of membrane;
- pH;
- curvature of the lipid membrane;
- surfactants affinity of the cell membrane.

15.10 Research work on the role of surfactants in hemolysis

In the early 19th century, the studies were carried out using artificial lipid membranes in place of erythrocyte membrane [3,7,11,12]. The ion channel formation ability of *Holothuroidea echinata* derived lectins was assessed. Additionally, the pore formation by CEL-III was also studied performing carboxyfluorescein leakage.

It was revealed that CEL-III has the ability of smaller pore formation in lipid membranes made up of human goboside or diphytanoylphosphatidylcholine [36]. To explore the surfactant-induced effects, the red blood cell membrane models are being commonly utilized. Several studies were conducted to predict the hemolytic potency of various surfactants. The hemolytic potency of biogenic surfactants depends upon various parameters such as hydrophile-lipophile balance, critical micellar concentration, and surfactant membrane/water partition coefficient. Researches emphasized how surfactant erythrolytic potency and their physicochemical parameters interact to bring about the changes in regards to hemolysis. The critical micellar concentration would determine the surfactant lytic capacity of nonionic surfactants [3,37–39].

Hemolysis of red blood cells has also been the basis for assays of some biosurfactant levels and measures of toxicity. A modified sheep RBC suspension assay and fish assay were compared for surfactin and its genetically modified variant fatty acyl glutamate. The study demonstrates the potential for screening for toxicity using this method [3].

The *Rhodococcus* sp. produces a succinoyl trehalose lipid, a biosurfactant that carries various activities including separation into the membrane of phospholipid. It would be of great significance and consequence to understand the nature of the interaction of trehalose lipid with biological

membranes. Initially, the trehalose lipid leads to the swelling of erythrocytes, and then hemolysis occurs when the concentration gets lower than the critical micellar concentration. It is demonstrated that in the process of hemolysis of human erythrocytes, the biosurfactant form pores within the erythrocyte membrane thus bringing about the creation of permeability zones in the red cell membranes by a colloid-osmotic mechanism. With the help of scanning electron microscopy, it is revealed that the trehalose lipid causes various changes in red blood cells such as echinocytosis or spherocytosis to support the bilayer-couple hypothesis. This phenomenon explains that the actions of trehalose lipid biosurfactants have a molecular base [7].

15.11 Research on the role of biosurfactants in pore formation and membrane lysis

Research work was carried out on lysis and leakage of vesicles using surfactin with isothermal titration calorimetry and calcein fluorescence de-quenching. *Bacillus subtilis* produces surfactin which is basically a lipopeptide. The diverse permeabilization behavior observed is attributed to surfactin-rich clusters, which can induce leaks and stabilize them by covering their hydrophobic edges. The detergent-like effects of antibiotic peptides on membranes characterize its hemolytic and antibacterial activity [1,4,40].

A study by Zakharova et al. evaluated the ability of fengycin to form ion-permeable pores in model lipid membranes mimicking the target fungal membranes. It is observed that the membrane conductance induced by fengycin enhanced the concentration at the second lipopeptide aqueous concentration. The existence of negatively charged species in the lipid bilayer appears to influence the pore formation ability of fengycin. While the molecular shape of membrane lipids appeared to have a lesser impact on pore formation [41].

Moreover, the selectivity of fengycin has been significantly influenced by the lipid composition of membranes of the target cell. The aggregation of fengycin on the membrane surface and the fatty acyl chains of lipids in the target cell membranes produce cell membrane disordering [41–43].

Researches were also carried out to search the lipopeptide-based drugs to lead to the development of daptomycin, the cyclic lipopeptide that works to target the cell membrane in cases of microbial resistance. The lipopeptide can act in two ways on the membrane to bring about its effect. It causes permeability changes by creating the transmembrane pores and also, it causes solubilization of lipid bilayer by acting as a detergent [40,44–49].

A study demonstrated how the solubilization kinetics and membrane properties affect the dynamics for short and long-lived pores. It was observed that the cyclic short-lived pores occur at a period inversely proportional to the solubilization rate. Biosurfactants induce pore formation through reduction of membrane surface area and the addition of membrane tension [5].

Awasthi et al. conducted a study on simulations of pore formation in lipid membranes. Although the transmembrane pores are performing a vital role in many biological processes, the mechanism of pore formation is poorly understood in terms of their free energy excursion which depends upon the potential mean force as per the reaction coordinate. It was observed that the reaction coordinates used in their study successfully led to pore formation however, there is a lag in the process of pore formation due to the occurrence of pore closing simulations. The reaction

coordinates may have their action on lipid as well as on water molecules, however acting on lipids they will have prominent effects in terms of hysteresis, the formation and disruption of a continuous hydrogen-bonding network across the membrane correspond to the magnitude of pore formation. Thus the study provides molecular insights into pore formation and thus may direct the development of pore formation efficiently in terms of favorable influences reaction coordinates [23].

15.12 Conclusion

Biomedical Applications of Biosurfactant is the burning area of research these days. Solubilization, permeabilization, and lysis of membrane are important phenomena in many biological applications wherein the biogenic surfactants and detergents have major contributions in various phases. The surfactants derived from natural sources may have key functions to perform in hemolysis and the formation of pores or channels in lipid membranes. However, the molecular basis of these biological processes in regards to the role of lipid biosurfactants is not fully understood and needs further exploration.

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CHAPTER

Biosurfactant as a vehicle for targeted antitumor and anticancer drug delivery

16

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16.1 Introduction

Biosurfactants are amphibious compounds, synthesized both intracellularly or extracellularly by microorganisms. The various chemical structures of biosurfactants include lipopeptides, glycolipids, polysaccharide-protein complexes, neutral lipids, fatty acids, and phospholipids [1]. Microorganisms that produce the biosurfactant are ubiquitous, found in freshwater, groundwater, and sea, and also in sludge, soil, and sediment [2]. Due to their amphibious characteristic, surfactants are active substances on the surface that normally align between the interfaces of the two phases with varied hydrogen bonding and polarities in air/water or oil/water interfaces [3]. Surfactants have both hydrophilic and hydrophobic groups where a hydrophobic group generally contains hydrocarbons, whereas the hydrophilic group is nonionic, amphoteric, and positively or negatively charged component [1]. Biosurfactant may therefore possess a hydrocarbon ring and a polar amino acid head [1]. As shown in Fig. 16.1, biosurfactants have diverse applications in food industries, agriculture, cosmetics, biomedical, petroleum industries, and bioremediation [2]. As nanosized drug carriers, biosurfactants are more advantageous than their chemical counterparts for targeted delivery to many disease sites [2,5]. Surfactin can be produced by *Bacillus* strains during the stationary phase with limited oxygen and nutrients in the culture media [2]. A biosurfactant composed of glycoprotein, synthesized by a probiotic, *Lactobacillus paracasei*, with the composition of the biosurfactants commonly produced by lactic acid bacteria, could be applied in human cells, for its antimicrobial activity against several pathogenic bacteria [6].

Biosurfactants are mainly secondary metabolites, playing crucial roles for survival especially in nutrient transport, microbe-host interactions, and quorum sensing, or for use as biocide agents [7]. Biosurfactants are superior to synthetic surfactants as they are eco-friendly, of microbial origin, and have low toxicity. These are advantageous for industrial applications such as food and cosmetics, enhanced oil recovery, and biological treatment [7]. Biosurfactants can be classified into high (such as polysaccharides, proteins, and lipoproteins) and low molecular weight (such as glycolipids and lipopeptides). With simple structures, low molecular weight biosurfactants have effective surface-active features [8]. Biosurfactants assist microbial adhesion especially at the interfaces of the fluid phase with polar and hydrogen bonding interactions. Biosurfactants may damage the cell

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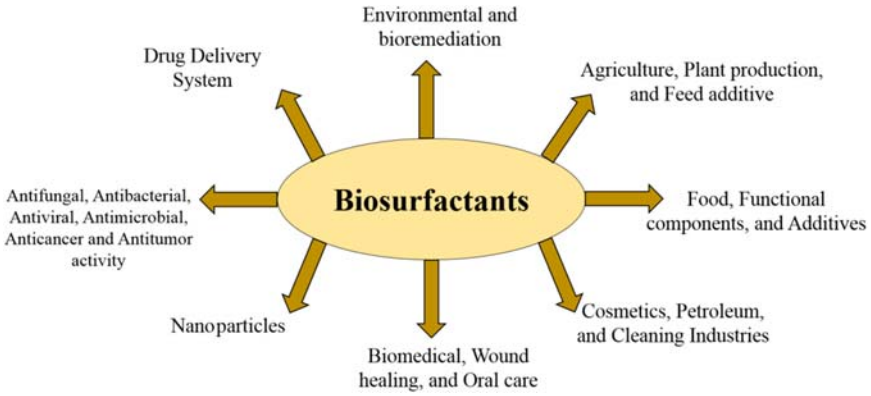


FIGURE 16.1

Biosurfactant applications.

Modified from P.J. Naughton, R. Marchant, V. Naughton, I.M. Banat, Microbial biosurfactants: current trends and applications in agricultural and biomedical industries, J. Appl. Microbiol. 127 (1) (2019) 12–28. https://doi.org/10.1111/jam.14243 [4].

membranes resulting in cell degradation, increasing the membrane permeability, and finally releasing the metabolites [9]. Physical membrane structural changes or alterations in the protein specificity will modify vital functions of the membrane including energy generation and transportation [10]. These tunable properties can be tailored toward specific applications in industries.

16.2 Properties of biosurfactant

The unique properties of biosurfactant stem not only from its surface activity at the interfaces, but also its low toxicity, high biodegradability, moderate conditions for its production, biocompatibility, high selectivity, and specific activities even at high salinity, temperatures, and pH [5]. However, contrary to synthetic surfactants, biosurfactants have a comparatively unclear boundary between their lipophilic and hydrophilic groups. The highly complex origin of the head groups (saccharides in glycolipids and amino acids in lipopeptide) makes it difficult to determine their exact structures, as these may vary even with a slight change in the environment. For example, in the presence of 1 and 2 carboxylic acids in the surfactin and di-rhamnolipids, the biosurfactants may become anionic with increased acidity, and nonionic at lower acidity [8]. The amphibious nature of the biosurfactants however promotes diversity of surface activities for applications in many areas related to foaming, emulsification, disinfection, dispersion, wetting, and dissolvation [11].

Surface tension is the measure of biosurfactant efficiency which is the amount of free energy to transfer a molecule from the bulk phase to the surface [1,12]. Biosurfactants reduce the interfacial free energy and decrease the interfacial tension by changing the bulk molecules to higher energy. A biosurfactant can effectively reduce the surface tension of water by reducing the water and air surface tension (from 72 mN/m to lower than 30 mN/m) [2]. Surfactin decreases the surface tension of water at 10 μM concentration to 27 mN/m [13], as similarly reportedly attained by the rhamnolipids [14].

The sophorolipids synthesized by *Candida bombicola* decrease the surface tension to 33 mN/m, while trehalose lipids and mannosylerythritol lipids (MELs) could reduce to lower than 30 mN/m [2]. The change in the surface tension depends on the critical micelle concentration (CMC). The CMC is the minimum concentration of surfactant to start micelle production, and the added extra-surfactant to promote micelle formation. It suggests the maximum biosurfactant monomer concentration in water and can be affected by temperature, ionic strength, and pH [1,13]. Hydrophilic lipophilic balance (HLB) describes the contribution of lipophilic and hydrophobic groups of surfactants in the emulsion which affects the emulsion stability. Low HLB (from 3 to 6) suggests the formation of water/oil microemulsions, while high HLB (from 8 to 18) suggests the oil/water microemulsions. The surfactants with very high HLB (more than 20) are necessary to be applied with a cosurfactant to decrease the active HLB. HLB is however only usable for nonionic surfactants. For ionic surfactant, it has to be measured empirically on a relative basis [2].

To reduce the contact angle of water with the hydrocarbon chains, the biosurfactant head in the aggregate formation in solutions will interact within the water phase while the hydrocarbon chain will be orientated on the inside compartment of the aggregates. Besides the micelles, the type of aggregates is defined based on the size of a hydrophobic carbon chain relative to the biosurfactant group and its water behavior. The critical packing parameter (CPP) explains this phenomenon and it is highly affected by various moieties of the biosurfactant and environmental factors. Thus, the biosurfactants showing different CPP will tend to orientate in different ways [2]. The aggregation can be described by spontaneous curvature (H_o) from the film of the surfactant. The significance of H_o is related to the CPP as the band will also be based on the relative ratio of the nonpolar and polar sizes. The hypothesis depends on the structural features of the whole film. In general, the CPP describes the individual molecules, while the H_o describes the chain with universal physical features [2]. The total reaction ability ratios (per unit interfacial area) of the surfactant for oil and water phases are called the ratio of Winsor-R. There are 3 potential states: (1) $R < 1$, which indicates that the reaction of the water surfactant is higher than the reaction of the oil surfactant and produces Winsor microemulsions Type 1; (2) $R > 1$, indicates that the strength of interaction between the oil-surfactant is higher than with the water-surfactant, and produces the Winsor microemulsions Type 2; (3) $R = 1$, represents the state in balanced interactions which leads to the production of Winsor microemulsions Type 3 [2].

16.3 Antitumor and anticancer properties of biosurfactants

Biosurfactants can be effective therapeutic agents and are viable alternatives to synthetic drugs and antimicrobials. The therapeutic applications of biosurfactants include as antigen adjuvants, for gene transformation, as inhibitors of fibrin-clot formation, or activators of fibrin-clot lysis, coating for biomaterials, additive to probiotics to counter urogenital tract infection, for immuno-pulmonary treatment, and as anticancer [8], and antitumor agents to cancer progression [6]. The discovery of biosurfactants with antitumor activities has generated interest in the search of novel compounds as well as their mechanisms of action [6]. Antitumor and anticancer activities of biosurfactants are shown in Table 16.1. Biosurfactants involve in many intracellular molecular reactions such as signal transduction, cell differentiation, and immune responses [32]. The applications of succinoyl-trehalose lipids (STLs) and MELs cause cell-growth arrest and apoptosis in cancer cells [33]. Glycolipids (the

Table 16.1 Antitumor and anticancer activities of biosurfactants against different cancer cell lines.

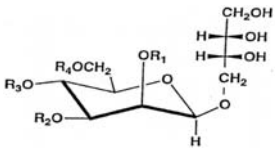
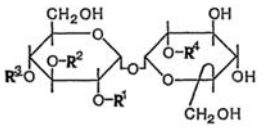
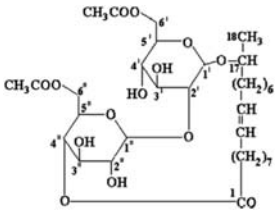
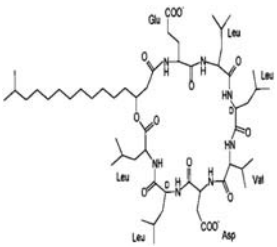
Biosurfactants	Cancer cell line	Results	Structure	References
Mannosylerythritol lipids (MELs)	Myelogenous leukemia (K562)	MEL (5.0 μM for 3 days) down-regulates the tyrosine kinase activities in the cancer cell (K562 cells) to prevent the cell growth and promote differentiation		[15]
Succinoyl-trehalose lipids (STLs)	Promyelocytic leukemia (HL60)	Significant effects of STL-3 (3 μM for two days) and its analogs on inhibition of cell growth, but only induce the differentiation of HL-60 cells.		[16]
	Human monocytoid leukemic cell-line (U937)	STL-1 is responsible in inducing differentiation of U937 into monocyte-macrophage, leading to cytotoxic substances production		[17]
Sophorolipids	Promyelocytic -leukemia (HL60)	Interaction with plasma membrane		[18]
	Liver cancer (H7402)	Sophorolipid (28.66/25.45 μg/mL) can induce cell death in H7402 cells by cell cycle arrest at the G1 phase and partially at S stage, with caspase 3 activation, and enhanced concentration of Ca^{2+} in the cytoplasm		[19]
	Lung cancer (A549)	Apoptosis induction with IC_{50} of 36.82/34.58 μg/mL		
	Pancreatic cancer (HPAC)	Sophorolipid (50 μg) show cytotoxic activity against HPAC cell lines by induction of necrosis, with no effect on normal human cells		[20]

Table 16.1 Antitumor and anticancer activities of biosurfactants against different cancer cell lines. ***Continued***

Biosurfactants	Cancer cell line	Results	Structure	References
	Esophageal cancer (KYSE109/ KYSE450)	Sophorolipid at 30 μg/ mL shows strong growth inhibition of esophageal cancer cells		[21]
Surfactin or Surfactin-like biosurfactants	Breast cancer (MCF-7)	Surfactin biosynthesized by *Bacillus subtilis* natto TK-1 inhibit MCF-7 cells at IC_{50} of 82.6, 27.3, and 14.8 μM after 24, 48, and 72 h treatment, respectively, by inducing apoptosis with increased Ca^{2+} and cell cycle arrest at G2/M phase.		[22]
		Surfactin-like compound purified from *Bacillus subtilis* CSY191 inhibits MCF-7 cells after 24 h at IC_{50} of 10 μg/ mL.		[23]
	K562	The cyclic lipopeptide (CLP) (2−64 μg/mL) produced by *Bacillus subtilis* natto T-2 inhibits proliferation in K562 cells by inducing cell death through cell cycle arrest at G1 phase.		[24]
	Colon adenocarcinoma (LoVo)	Surfactin at IC_{50} of 26 μM after 48 h treatment strongly inhibits the growth of LoVo cells by promoting pro-apoptotic agents and cell-cycle arrest		[25]
	Colorectal cancer (HepG2)	Surfactin produced by *Bacillus* natto TK-1 can induce apoptosis in HepG2 cells by		[26]

(*Continued*)

Table 16.1 Antitumor and anticancer activities of biosurfactants against different cancer cell lines. ***Continued***

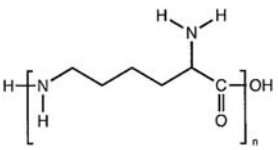
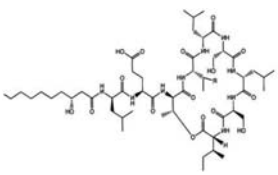
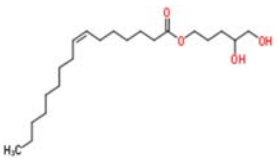
Biosurfactants	Cancer cell line	Results	Structure	References
		reactive oxygen species (ROS)-activated endoplasmic reticulum stress (ERS), and increased Ca^{2+} concentration		
	Colon cancer (HCT15/HT29)	The lipopeptides show a significant antiproliferative activity against the HT-29 cells (IC_{50} 120 μg/mL) and HCT-15 (IC_{50} 80 μg/mL).		[27]
	Breast cancer (MDA-MB-231 and T47D), and normal fibroblast cell line (MC-3 T3-E1)	Surfactin purified from *Bacillus subtilis* 573 has been evaluated against T47D, MDA-MB-231, and MC-3 T3-E1, and exhibits inhibition of cell growth and cell cycle arrest at G1 phase.		[6]
e-poly-L-lysine	Cervix adenocarcinoma (HeLaS3), HepG2 and Colorectal cancer (CaCo)	A e-poly-L-lysine biosynthesized by *Bacillus subtilis* SDNS is tested against HepG2, CaCo, and HeLa S3. The maximum inhibition activity achieved against HeLa S3 cell line (77.2%), with no activity against CaCo cell line (9.8%) after 72 h treatments.		[28]
Viscosin	Metastatic prostate cancer (PC3M)	Viscosin produced by *Pseudomonas libanensis* M9−3, inhibits migration of the PC-3M, without any toxicity.		[29]
Serratamolide	B-Chronic lymphocytic leukemia (BCLL)	Serratamolide purified from *Serratia marcescens* inhibits BCLL cell by		[30]

Table 16.1 Antitumor and anticancer activities of biosurfactants against different cancer cell lines. ***Continued***

Biosurfactants	Cancer cell line	Results	Structure	References
		inducing intrinsic apoptosis at IC_{50} of 13 μM through cytochrome *c* release and caspase-9 and -3 activation, but without caspase-8 activation, and with no effect on normal peripheral blood lymphocytes (PBL).		
Monoolein	HeLa, U937, and Vero	Monoolein isolated from palm-oil contaminated soil show antiproliferative activity against U937 and HeLa cell lines in a dose-dependent manner with no activity against normal Vero cells even at higher concentrations. Cell and DNA morphological changes (cell size reduction, blebbing of the membrane, and DNA fragmentation) are obtained in both cancer cells		[31]

Modified from E.J. Gudiña, V. Rangarajan, R. Sen, L.R. Rodrigues, Potential therapeutic applications of biosurfactants, Trends Pharmacol. Sci. 34 (12) (2013) 667–675. https://doi.org/10.1016/j.tips.2013.10.002.

amphipathic molecules containing lipids with attached carbohydrates) participate in the cell-cycle arrest and induction of apoptosis in mouse malignant melanoma (B16) cells. Increased concentration of MELs lead to the aggregation of B16 cells at G0/G1 subphase (a sign of apoptotic induction). A series of programmed cell death events have been observed such chromatin condensation, and DNA damage, suggesting the ability of MELs to induce apoptosis in the B16 cells [33]. The study further suggests that the organization of protein kinase C (PKC) activity may be related to the programmed cell death caused by the MELs. PKC activation is one of the first steps in signal transduction, resulting in multiple cellular responses. The PKCs are important in cell growth control, differentiation, and death. The MELs could induce human leukemia cells (HL60) differentiation to granulocytes, suggesting that they can activate both apoptosis and differentiation [18].

Glycolipids, such as the crude glycolipid produced by *Sphingobacterium detergens*, exhibit antitumor promoting action against human colorectal cancer (Caco2) cells [34]. The sophorolipids synthesized by *Wickerhamiella domercqiae* show antitumor effects against human liver cancer cells (H7402) by induction of apoptosis through the cell-cycle arrest at the G1 phase, with Caspase 3 activation and increased concentration of Ca^{2+} in the cytoplasm [19]. Several lipopeptides synthesized by *Pseudomonas*, *Serratia*, and *Bacillus* species also show antitumor activities against different cancer cell lines [8]. Monoolein biosurfactant synthesized by the *Exophiala dermatitidis* SK80 (dematiaceous fungus) prevents the development of leukemia (U937) and cervical cancer (HeLa) cells in a dose-dependent manner, with no toxicity against normal cells even at high concentrations [8]. Surfactin (lipopeptide) could prevent cell proliferation by stimulating the pro-apoptotic factors and cell cycle arrest. Surfactin prevents the PI3K/Akt signaling pathway, and plays a key role in controlling proapoptotic mechanisms including the cell cycle arrest, making Surfactin capable of regulating the cell cycle and suppressing the cancer cell progression [8].

A degree of saturation of sophorose, and to a lesser degree of hydroxyl fatty acids and lactone has produced new compounds which could cause morphological changes of cells and DNA [31]. Glycolipids such as α-myrmekioside or trikentroside and galacosylceramide (KRN7000) are involved in cell growth inhibition and cell death of numerous cancer cells. KRN7000 contains a fraction of sugar bound to a sphingosine-base and a fatty acid chain which exhibits anticancer activity against lung, colon 26 adeno-carcinoma, liver, EL-4T cell lymphoma, sarcoma, and EL-4 lymphoma [11]. Myrmekioside and its derivatives (E-1, E-2, and E-3) isolated from *Myrmekioderma dendyi* show anticancer activity against A549 and NSCLC-N6 cell lines, while trikentroside produced by *Trikentrion* sponge shows cell cycle arrest of A549 [35]. The anticancer effects of surfactin or surfactin-like biosurfactants on pancreatic (SW1990), rat melanoma cancer (B16), and human oral epidermoid carcinoma (KB-3-1) have also been reported using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Surfactin-like lipopeptide exhibits cytotoxicity against SW1990, KB-3-1, and B16 cell lines after 24 hours treatments with IC_{50} of 58 ± 1.6, 57 ± 2.6, and 20 ± 1.6 μM, respectively [36]. Iturin A biosurfactant synthesized by *Bacillus megaterium* shows significant antiproliferation activity by inhibiting the Akt signaling pathways, resulting in apoptotic induction in breast cancer cells (MCF-7 and MDA-MB-231) and inhibit tumor growth in breast cancer xenograft model [37]. Iturin A can inhibit the epidermal growth factor (EGF) caused by Akt phosphorylation and also targets proteins including Glycogen Synthase Kinase 3 (GSK3β) and Forkhead family of transcription factor (FoxO3a) [11]. Hallobacillin purified from *Bacillus* sp. shows anticancer activity on HCT-116 cell line (IC_{50} 0.98 μg/mL), while Mixirins (A, B, and C) also produced by *Bacillus* sp. exhibit anticancer activity on colon cancer cells, especially type A. Mixirins are cyclic-octapeptides consisting of a combination of D and L amino acids with abnormal β-amino alkanoic acid [38].

Other biosurfactant molecules that have exhibited anticancer activities include fellutamides, somocystinamide A, apratoxin, and rakicidin. Fellutamides A and B are linear lipopeptides produced by *Penicillium fellutanum* (fish-derived fungus) exhibit cytotoxic activities on murine leukemia cells (P388, L1210) and human epidermoid carcinoma cells (KB) [39]. Fellutamides C and F are produced by *Aspergillus versicolor* which exhibit cytotoxicity against XF498 CNC cancer, A549, colon cancer (HCT-15), ovarian cancer (SK-OV-3), and skin cancer (K-MEL-2) [40]. Somocystinamide A produced from *Lyngbya majuscula* (cyanobacteria) has demonstrated significant cellular toxicity against lung, leukemia, prostate, myeloma, and breast cancer at IC_{50} between 3 nm and 5.8 μm, based on the cancer type [41]. It is considered a multipotency inhibitor of angiogenesis and tumor cell proliferation

and can cause programmed cell death in leukemia and Jurkat cells by activating poly (ADP-ribose) polymerase (PARP) cleavage and caspase-8 [11]. Apratoxins (derivatives A to G) are purified from cyanobacteria, and apratoxin A is reported to induce apoptosis by activating caspases and blocking the IL-6 signaling pathway in U2OS human-bone osteosarcoma cells [42]. Rakicidins, the lipopeptides isolated from *Micromonospora* 9 marine bacteria, exhibit hypoxia-selective cellular toxicity on cancer cell lines such as PANC-1 and HCT-8. Hypoxia is normally found in solid tumors which can escape apoptosis, leading to angiogenesis, invasion, and metastasis [43].

There has been a progress in the drug-vector development based on surface-active molecules, such as biosurfactants that react with lipids of the cell membrane; or design factors that react with other lipids participating in many cellular developments. Biosurfactants can be applied to impact the sphingolipids activity as these lipids are effectors that regulate different parts of cell growth and reproduction, and are therefore potential anticancer therapies [44]. Surfactin carries long-chain fatty acids that could efficiently permeate in the membrane of the cancerous cells. The anticancer activity of surfactin may be due to the hydrophobic nature of the fatty acid that can react with the acyl chain of the phospholipids in the membrane. At the same time, the peptide portion reacts strongly with the polar heads of the membranous lipids in cancerous cells [11,36]. The potential mechanisms of antitumor activity can therefore be attributed to the ability of biosurfactants to disrupt the cell membranes [45]. As shown in Fig. 16.2, biosurfactants as anticancer agents interfere with cancer development [11]. These may involve molecular intercellular interactions that include cell differentiation, signal transduction, and cellular immune responses [32]. The anticancer activity mechanisms of biosurfactants include delaying the cell cycle progression, stimulation of apoptosis via the death-receptors in cancer cells, prevention of critical signaing pathways (such as Akt, Kinase/Signal-transducer, extracellular signal-regulated-Kinase/c-Jun-*N*-terminal-kinase (ERK/JNK)), and transcription activator (JAK/STAT), reduction of angiogenesis, activation of natural killer T (NKT) cells, and disruption of cell membranes, leading to cell lysis, enhanced permeability of the membrane, and metabolite leakages [45].

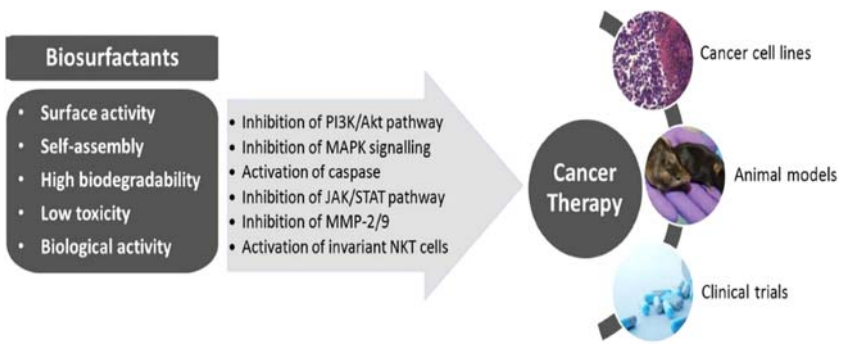


FIGURE 16.2

Anticancer properties of biosurfactants for new cancer treatment strategies.

From E.J. Gudiña, J.A. Teixeira, L.R. Rodrigues, Biosurfactants produced by marine microorganisms with therapeutic applications, Mar. Drugs 14 (2) (2016). https://doi.org/10.3390/md14020038 [11] with permission from MDPI (Creative Commons by Attribution (CC-BY) license).

The anticancer activity of surfactin is related to apoptosis, suppression of cell growth, cell cycle arrest, and inhibition of metastasis. Surfactin inhibits cancer cells by interfering with the cell survival signaling pathways, and also by controlling the cell-cycle regulatory proteins. The intrinsic mitochondrial or caspase pathway induced by surfactin, namely JNK/19 m/Ca^{2+}/Bax-to-Bcl-2 ratio/cyt *c* and ERS/Ca^{2+}/ERK1/2 pathways, may be activated by high ROS production. Surfactin induction of apoptosis is related to the modified phospholipid composition, resulting in a significant reduction in the unsaturated cellular fatty acids. Surfactin also prevents migration, invasion, and colony generation of cancer cells based on metalloprotenaise-9 (MMP-9) expression which interferes with the ERK1/2, PI3K/Akt, and NF-κB, AP-1 signaling pathways as shown in Fig. 16.3 [1]. The surfactin lipopeptide also induces apoptosis in the

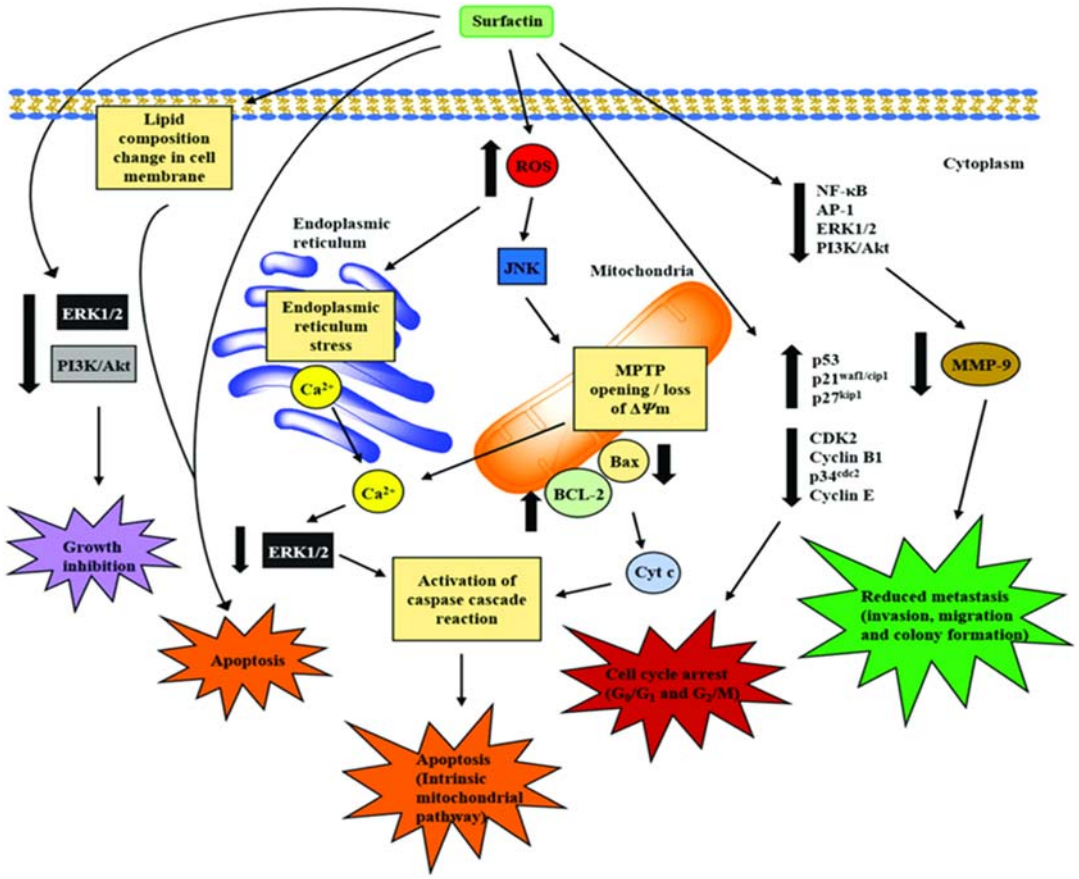


FIGURE 16.3

Potential mechanisms involved in the anticancer activity of surfactin.

From Y.S. Wu, S.C. Ngai, B.H. Goh, K.G. Chan, L.H. Lee, L.H. Chuah, Anticancer activities of surfactin potential application of nanotechnology assisted surfactin delivery, Front. Pharmacol. 8 (2017) 1–22. https://doi.org/10.3389/fphar.2017.00761 [1] with permission from Frontiers Media S.A. (Creative Commons by Attribution (CC-BY) license).

MCF-7 cells by ROS/c-Jun N-terminal kinase via mitochondrial-mediated caspase pathway, and enhances the production of ROS, leading to mitochondrial permeability and possibly membrane damage, which ultimately increases the concentration of Ca^{2+} in the cytoplasm [46]. The release of cytochrome *c* to the cytoplasm leads to the activation of caspase-9, and finally apoptotic induction. Surfactin has been shown to reduce the growth of MCF-7 cells by cell cycle arrest at the G2/M phase by controlling their cell cycle factors. Surfactin stimulates the p53 expression (tumor inhibitor) and the cyclin-kinase inhibitor p21waf1/cip1, and controls the activity of the G2 specific-kinase, cyclin B1/p34cdc2 [8].

16.4 Biosurfactants as drug carriers

The development of novel therapeutics and drug delivery systems (DDSs) with enhanced activities has important impacts on the ability to treat diseases such as cancer [47]. The optimal DDS should have two major characteristics: (1) optimum drug loading capacity, which increases the bioavailability of the drug to reach the desired target; and (2) targeted and controlled release of the drug. To achieve these, drug carriers such as particulate, polymeric, cellular, and macromolecular structures have been developed. Particulate types are found in spread colloidal forms such as nanoparticles, lipid particles, micelles, microspheres, and vesicular systems such as liposomes, noisome, virosomes, and sphingosomes [48]. The use of biosurfactants as a DDS provides attractive features such as passive delivery, especially when the drug therapy activity is limited. Candidiasis treatment for instance is complex as a limited number of antifungal drugs have shown no toxicity or side effects in humans. The use of controlled delivery by combining the antifungal drug within the different drug delivery agents has been proposed as the solution [4]. However, the limitation of DDS ranges from poor activity during drug delivery to sedimentation attributable to the dilution with biofluids before arriving at the target location. The most pertinent requirement is the use of biologically compatible and biodegradable therapeutic agents as their components [8]. Biosurfactants are unique in meeting all these as effective DDS.

16.4.1 Microemulsions

Self-emulsifying DDSs are nanosize and spherical in shape and could solubilize hydrophobic drugs, to facilitate the adsorption of drugs along the intestinal lymphatic pathways [2]. Microemulsions as new DDSs are suitable for oral, ocular, nasal, transdermal, local, parenteral, and intravenous methods of drug administration. Although microemulsions have generated great interest because of their ease of formulation, preclinical and systematic studies are still necessary for the optimal formulation, safety, and efficacy standards for any particular route of drug [8]. Microemulsions produced by using biosurfactants are thermo-dynamically stable and are promising as DDSs as they are easy to prepare and highly soluble, forming an isotropic system with long-term stability [49]. Liquid-self-emulsifying DDS formulations may have cosurfactants, oils, surfactants, and/or cosolvents. The surfactants are necessary to help dissolve the drug if it is used in relatively high quantities, and not only because of the low interstitial tension, but also to increase the drug permeability [2]. Fig. 16.4 shows the difference between an emulsion and a microemulsion [50,51]. A microemulsion-based colloidal DDS usually involves an aqueous phase, an oil phase, a surfactant, and possibly also a cosurfactant or a cosolvent. The main component of a microemulsion system is

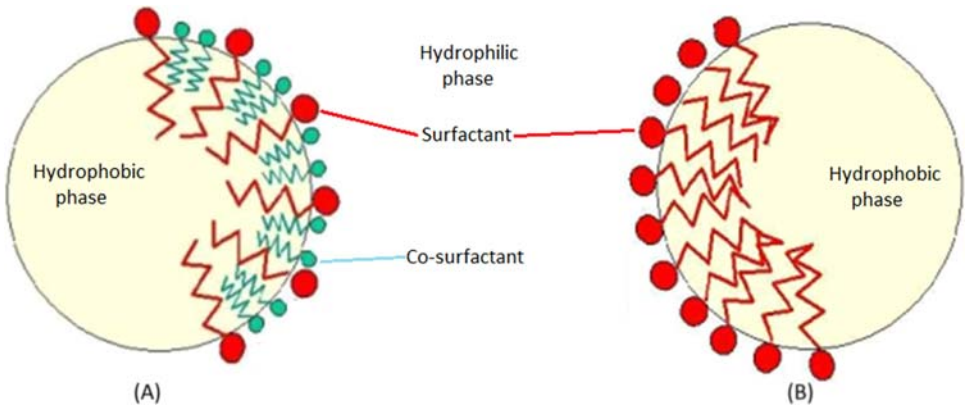


FIGURE 16.4

(A) Emulsion (droplet diameter 1–20 mm); (B) microemulsion (droplet diameter 10–100 nm).

Modified from A. Mishra, R. Panola, A.C. Rana, Microemulsions: as drug delivery system, J. Sci. Innovative Res. 3 (4) (2014) 467–474 [50].

a surfactant that self-aggregates to form molds of different constructions. These constructions can wrap or melt a hydrophilic or hydrophobic drug in a diffusing phase [water in water/oil (W/O) and oil in oil/water (O/W) microemulsions] in the center (spherical shape), thus separating the diffused stage from the continuous stage [8,52].

Pharmaceutically suitable excipient for the design of safer microemulsions is of paramount importance. Normally synthetic hydrocarbon oils are used such as cyclic oils (cyclohexane), dodecane, heptane, and surfactants with hydrophobic carbon chains like stetraethylene-glycol-monododecyl ether and sodium-dodecyl sulfate, but the formulations may have biocompatibility issues with some side-effects [53]. The use of lecithin and nonionic surfactants such as Brijs, Arlacel 186, Spans, AOT, and Tweens, which are amphiphilic compounds (microemulsion system components), exhibit higher biocompatibility [8]. Sophorolipids and rhamnolipids can also be mixed with lecithin to synthesize biocompatible microemulsions where phase behavior is virtually insensitive to changes in salt concentration and temperature, thereby making them attractive for DDS and cosmetic applications [2]. Rhamnolipids as a cosurfactant changes the HLB level of a renewable methyl-ester-ethoxylate surfactant, to form a microemulsion with the O/W limonene system, while the oleyl-alcohol serves as a hydrophilic link. The micellization and intersectional behavior of a surfactin and sodium dodecyl-benzyl sulfonate mixture indicate that the synthesis of mixed micelles is thermo-dynamically feasible. The advantageous biosurfactant properties in the mixture of biosurfactants with synthetic surfactants represent environmentally friendly and more sustainable formulation as compared to the conventional systems and also at a reduced cost with limited use of biosurfactants. This could spur the formation of a more effective and eco-friendly formulation [54].

16.4.2 Nanoparticles

Nanoparticles (NPs) are distinguishable by their nanosizes, fixed shapes, and unique geometries. The production of NPs is still challenging due to the high cost of the currently available

technologies, also it generates hazardous wastes and unstable NPs with lower target specificity. Cleaner, nontoxic, and eco-friendly synthesis of the NPs are critical to promoting the widespread applications of NPs in biotechnology and targeted drug delivery, such as the use of molds for the synthesis of NPs [2,8]. Also, the release of a drug in the lipid polymer-coated-hybrid NPs is stimulated by the rhamnolipids isolated from *P. aeruginosa* biofilm. Phosphatidyl-choline (PC) and poly (lactic-*co*-glycolic-acid) (PLGA) are used as the polymer-NP lipid coating and core, respectively. The drug could be released near the colonies of *P. aeruginosa*, thereby enhancing the antibacterial activity of those NPs. The release of a rhamnolipid-stimulated drug by *P. aeruginosa* biofilm culture implant in sputum under in vivo conditions proves that biosurfactants could also be used in the DDSs (Fig. 16.5) [55]. In another study, the gold NPs (AuNPs) and silver NPs (AgNPs) have been synthesized using *B. subtilis* bacteria. The AuNPs are produced inside and outside the cell, while the AgNPs are synthesized exclusively outside the cell. The results indicate that the NPs are stabilized by the active molecules on the surface, which are the surfactin or other biomolecules released by *B. subtilis* [56]. AuNPs exhibit great potentials for gene delivery, imaging technologies, targeted therapy [57], and therapeutic applications such as antiangiogenesis, antiHIV, antimalarial, and antiarthritis activities. AgNPs also exhibit antiinflammatory, antifungal, antiviral, antiplatelet and antiangiogenesis activities [58]. Rhamnolipids isolated from *P. aeruginosa* have been used to synthesize the AgNPs which exhibit activities on *Candida albicans* and also the Gram-negative and Gram-positive bacteria, indicating their broad spectrum of antimicrobial activity [59]. The antibacterial activity of sophorolipid-coated AgNPs and AuNPs against Gram-negative and Gram-positive bacteria has also been demonstrated. However, the sophorolipid-coated AuNPs are more cyto and geno-compatible than the AgNPs [2].

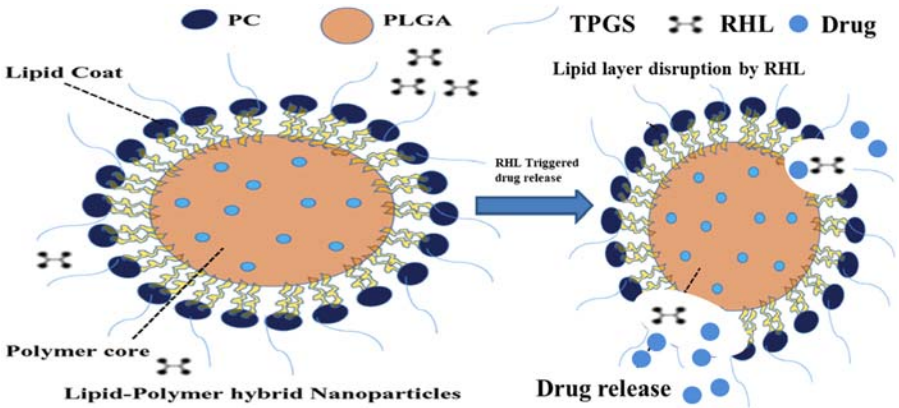


FIGURE 16.5

Rhamnolipid (RHL)-triggered drug release (TPGS: *d*-α-tocopheryl poly(ethylene glycol) 1000 succinate).

Modified from W.S. Cheow, K. Hadinoto, Lipid-polymer hybrid nanoparticles with rhamnolipid-triggered release capabilities as antibiofilm drug delivery vehicles, Particuology 10 (3) (2012) 327–333. https://doi.org/10.1016/j.partic.2011.08.007[55].

16.4.3 Vesicles

Biosurfactants contain lipopeptide that could enhance the immune response, making them potential adjuvants in vaccines [4]. Vesicular DDSs, which include noisomes and liposomes, are potentially important for targeted drugs delivery with reduced unwanted toxicity [60]. Noisomes are nonionic surfactant-based vesicles, consisting of a hydrophobic chain, making them highly appropriate as carriers in DDS (Fig. 16.6A). Noisomes are formed by hydration, with or without the addition of cholesterol or other fats. Noisome-hydrophilic core is considered an ideal environment for hydrophilic drugs where the drugs are primarily confined in the hydrophilic areas below the oily layer [4]. Liposomes consist of 2 hydrophobic tails (Fig. 16.6B), and with or without cholesterol in the structure [63]. They have been used as promising vehicles, depending on the drug delivery pathways, with broad applicability including vaccination. MEL-A, a type of biosurfactant glycolipid contains cationic-liposomes that could increase the efficiency of gene transfer by five to sevenfold in cultured mammalian cells [64]. Liposome carriers having B-sitosterol B-D-glucoside biosurfactant complexed-DNA has been used in gene therapy of the herpes simplex virus thymidine kinase. The nanocarriers of biosurfactant could enhance the effectiveness of the in vitro and in vivo transfection of genes [8]. The improved delivery of small interfering-RNA (siRNA) in HeLa cells using surfactin cationic liposomes has been achieved, better than the free surfactin. The surfactin-liposomes with their higher biocompatibility may enhance specific silencing of gene, thus more efficient delivery system, resulting in increased cell uptake of siRNA, thereby increasing the effect of a specific knockout [65]. Patents based on Rhamnolipid-liposomes as DDSs have been filed or granted as small capsules for nucleic acids, drugs, dyes, proteins, and other compounds. These are considered as safe and biodegradable, with acceptable bioaffinity, stability, and longer duration of shelf-life [2]. Rhamnolipids have been used to increase the oral drug absorption, which could improve, even at low concentrations, the para-cellular and trans-cellular transport mechanisms in

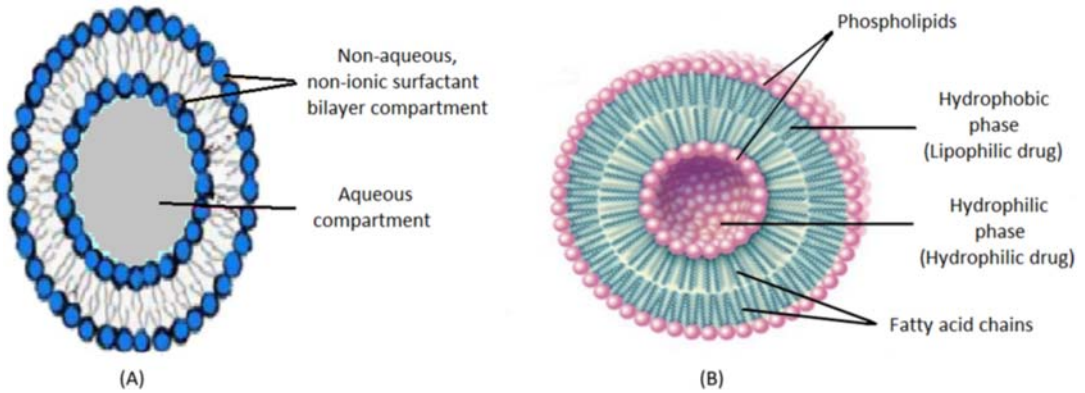


FIGURE 16.6

(A) Nonionic surfactant vesicle (Niosome) and (B) liposome.

Modified from G.P. Kumar, P. Rajeshwarrao, Nonionic surfactant vesicular systems for effective drug delivery – an overview, Acta Pharmaceutica Sin. B. 1 (4) (2011) 208–219 [61] and M. Fahim Uddin, Liposomes, 2014. https://www.slideshare.net/bharathpharmacist/liposomes-39686019. (Accessed 30 March 2021) [62].

the Caco-2 cells, and prevent the P-glycoprotein (P-gp) activities. The rhamnolipids also exhibit low cytotoxicity against Caco-2 cells and erythrocytes [66].

16.5 Conclusion and future outlook

Biosurfactants synthesized from microorganisms have shown antiviral, antimicrobial, antitumor, and anticancer activities, which could therefore be used as alternatives to conventional treatments. Biosurfactant molecules are an excellent alternative to synthetic surfactants due to their low toxicity and biodegradability, and also of microbial origin. These are advantageous for applications in the food and cosmetic industries, improved recovery of oil extraction, and biological treatment. The antitumor and anticancer activities can be attributed to the inhibition of tumor/cancer cell proliferation, cell death (apoptosis), cell cycle, and metastatic arrest. The hydrophobic nature of the fatty acid component in the biosurfactants can react with the acyl-chain in the phospholipid membrane, and the peptide portion reacts strongly with the polar heads of the cancerous cells membranous lipids. The biosurfactants could therefore disrupt the cancer cell membranes resulting in cell lysis, enhanced membrane permeability, and leakage of metabolites. Biosurfactants have found applications in cosmetics and as antibiotics and have also met the requirements of drug control bodies around the world for being biologically compatible and nontoxic which provide the impetus for successful application in the formulations of drug delivery. The types of DDSs that can be developed include the particulate, polymer, cellular vectors, and macromolecular carriers. A particulate type includes the microspheres, NPs, liposomes, and micelles. Further research on their mechanisms of action, in vivo, and clinical study is however required for implementation during cancer treatments and biomedical applications.

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CHAPTER

Biosurfactants in the pharmaceuticalsciences

17

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17.1 Introduction

The pharmaceutical sector shows great annual growth, even in the midst of global crises. This is because it is a sector considered essential that, at any time, is fundamental to guarantee the population's life, is related to food, personal and beauty care, diagnosis, prevention, and treatment of diseases.

In addition, obviously, to the service provided to the population by professionals in the sector (for example by the pharmacists), this care is achieved through pharmaceutical products, including medicines and cosmetics. A multitude of these products have surfactants in their composition and many depend on them for their effectiveness and/or stability. As examples of the use of surfactants in the composition of pharmaceutical products, it is possible to highlight the preparation of emulsions for the most diverse routes of administration, and also, its use in conditioners, shampoos, soaps, toothpastes, mouthwashes, among others. Surfactants also find wide application in the manufacture of nanostructured systems, such as liquid crystals.

Furthermore, for the good functioning of this sector, good manufacturing, and handling practices are indispensable, including care with the sanitization of environments, surfaces, accessories, and machinery, and, among other chemicals, surfactants are abundantly used for this purpose. Surfactants are also used in the quality control practices, for example, to inactivate some preservatives, or to favor the solubilization of a sample in the culture medium.

It is noted, therefore, that these surfactants have great versatility and relevance, including economic, in this sector.

In relation to their chemical characteristics, they are described as amphiphilic molecules, as they present a hydrophilic and a hydrophobic portion (Fig. 17.1), which guarantees their affinity for compounds of different polarities. This particularity is what makes these molecules so necessary, as they are capable of reducing the interfacial and surface tensions, allowing the formation of micelles, mixtures of immiscible compounds, and, even, the disorganization of biological membranes, such as bacterial membranes.

Thus the need for surfactant molecules that are less aggressive to the environment is a reality that becomes increasingly relevant when we consider the varied and significant use of these molecules. Most surfactants are derived from petroleum, or even when they are not, they correspond to

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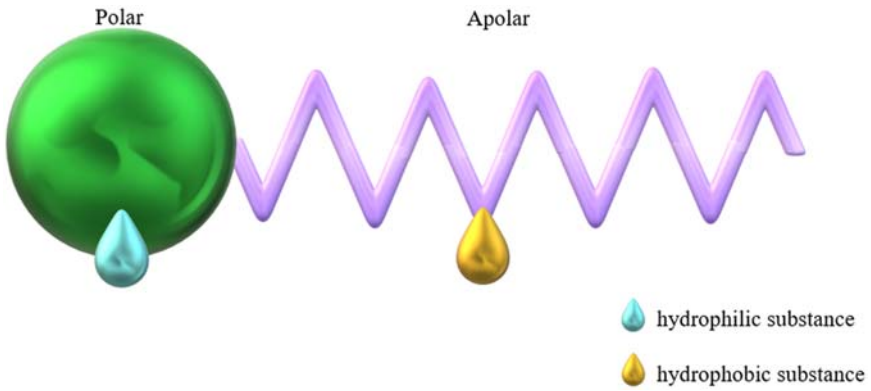

FIGURE 17.1

Schematic representation of a surfactant molecule and its affinities.

pollutants. Thus, biodegradable molecules of natural origin have been studied aiming their production with less environmental impact and, also, a safer discard and less pollutant. Is in this context that the biosurfactants should be mentioned, considered as molecules of natural origin, as they are synthesized by microorganisms, also capable of exerting a surface effect, reducing surface/interfacial tension [1].

Considering the above, this chapter aimed to discuss the use of biosurfactants in the pharmaceutical sciences. However, the work initially discusses general uses of surfactants in relation to pharmaceutical products and services to facilitate the understanding of the relevance of researching and applying biosurfactants in the pharmaceutical sector. The state of the art regarding the use of biosurfactants for health care was also presented.

17.2 Main uses of surfactants in the pharmaceutical industry

Surfactants or even receiving other denominations depending on the use to which they are assigned (e.g., detergents, emulgents), are a diversity of chemical molecules but which have in common a polar portion (water-soluble) and a tail of nonpolar hydrocarbon which therefore has a greater affinity for fatty substances, as previously described (Fig. 17.1). Due to this characteristic, they are used in massive quantities in residences every day, as they exhibit solubility and cleaning properties, being part of the composition of products for sanitization [2]. In industries in the pharmaceutical sector, as well as in pharmacies, this massive use is also observed.

For an in-depth look at these molecules, it is worth noting that surfactants can be classified into four types according to their chemical behavior in the presence of water. Each of these classes has particularities of use, as shown in Table 17.1.

As was mentioned in Table 17.1, surfactants are usually used in the composition of pharmaceutical products, including medicines that contain drugs that are poorly soluble in water, and in this

Table 17.1 Classification of surfactants based on their behavior in the presence of water [2,3].

Classification	Definition	Functional groups	Main uses
Anionic surfactants	Molecules containing a polar group that carries a negative charge in a slightly acidic, neutral, or alkaline aqueous medium.	Carboxylate, sulfate, sulfonate, or phosphate.	• Industrial processes (sanitization of accessories and equipment); • Pharmaceutical products and formulations - as part of the composition, with a detergent, emulgent, foam-promoting purpose, favoring the disintegration of solid pharmaceutical forms, or even improving the adsorption/absorption and the partition of drugs between hydrophobic and hydrophilic compartments in organs and in the body.
Cationic surfactants	Molecules containing a polar group that carries a positive charge. At least one hydrocarbon chain is found attached to a nitrogen	Alkylamines, alkyl imidazolines, quaternary ammonium compounds, ethoxylated alkylamines, and esterified quaternaries. The main surfactants in this group are quaternary ammonium compounds (QAC).	• Hair products such as hair conditioners and antistatic agents; • Emulgents; • Disinfectants acting as antibacterial (Gram-negative and Gram-positive) substances (QACs), • Antifungal and antiprotozoal (QACs); • Mouthwash products and oral antiseptics (suitable for topical); • Should not be applied systemically as they are toxic to mammalian cells.
Nonionic surfactants	The surface activity is derived from an equilibrium between the hydrophobic and hydrophilic structures of the molecule. They do not dissociate in the aqueous media, being the solubility provided by their groups of polar heads. With their lack of charge, nonionic surfactants are compatible with cationic and anionic surfactants.	The hydrophobic part of these nonionic surfactants are generally derived from an alkylated phenol, fatty acid or long-chain linear fatty alcohol. An ethylene oxide chain is generally the hydrophilic portion.	• In several biotechnological processes, to facilitate solubilization and increase the stability of the drug carrier; • Good compatibility with the skin and eyes; • Used as emulsifiers, mainly in cosmetic products for sensitive skin, baby's skin, as well as for daily skincare. Able to stabilize the foam.

(*Continued*)

Table 17.1 Classification of surfactants based on their behavior in the presence of water [2,3]. ***Continued***

Classification	Definition	Functional groups	Main uses
Amphoteric surfactants	Their surfactant properties are highly influenced by pH, exhibiting a positive or negative charge, while showing a zwitterionic form (dipolar ion) at an isoelectric point depending on Amphoteric surfactants pH.	An important example are the amino oxides (AOs), which are products of the reaction of tertiary amines and hydrogen peroxide. Alkylamides, alkylamines, and substituted alkyl amino acids are widely employed in cosmetics.	• Foam stabilizers and thickening agents, however, in the presence of acidic substances they tend to lose these properties; • Used as soft surfactants for child products; • In deodorants as an antibacterial agent; • They are compatible with the skin and are generally associated with other surfactants. Considering their zwitterionic property can be used with anionic surfactants.

case, the use of surfactants serves to reduce the interfacial tension between the medium and the poorly soluble drug, favoring solubilization [4].

In the preparation of low-viscosity, therefore liquid formulations, surfactants are used as solubilizers to promote the dissolution of drugs, active substances, or compositions based on plants, vitamins (such as vitamin E), fragrances, and other hydrophobic ingredients [4].

Surfactants can also contribute to the absorption of drugs administered topically, for example, when used in rectal, vaginal, and urethral dosage forms, they increase the percutaneous absorption rate. They have the same effect when used in dentistry, in oral or even sublingual dosage forms [4].

For many centuries these molecules have been used in the food sector. In this context, it should be remembered that products such as whole milk and milk cream are emulsions, as well as butter, margarine, and ice cream. Naturally occurring surfactants, such as egg yolk lecithin and various milk proteins, are used for food preparation, such as mayonnaise, salad creams, sauces, and desserts. Polar lipids, such as monoglycerides, can be used as emulsifiers for food products. In addition to the natural ones, synthetic surfactants such as sorbitan esters and sucrose esters have been used in food emulsions [5].

Three main types of emulsions are described and relevant in the pharmaceutical sector, including considering food technology. Oil-in-water (O/W) emulsions, where oil droplets are suspended in a continuous aqueous phase, this being the most versatile of the emulsion types, verified in mayonnaise, cream liqueur, creams, whipping toppings, mixtures of ice cream, having their properties controlled both by the surfactants employed and by the components of the aqueous portion of the emulsion. This kind of emulsion can be further divided into other three groups. The first group (coffee cream and cream liqueur) needs to be stable in relation to the formation of the cream and coalescence during its useful life. The second group corresponds to the emulsions that can be used as ingredients to the formation of more complex products, for example, proteins and polysaccharides can be used to form a matrix

to entrapped fat globules or with which these globules interact (yogurt and melted cheese). Finally, the third kind of emulsions is based on droplets necessary to elaborate new structures during processing, as in ice cream, where the emulsion is destabilized and still interacts as a means to create a structure [5].

The other type is the water-in-oil (W/O) emulsion, where water droplets are suspended in a continuous oily phase, that is observed in butter, margarine, and fatty pastes. In this case, the emulsion stability is more dependent on the properties of the oily components, therefore on the grease phase, but also the surfactant used [5].

There is also a third type, the water in oil in water emulsion (W/O/W), a multiple emulsion, which is actually oil in water emulsion, whose droplets themselves contain water droplets [5]. Independent of the type of emulsion, all of them are traditionally prepared with surfactants.

In the personal care, hygiene, and cosmetic products industry, surfactants are mainly used for stabilizing emulsions (both A/O and O/W) and also to impart properties that include thickening, changing flow characteristics, emollience, conditioning, solubilization, foam stabilization, and detergency to products [6].

A shampoo, for example, must meet the following criteria: detergency, smoothness, foaming power, good conditioning (overfatting the hair fibers), stability, adequate viscosity and, still, be esthetically attractive. To meet all these needs surfactants are used [7].

The stability of cosmetic emulsions is a matter of great concern and the subject of several studies. Although it seems to be something simple, obtaining stable, long-lasting and pleasantly sensible emulsification from pharmaceutical products is a complicated task, suffering interference from several factors. One of the most important points is the choice of the correct concentration of surfactants, called critical micellar concentration (CMC), in order to form a kind of barrier that surrounds the droplet of the internal phase of the emulsion, serving as an obstacle to coalescence, a term used to determine the union of several droplets, which can culminate in phase separation (Fig. 17.2). If

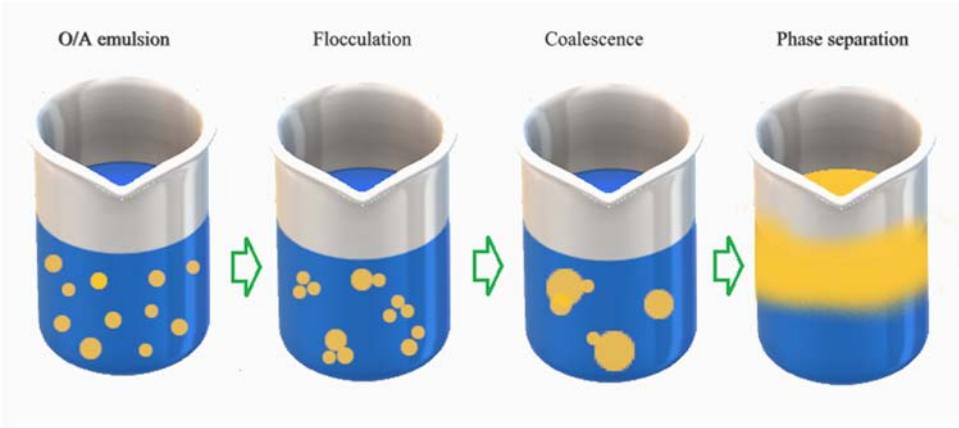

FIGURE 17.2

Representation of flocculation, coalescence and phase separation phenomena, indicators of emulsion instability.

CMC is not reached, the surfactants, in aqueous solution, behave as free monomers and not as micelles [8].

In this phenomenon of emulsification, there is also interference from the speed and duration of agitation, the temperature of emulsification, and even the use of stabilizers in the form of cosurfactants (for example, alkyl polyglycosides) to extend stability. The agitation is noteworthy, as it contributes to the reduction of the droplet diameter of the internal phase of the emulsion, to hinder coalescence [7].

In O/W emulsions, the addition of hydrophilic thickeners, with the ability to increase the viscosity of the aqueous phase, is a resource capable of reducing the fat content of the emulsion, when a lighter touch is desired. It also contributes to the stability of the emulsion, since the higher viscosity serves as a physical barrier to coalescence [7].

In addition to the various factors mentioned that influence the stability of emulsions, there is one more that deserves attention, the hydrophilic−lipophilic balance (HLB). It corresponds to a scale according to which the required HLB is calculated based on the concentration and polarity of the components of the emulsion oily phase. Then, at least one pair of surfactants, emulsifier and coemulsifier, is selected, in appropriated concentrations to meet the required HLB [7].

Surfactants are also used to clean environments and machinery. They assist in the removal of dirt, including those resulting from previous operations and even in the removal of biofilms. These biofilms correspond to aggregates of microorganisms that multiply and produce a viscous matrix of extracellular polymeric substances, which protect cells. Biofilms are mainly made up of water (95%) and most of the organic matter consists of exopolysaccharides and microorganisms. They must be efficiently removed when sanitizing surfaces and equipment, because otherwise, some of them may come off and contaminate other surfaces or products. The cells detached from biofilms are important sources of contamination that alter the quality and safety [8,9]. Surfactants act by reducing the surface tension of aqueous fluids, allowing better contact between cleaning agents and residues to be removed [8].

In this context, it can still be said that surfactants are a strategy to control microbial contamination. The chemical nature of these compounds causes a change in the properties of the surfaces on which they are applied, reducing their surface tension, preventing their adhesion, and promoting the separation of these microorganisms from the surface [10−12].

17.3 Biosurfactants

As is known, this chapter aims to discuss the importance and applications of biosurfactants in the pharmaceutical sector. Therefore, after understanding the wide range of applications of surfactants in general in the sector, it is convenient to present the biosurfactants briefly. Biosurfactants, as well as surfactants, are amphiphilic molecules, exhibiting a basic structure composed of a hydrophobic portion, often a hydrocarbon chain of one or more fatty acids linked to a hydrophilic portion that can be an ester, a hydroxyl group, phosphate, carboxylate, or carbohydrate [13].

They are surfactants biologically and naturally produced by bacteria, fungi, and yeasts. Therefore, they can be understood as a promising and useful type of surfactant in the various

pharmaceutical applications previously described, including the use in cosmetics, food, medicines, and, even, in environmental remediation [14].

Restricted data are available for the use of biosurfactants in pharmaceutical applications. However, some already described properties, such as emulsification, foaming capacity, ability to change the flow properties, and consistency of the product, demonstrate that they can be used efficiently by the industries in the sector [15].

In addition to being simply another source of new raw materials, which is already valid and allows diversification in the properties of products, the use of biosurfactants is even more relevant considering the increasing concern of consumers with the environment, as well as, considering the recent environmental control legislation.

This context has motivated the scientific community to seek the development of sustainable formulations in different sectors of modern industry [16,17]. As a result, there is also a tendency to replace synthetic surfactants with biological ones (ecologically less aggressive), such as biosurfactants that began to be studied in the mid-1960s [1,18].

This tendency can be confirmed by means of data from the biosurfactants market. It is estimated that the global market for biosurfactants in the year 2016 was 2 billion dollars and that it can reach something around 2.7 billion in the year 2022 [19]. Another source reports a similar scenario, indicating 1.5 billion dollars in 2019, with an expected growth of 5.5% (Compound annual growth rate - CAGR) between the years 2020 and 2026 [20]. These values are based on the results of companies that already operate in this sector such as BASF Cognis, Croda International PLC, Ecover, Evonik Industries AG, Jeneil Biotech, Saraya, Synthezyme LLC, Urumqui Unite Bio-Technology Co. Ltd., AGAE Technologies, MG Intobio, Henkel Ag & Co. Kgaa, Kemin Industries Inc, Givaudan SA (Soliance), among others [19,20].

In nature, several living organisms can produce surfactants, with different biological functions, such as the digestion of fats promoted by bile salts and, in the case of vegetables, protection against external agents through saponins, a secondary metabolite produced by this kingdom [21,22].

When considering biosurfactants, therefore, resulting from microbial synthesis, by bacteria, yeasts or fungi, there are indications that their biological function is associated with the survival of these individuals, more specifically:

- Anchoring or releasing the cell from substrates according to the availability of nutrients and survival conditions;
- Hydrocarbon solubilization and transport;
- Antibiotic action stimulates the competitiveness and survival of microbial populations that produce biosurfactants [23,24].

Researches indicate that these by-products have emulsifying properties and are capable of reducing surface and interfacial tensions through the same mechanism of action as chemical surfactants. In addition, reports are indicating that biosurfactants have antimicrobial and antiviral actions and can even be used for the treatment of certain pathologies [25–27].

Among the advantages that make these compounds more interesting technological options with their synthesized equivalents, can be mentioned:

- *Biodegradability*: Compounds produced by microorganisms have inherent ecological acceptability and are easily degradable [28].

- *Low toxicity index*: In general, biosurfactants are considered to be low toxic products or without any degree of toxicity. In addition, they demonstrate good biocompatibility and digestibility, which allows its application in the food industry, in cosmetics, and pharmaceutical products [27,29].
- *Superficial and interfacial activity*: The efficiency of biosurfactants is related to the possibility of reaching the CMC with lower concentrations of these molecules. Studies indicate that lower concentrations of biosurfactants cause greater effectiveness in decreasing surface tension, due to their lower CMC compared to their synthetic counterparts [24,30]. In addition to the economic issues involved in the need for lower concentrations of surfactants, the lower risk of toxicity with the use of smaller amounts of these molecules can also be mentioned.
- *Specificity*: As they are complex organic molecules with specific functional groups, they consequently demonstrate specificity in their action, generating industrial interest in several sectors [31].
- *Tolerance to temperature, pH, and ionic strength*: The activity of many biosurfactants is resistant to environmental factors such as temperature and pH, remaining active at elevated temperatures and pH values ranging from 2 to 12 [32]. The ionic stability of biosurfactants also deserves to be highlighted, supporting concentrations of up to 10% of salts, while a concentration of 2% of sodium chloride is able to inactivate a synthetic surfactant [33,34]. This offers to the formulators of cosmetic products and medicines an easier way to formulate products with these more extreme characteristics or that have to be subjected to them.
- *Anti-adherent agents*: The formation of biofilm, as already mentioned, a matter of great concern regarding the microbiological quality of pharmaceutical products, begins with the process of adhesion of microorganisms to biotic and abiotic surfaces. This adhesion depends on several factors, such as the type of microorganism, hydrophobicity, electrical charges on the surface, and environmental conditions [35,36]. Biosurfactants can be used to reverse this adhesion, changing the hydrophobicity of the surface, affecting the adhesins of the biofilm cells, impairing their adhesion on the substrate [31].

The classification of biosurfactants generally occurs through chemical composition and also by their microbial origin, unlike chemically synthesized ones that are classified according to their polar groups. The main classes of biosurfactants are glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids, polymeric surfactants, and particulate surfactants [13,37–40].

These biosurfactants can also be divided into other two categories according to the molecular weight:

- *Low molecular weight surfactants*: Are efficient in the surface and interfacial tension reduction (glycolipids, lipopeptides, and phospholipids) [41].
- *High molecular weight surfactants*: Effective as emulsion stabilizing agents, represented by polymeric and particulate surfactants [41].

The most well-known and studied biosurfactants are the glycolipids, basically, carbohydrates linked using an ester/ether group in long-chain aliphatic acids, such as rhamnolipids, trehalolipids, and sophorolipids [24,42].

Lipopeptides and lipoproteins are a class of biosurfactants that have demonstrated antimicrobial action against some bacteria, fungi, and viruses, being promising molecules for use in clinical practice and in some industrial sectors. Consists of a lipid-linked to a polypeptide chain [43,44].

Fatty acids, phospholipids, and neutral lipids are secreted by a range of bacteria and yeasts and have satisfactory surfactant activity and, in the case of neutral lipids, significant emulsifying action. These biosurfactants are essential for medical applications [45].

Emulsan and liposan are polymeric biosurfactants extensively researched. They are polysaccharide-protein complexes produced by several microbial genera. These molecules are not directly responsible for reducing surface tension but act as emulsifiers, being used in the food and cosmetics industries [31,46].

Particulate biosurfactants are extracellular vesicles that assist in the absorption of hydrocarbons by cells. In general, they are composed of proteins, phospholipids, and lipopolysaccharides, exhibiting good emulsification capacity [47].

17.4 Reports of biosurfactants employed in the pharmaceutical sector

It is necessary to start this discussion citing Satpute [48] who states that, currently, the application of biosurfactants, which was previously inaccessible, can be achieved and this promotes the innovation of formulations. According to Cameotra and Makkar [49], there are more economical ways of producing biosurfactants, and they provide access to these products. The ways to reduce costs are based on changing some fermentation processes to reach higher yields. Therefore, there must be an optimization of the process at the biological level so that the production of biosurfactants is commercially viable. Considering the use in pharmaceutical products, even a higher cost of the biosurfactant in comparison to conventional surfactants can be compensated by using more effective biosurfactants in small quantities. Also, they allow the offer of less "synthetic" products to consumers, increasing the added value.

As previously discussed, biosurfactants have many advantages when compared to synthetic surfactants, including being used in several areas of the industry. Well-described properties for these molecules are: antifungal, antiviral, antibacterial, antitumor, and anticancer [50].

Starting the reports of properties of interest in the pharmaceutical sector for biosurfactants, we can mention the glycolipids, which are presented as carbohydrates in combination with long chains aliphatic acids, such as trehalose lipids produced by *Mycobacterium*, the rhamnolipids by *Pseudomonas* sp. and sophorolipids by yeasts [49]. The antimicrobial effect of seven rhamnolipids synthesized by *Pseudomonas aeruginosa* AT10 using soybean oil residues was evaluated. They showed antifungal activity in concentrations ranging from 16 to 32 μg/mL, demonstrating the viability of these agents for biological control [49].

Mannosylerythritol lipid (MEL) is also a glycolipid biosurfactant produced by strains of *Candida* using vegetable oils. It exhibits antimicrobial, neurological, and immunological properties [49].

Shibahara [51] and Rodrigues [52] highlight the effects of bacterial glycolipids on the initiation of neurites in PC12 cells. After being treated with the MEL-A and MEL-B glycolipids, significant growth of neurites was observed. MEL-A similarly increased acetylcholinesterase activity to nerve growth factor (NGF), but the development of neurites induced by MEL-A after treatment of PC12 cells, blocked the action of NGF. Therefore, it is concluded that NGF and MEL-A induce differentiation of PC12 cells by different mechanisms.

The effects of seven glycolipids (MEL-A, MEL-B, polyol, rhamnolipid, sophorose lipids, STL (Liposucto-trehalose-sucroil)-1 and-3) in human promyelocytic leukemia cell lines were also evaluated [50]. Except rhamnolipid, all of them promoted cell differentiation, rather than cell proliferation. There was a significant increase in the differentiation characteristics in monocytes and granulocytes, and this activity is not only due to the surfactant effect but also to a specific action on the plasma membrane [49].

The immunological properties of glycolipids were tested in mice with malignant melanoma. It was noted that glycolipids exert effects in blocking the growth, apoptosis, and differentiation of melanoma cells. After exposure of cells to glycolipid, chromatin condensation, DNA fragmentation, and the sequence of events related to apoptosis occurred. It was found that the glycolipid is capable of inhibiting cell growth in a dose-dependent manner [52].

There are reports that sophorolipids (glycolipids) are immune response modulators, capable of decreasing mortality from sepsis and also the production of IgE in U266 cells in vitro. Thus, these data indicate the possibility of using sophorolipids as antiinflammatory agents and, also, in the therapy of diseases with altered IgE regulation [50].

There are indications of the ability of biosurfactants to prevent oral infections. Research carried out using a biosurfactant produced by *Streptococcus mitis* demonstrated the ability to inhibit the adhesion of *S. sobrinus* and *S. mutans* to the healthy tooth enamel, as well as the inhibition of the adhesion of *S. sobrinus* to salivary films. Similar effects were observed with biosurfactants synthesized by *Lactococcus lactis* 53 and *S. thermophilus* A, probiotic microorganisms, which demonstrated high antimicrobial activity even at low concentrations [50].

The role of biosurfactants as protectors of vaginal infections has been described by Lepargneur and Rousseau [53]. In the premenopause period, women tend to suffer from a decrease in the microbiota that is mainly composed of *Lactobacillus*. It is known that one of the factors that make these microorganisms protect the vaginal mucosa is related to the production of biosurfactants [49].

The increase in the incidence of the human immunodeficiency virus (HIV) in women aged 15 to 49 years has stimulated the realization of researches looking for ways to present an effective and safe control. The sophorolipid produced by *C. bombicola* and its analogs, showed interesting activities such as spermicide, antiHIV, and cytotoxic activities. These studies proved that the ethyl ester derivative of sophorolipid diacetate is a potent virucidal spermicide [50].

Lipopeptides are microbial biosurfactants produced by a wide spectrum of microorganisms and their structural characteristic is a fatty acid combined with an amino acid. Lipopeptides have activity as antibiotics, antivirals, immunomodulators, and enzyme inhibitors. An example of a lipopeptide with antiviral action is surfactin [49]. The production of these lipopeptides by *Bacillus* is the main mechanism for inhibiting the growth of pathogens in the gastrointestinal tract. According to reports, after isolation and purification of the lipopeptide biosurfactant produced by *Bacillus circulans*, there was an antimicrobial potential, with the ability to inhibit Gram-positive bacteria such as *B. pumilis*, *M. flavus*, *Mycobacterium smegmatis*, and Gram-negative bacteria like *E. coli*, *P. vulgaris* and *P. mirabilis* [54]. Others surfactin activities in the pharmaceutical sector include: the ability to inhibit clot formation, antibacterial activity, formation of ion channels in membranes, antifungal, antiviral and antitumor activity [1].

As biosurfactants are capable of effectively inhibiting the adhesion of pathogens to surgical instruments, a surfactin solution could be used, for example, in urethral catheters as a precoating, resulting in the reduction of biofilm formation by Gram-negative bacteria such as *S. typhimurium*, *S. enterica*, *E. coli* and *P. mirabilis* [50]. This colonization of medical instruments with microorganisms increases the spread of serious nosocomial infections, and even with hygiene measures this type of infection remains an important problem in hospitals [55].

Iturin, produced by *B. subtilis*, affects yeast cell membrane morphology and structure, demonstrating antifungal activity [1].

Biosurfactants also have several cosmetic applications due to their surface properties and skin compatibility and can also be used in personal hygiene formulations. According to Nitschke and Pastore [1], some companies have developed products using biosurfactants as facial creams, makeup products, lipsticks, and hair products. In these formulations, the most used glycolipid are sophorolipids, rhamnolipids, and mannosileritritol lipid [14]. According to Kosaric [15], biosurfactants have other properties that can also be explored in the cosmetic sector; such as water affinity, foam formation, ease of spreading, and moistening.

Sophorolipids also have excellent moisturizing properties and are compatible with the skin; rhamnolipids can replace petrochemical surfactants used in cosmetics because they are natural emulsifiers and biosurfactants. Glycolipids can also be used in antidandruff, antiwrinkle and antiaging products, nail care products, toothpastes, among others, due to their high surface activity [14]. Thus, surfactants conventionally used in products such as creams, lotions, pastes, powders, gels, and sprays can be replaced by biosurfactants. Some examples of formulations using biosurfactants in the cosmetic industry are insect repellents, antacids, makeup (lipstick, mascara, eyeshadows), lubricated condoms, hygiene products, depilatory and shaving products [50].

Another application of biosurfactants is in the food industry, with the role of promoting emulsification giving consistency and texture to foods, as well as promoting the solubilization of aromas, dispersion of phases and influence on the rheology of raw materials, such as flour [1]. The population is increasingly concerned and demanding about the quality and safety of their food, so surfactants have been widely used in the formation of food products such as dairy products, fermented products, bakery products, and breweries [48].

In a study by Guerra-Santos et al. [56], a glycolipid was able to enrich the properties of salad dressings and preparations made in confectionery sweets, emulsified partially digested meat fat molecules, and also played an important role in preventing the formation of harmful microbial biofilms.

Another study was carried out evaluating the biotechnological potential of the *Lactobacillus casei* (MRTL3) strain verifying its ability in the production of biosurfactants. This production was verified associated with the ability to decrease surface tension and inhibitory effect against foodborne pathogens. Thus, it is concluded that the biosurfactant produced by *L. casei* (MRTL3) can be used as a source of biopreservatives in food processing, being an alternative to chemical and conventional preservatives [57].

Table 17.2 presents a survey regarding publications that indicate the use of biosurfactants in the pharmaceutical sector.

Table 17.2 Use of biosurfactants in the pharmaceutical sector according to the literature.

Authors	Year of publication	Biosurfactant studied	Produced by	Purpose of use
Silva et al. [58]	2019	Several	*Enterococcus*, *Lactococcus*, *Lactobacillus*, *Lactosphaera*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Vagococcus* e *Weissella*	Cosmetic industry, acne control
Bueno et al. [59]	2008	Carbohydrate-protein-lipid type	Bacterias of the genus *Bacillus* (*Bacillus pumilus*)	Emulsification (suggestion: salad dressing emulsification)
Nitschke et al. [1]	2002	Iturina	*B. subtilis*	Pharmaceuticals and cosmetics
Brown et al. [60]	1991	Peptidelipid, lipopeptide, rhamnolipid, glycolipid, cellobiose lipids, trehalose-tetraester, sophorolipids, spiculisporic acid, lipopeptide, phospholipids.	*Bacillus subtilis*, *Bacillus licheniformis*, *Pseudomonas aeruginosa*, *Arthrobacter* sp., *Ustilago maydis*, *Rhodococcus erythropolis*, *Torulopsis bombicola*, *Penicillium spiculisporum*, *Corynebacterium lepus*	Cosmetics
Varvaresou et al. [61]	2015	Rhamnolipids, sophorolipids, mannosylerythritol lipid, trehalipids, xylolipids and lipopeptides	*Pseudomonas aeruginosa*, *C. bombicola*, *Pseudozyma antarctica*, *Pseudozyma aphidis*, *Pseudozyma rugulosa, Pseudozyma parantarctic*, *Micrococcus luteus*, *Rhodococcus erythropoli*, *L. lacti*, *Pseudomonas* sp., *Arthrobacter* sp.	Cosmetics and biopharmaceuticals
Vecino et al. [62]	2017	Lipopeptide, mannosylerythritol lipids, rhamnolipids, glycolipopeptide, trehalose lipids, glycolipids	*Pseudomonas fluorescens*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus mojavensis*, *Enterobacter*, *Corynebacterium xerosis*, *Pseudozyma*, *Pseudomonas aeruginosa*, *Burkholderia kururiensis*, *Lactobacillus pentosus*, *Pseudomonas strains*, *Rhodococcus pyridinivorans*	Cosmetics
Rodrigues et al. [52]	2006	Rhamnolipids	*Pseudomonas aeruginosa*	Cosmetics

Fakruddin et al. [63]	2012	Rhamnolipids, sophorolipids and trealolipids	*Acinetobacter* sp., *Bacillus* sp., *C. antartica*, *Pseudomonas aeruginosa*	Emulsifiers, foaming agents, solubilizers, wetting agents, detergents, antimicrobial agents, mediators of enzymatic action, in insect repellents, antacids, bath products, acne care, anti-dandruff products, solutions for contact lenses, baby products, mascara, lipsticks, toothpaste
Kosaric et al. [15]	1992	Glycolipid (sophorose lipid), glycolipid (rhamnose lipid), lipoprotein (surfactin), glycolipid, polysaccharide-fatty acid	*Torulopsis bombicola*, *Bacillus licheniformis*, *Bacillus subtilis*, *Pseudomonas* sp., *Arthrobacter paraffineus*, *Arthrobacter*, *Pseudomonas fluorescens*, *Torulopsis petrophilurn*, *C. tropicalis*	Emulsifiers, demulsifiers, wetting agents, spreading agents, foaming agents, functional food ingredients and detergents
Akbari et al. [64]	2018	–	–	Pharmaceutical and cosmetic products: detergents, humectants, emulsifiers, foaming agents, solubilizers
Shoeb et al. [14]	2013	Glycolipids, trehalose lipids, sophorolipids, surfactin	*Pseudomonas aeruginosa*, *Torulopsis bombicola*, *Rhodococcus erythropolis*, *Bacillus subtilis*	Pharmaceutical products
Silva et al. [65]	2015	–	–	Pharmaceutical products
Lima et al. [66]	2014	Sophorolipids, raminolipids and mannosileritirol lipids	*C. glabrata*, *C. apicola*, *Pseudomonas aeruginosa*, *C. antartica*	Cosmetics: detergency and sparkling property
Pinto et al. [67]	2008	Surfactin	*Bacillus subtilis*	Pharmaceutical applications
Carvalho et al. [68]	2014	Glycolipids, phospholipids and neutral lipids	Spirulina (microalgae)	Pharmaceuticals and cosmetics
Matsuura et al. [69]	2004	Surfactin	*B. subtilis*	Pharmaceutical products
Medeiros et al. [70]	2017	Glycolipids	*Yarrowia* sp.	Cosmetics

17.5 Final considerations

Finally, it is noted that the literature provides relevant information related to the use of biosurfactants in the pharmaceutical sector, produced by a wide range of microorganisms, including those that are considered probiotics, with other beneficial properties associated with their use. They have properties such as foaming, emulsifying, detergent power, but also biological activities, such as antibacterial and antifungal. So many properties and benefits, including the possibility of meeting the call for lesser environmental impacts, indicate a great growth in the use of these products, including positive and significant impacts on the pharmaceutical market.

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CHAPTER

Naturally occurring bioactive biosurfactants

18

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18.1 Introduction

Biosurfactants are amphiphilic compounds that show surface-active properties as they constituted hydrophilic heads and hydrophobic tails [1,2]. Biosurfactants are generally produced either as secondary metabolites of various plants, animals or by the enzymatic processes using hydrolytic enzymes [3]. Several microorganisms like bacteria, fungi, and yeasts can also produce various types of biosurfactants. Marine bacteria have been found as a rich source of biosurfactants [4]. Marine microorganisms are considered a huge natural source for the production of a wide variety of biosurfactants [2,5,6]. Table 18.1 represents some important biosurfactants produced from the different sources of marine microorganisms [7–13]. Based on their natural chemical structure and sources, biosurfactants are classified into the following five classes (1) glycolipids (2) lipopeptides (3) phospholipids (4) polymeric surfactants, and (5) particulate surfactants [14]. On many occasions, it was found that naturally occurring biosurfactants are much more advantageous than synthetic surfactants due to their nontoxic nature, better environmental compatibility, higher foaming ability and biodegradability, lower critical micellization concentration (CMC) values, etc. [3,15,16]. As a result, biosurfactants have gained tremendous attention during the last decade and have found a wide range of applications in cosmetics, agriculture, textile, painting, pharmaceutics, food, and many other industries [17]. Moreover, biosurfactants have been used as multifunctional agents which include moisturizing, wetting, stabilizing, emulsifying, antimicrobial, antiadhesive, etc., agents [18,19]. Biosurfactants are being used for the biodegradation of fluorine [20], remediation of phenanthrene-contaminated soil [21], biodegradation of diesel-contaminated water [22]. They can also be used for the bioremediation of contaminated soil [23] and biodegradation of hydrocarbons [24]. Recently, it was found that biosurfactants can also be used to remove heavy metals from soil and water [25,26]. Several biosurfactants reported possessing significant biological efficacies which include antimicrobial, antibacterial, antifungal, insecticidal, antiviral, immunomodulator, anticancer and anti-tumoral activities. Fig. 18.1 represents a glimpse of bioactive naturally occurring biosurfactants [4,27–29]. Biosurfactants showed potential activities against cancer treatment [30] and healing of wounds [31]. Non-ionic surfactants such as sucrose esters can form microemulsion which is very much helpful for drug delivery through the various routes of drug administration [27]. In agriculture, these can also be used as biological control of pests [32]. Biosurfactants are generally the amphiphilic type of compounds with hydrophilic/lyophobic heads consisting of amino

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Table 18.1 Important biosurfactants from marine microorganisms.

Type of biosurfactant	source	Activity	Ref.
Glycolipid	*Arthrobacter* sp.	Surface active agent	[7]
Glycolipid	*Alcanivorax borkumensis*	Surface active agent	[8]
Glycolipid	*Rhodococcus erythropolis*	Solubility enhancer of polycyclic aromatic hydrocarbons	[9]
Glycolipid	An unidentified marine bacterium MM1	Surface active agent	[10]
Lipopeptide	*Bacillus circulans*	Antimicrobial activity	[11]
Phospholipids	*Myroides* sp.	Surface active agent	[12]
Serrawettin	*Serratia marcescens*	Surface active agent	[13]

acids, peptides anions or cations, mono/di- or polysaccharides, and hydrophobic/lyophilic tail consisting of unsaturated or saturated fatty acids [33]. Based on the molecular weight, biosurfactants are usually classified as low (e.g., glycolipids and lipopeptides) and high (polysaccharides, proteins, lipoproteins, etc.) molecular weight biosurfactants. Generally, biosurfactants having low molecular weight show excellent surface-active properties due to their simpler structures as compared to the high molecular weight biosurfactants. In this chapter we have discussed various biological activities of naturally occurring biosurfactants.

18.2 Bioactivity of naturally occurring biosurfactants

18.2.1 Antimicrobial activity

Due to the ever-increasing resistance of pathogenic microorganisms against commercially available antimicrobial drugs, biosurfactants have gained considerable attention as an effective alternative [34–36]. Surfactin, isolated from *Bacillus subtilis*, is the most widely reported lipopeptide class of biosurfactant having significant antimicrobial activity [37]. Surfactin-type biosurfactants that interact rapidly with bacterial cell walls may come out as the next generation of antibiotics. Several other lipopeptides such as bacillomycins, fengycin, iturin, mycosubtilins, etc., isolated from *B. subtilis* [38], and lichenysin, pumilacidin, polymyxin B isolated from the other *Bacillus* strains reported to possess moderate antimicrobial efficacies [39–42]. Recently, lipopeptide based biosurfactants from other sources such as daptomycin (isolated from *Streptomyces roseosporus*) [43], viscosin (isolated from *Pseudomonas*) [32,44], rhamnolipids (isolated from *P. aeruginosa*) [44,45] and sophorolipids (isolated from *C. bombicola*) [46,47] have also showed potential antimicrobial activities. Mannosylerythritol lipids isolated from *Candida antarctica* showed specifically antimicrobial efficacy against Gram-positive bacteria [48]. In 1989, Lang et al. [49] reported for the first time that some biosurfactants possess moderate antimicrobial efficacies. In 2015, Rienzo et al. [50] showed that some biosurfactants *viz*. sophorolipids and rhamnolipids can inhibit biofilms formed by various Gram-positive and Gram-negative microorganisms. It was also reported that bioactivity can be boosted up in the presence of caprylic acid. Self-assembled sophorolipids can facilitate drug

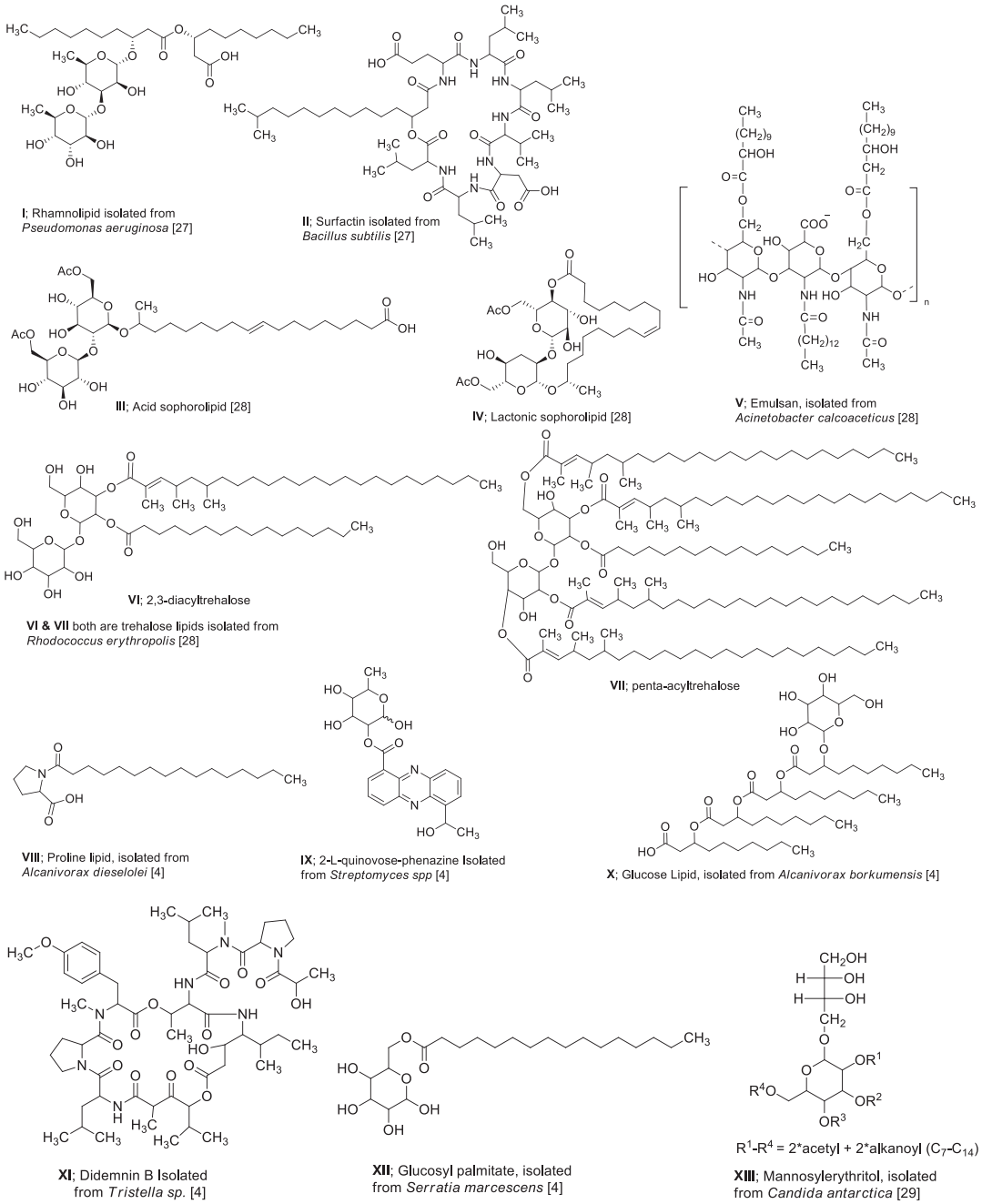

FIGURE 18.1

Glimpse of bioactive naturally occurring biosurfactants.

delivery procedure as it has the ability to span through the structurally alike bacterial cell membrane [51]. In 2020, another glycolipid biosurfactant isolated from seaweed *Sargassum myriocystum* was found to possess potent antimicrobial activities [52]. Parul Vatsa and coauthors [53] compiled a review related to the microbial activities of various rhamnolipid biosurfactants. Das et al. [11] demonstrated the potent antimicrobial activity of a lipopeptide biosurfactant derived from a marine *B. circulans*. Other lipopeptide biosurfactants such as surfactin and fengycin were able to inactivate endospores of *B. cereus* [54]. In 2009, Nitschke et al. [55] described the excellent antimicrobial activity of a rhamnolipid type of biosurfactant produced in soybean oil waste. Rhamnolipids produced by *P. aeruginosa* also showed antimicrobial activity [44,45]. Several other biosurfactants such as daptomycin, a cyclic lipopeptide isolated from *S. roseosporus* [43], sophorolipids obtained from *C. bombicola* [47], mannosylerythritol lipids [48] isolated from *C. antarctica* strain reported to possess significant antimicrobial activity. Viscosin, isolated from *P. fluorescens* is shown to possess antibiotic activity [56]. Antibacterial efficacies of lichenysin A, a biosurfactant obtained from *B. licheniformis* was reported by Yakimov et al. [40].

18.2.2 Antifungal activity

Antifungal activities of biosurfactants were reported long back [57]. Seven structurally different rhamnolipids were isolated from *P. aeruginosa* which showed excellent antifungal properties against several fungal strains [45]. Iturin A, a lipopeptide obtained from the strains of *B. subtilis*, showed potent antifungal activities [58]. Viscosinamide, a cyclic depsipeptide obtained from *P. fluorescens*, reported possessing significant antifungal activity [59]. In 2005, Mimee et al. [60] reported *in vitro* antifungal activity of flocculosin, a glycolipid isolated from *Pseudozyma flocculosa*. Several other biosurfactants of different categories such as glycolipids [61–63] rhamnolipids [64,65] and cyclic lipopeptides [66,67] showed antifungal activity against phytopathogenic fungi.

18.2.3 Antiviral activity

Various biosurfactants are found to possess antiviral efficacies. Among all, surfactin and its analoges showed the most significant antiviral efficacies [33]. It was found that biosurfactants are more effective against enveloped viruses, such as retroviruses, herpes viruses, etc., than nonenveloped viruses. Therefore it was proposed that the physicochemical interaction between the biosurfactant and the virus envelope is the main reason behind the potent antiviral behavior [68]. In 2005, Shah et al. [69] screened the antiviral efficacy of sophorolipids against the human immunodeficiency virus. Lipopeptides produced by *B. subtilis* fmbj also showed moderate antiviral activities against several viral strains [70]. In 2008, Remichkova et al. [71] reported the antiviral activity of a rhamnolipid obtained from *Pseudomonas sp*. strain against herpes simplex viruses. It was also observed that a small amount of rhamnolipid, even lower than the critical micelle concentration, is good enough to show a high suppressive effect against herpes simplex viruses.

18.2.4 Antibioflim activity

Among all biosurfactants, lipopeptides such as surfactins, polymixins, fengycins, fusaricidins, etc., are regarded as the most effective to disperse microbial biofilms [72–75]. Several other lipopeptides

were obtained from various *Bacillus* or *Paenibacillus* strain inhibit the biofilms formation [76–78]. In 2014, Banat et al. [79] compiled a review related to the antibioflim activity of the naturally occurring biosurfactants. A glycolipid type of biosurfactant, obtained from *L. fusiformis* S9, inhibited biofilm formation by *E. coli* and *S. mutans* [80]. It was also found that the ability to inhibit bioflim formation by naturally occurring biosurfactants is much higher than the commercially available chemical surfactants like SDS and CTAB. Sophorolipids can also be used as promising antibiofilm agents [81]. Rhamnolipids isolated from *P. aeruginosa* showed promising antibioflim efficacy even at a low concentration in the presence of caprylic acid [82]. Biosurfactants can affect the adhesion of microorganisms as they can partition at the interfaces of fluid phases through the hydrogen bonding and polarity differences [83–85]. Some noble biosurfactants produced by *Robinia pseudoacacia* and *Nerium oleander* were reported to possess antiadhesion activity against *C. albicans* biofilm [86]. It was found that sophorolipids, even at very low concentrations can able to disrupt biofilms constituted by the mixed cultures of *B. subtilis* and *Staphylococcus aureus* under static and flow conditions. Two biosurfactants produced by *B. subtilis* and *B. licheniformis* showed promising antiadhesion activity to prevent the formation of bioflim of human bacterial pathogens [75]. Before use, urethral catheters are generally rinsed with surfactin which reduces the chance of biofilm formation by *Salmonella typhimurium*, *S. enterica*, *Escherichia coli*, and *Proteus mirabilis* [87].

18.2.5 Anticancer activity

Recently, biosurfactants are also found effective against cancer cells [88]. Among all other biosurfactant known so far, surfactin is considered the most potential anticancer agent. In 2007, Kim et al. [89] found that surfactin can suppress the proliferation of the human colon carcinoma cells. In 2010, Cao et al. [90] reported induced apoptosis activity of surfactin against breast cancer cells. In 2013, Park et al. [91] reported anticancer activity of surfactin against breast cancer cells. In 2017, Wu et al. [92] observed significant cytotoxic efficacies of surfactin against breast cancer, colon cancers, leukemia, and hepatoma. Some other types of biosurfactants, such as lipopeptides and glycolipids showed selective inhibition of cancer cells via disruption of cell membranes [27]. Anticancer activity of surfactin against breast cancer cells was also studied by Duarte et al. [93] Biosurfactant extracted from an *Acinetobacter* M6 strain also showed promising anticancer activity against lung cancer [94]. Anticancer activity is also found in the biosurfactant isolated from *Leuconostoc mesenteroides* sp. [95]. Other biosurfactants such as iturin [96,97] and fengycin [98] were also reported to possess potent anticancer activity against breast cancer, colon cancer and lung cancer. Though, Jiang et al. [99] demanded that rhamnolipids exhibit similar cytotoxicity against both cancer cells as well as normal cell by reducing the surface tension of culture medium.

18.2.6 Antitumor activity

The most significant contribution of biosurfactants is their ability to control various human cell growths and therefore they can act as potent antitumor agents. Interestingly, various biosurfactants have been found to take part in several intercellular molecular recognition steps [100]. Surfactin and its analoges specifically showed potent antitumor activity against hepatocellular carcinoma [101], ehrlich's ascite carcinoma [102], myelogenous leukemia [103], colon adenocarcinoma [89], breast cancer [104,105], colorectal cancer [106], colon cancer [107] and many other cells.

Mannosylerythritol lipids showed excellent antitumor activity against the myelogenous leukemia K562 cell line [108]. Succinoyl trehalose lipids are found to possess antitumor activity against promyelocytic leukemia [109] and basophilic leukemia [110] cells. Saini et al. [30] reported the antitumor activity of viscosin against metastatic prostate cancer cell lines. Serratamolide showed antitumor activity against B-chronic lymphocytic leukemia cells [111]. Chiewpattanakul et al. [112] observed the growth inhibition activity of monoolein against cervical cancer as well as leukemia cancer cells. Iturin A, a lipopeptide biosurfactant, isolated from marine bacterium *B. megaterium* showed prominent antitumor activity [113].

18.2.7 Wound healing and antiinflamatory activity

Some recent studies revealed that some biosurfactants especially rhamnolipids possess moderate wound healing activities to treat ulcers and burns even at very low concentrations [31,114]. Kim et al., [115] screened the antiinflammatory activity of surfactin A, B, C, and D isolated from *B. subtilis* strain and in their study surfactin C showed the most prominent efficacies.

18.2.8 Antimelanogenic activity

Mannosylerythritol lipids, a glycolipid type of biosurfactants produced by various yeasts, are being used in cosmetics. In 2019, Bae [116] reported the antimelanogenic activity of mannosylerythritol lipids.

18.2.9 Antimycoplasmal activity

Vollenbroich et al. [117] reported antimycoplasmal efficacies of surfactin isolated from *B. subtilis*. They observed improved proliferation rates of mycoplasma-affected mammalian cells.

18.2.10 Anti-HIV activity

In 1994, Itokawa et al. [118] observed that surfactin can inhibit the growth of human immunodeficiency virus 1 (HIV-1). Another biosurfactant, that is, myramistin also showed potent anti-HIV activity [119,120].

18.2.11 Antithrombotic activity

Lim et al. [121] reported antithrombotic activity of surfactin isolated from *B. subtilis*. It was observed that surfactin has efficacy to inhibit platelet aggregation this leads to restricting the additional fibrin clot formation.

18.2.12 Antiproliferative activity

Biosurfactants isolated from *Lactobacillus casei* showed prominent antiproliferative activity [122]. Ohadi et al. [123] reported antiproliferative activity lipopeptide based biosurfactant produced by *Acinetobacter junii* B6.

18.2.13 Antioxidant activity

In 2016, Zouari et al. [124] demonstrated in vitro antioxidant efficacies of the biosurfactants isolated from *B. subtilis*. Next year, Merghni et al. [122] screened the antioxidant efficacies of other biosurfactants isolated from *Lactobacillus casei* and found moderate efficacies. Haque et al. [125] reported antioxidant efficacy of rhamnolipids isolated from *Marinobacter litoralis*.

18.2.14 Activity against Coronavirus disease 2019

Coronavirus disease 2019 (COVID-19), caused by a new strain of Coronavirus create a global pandemic situation. It is reported that the Coronavirus affected patients generally have high levels of cytokine storm [126]. A high level of cytokine storm badly affects the immune system and thereby kills the healthy cells [127]. Because of the immunosuppressive potential, it was expected that biosurfactants can be useful against this novel Coronavirus. Along with other bioactive compounds, the efficacies of several biosurfactants are also screened against Coronavirus. It was found that surfactin has the potential to reduce cytokine storms [36]. This indicates the use of biosurfactants would be a fruitful way to reduce the cytokine storm level of the COVID-19-affected patients [128]. On the other hand, it was also found that biosurfactants interact with the spike protein and lipid envelope of the Coronavirus this leads to rupture of the outer membrane and makes the virus inactive [129].

18.2.15 Larvicidal and pupicidal activity

Biosurfactants produced by different microorganisms are found to possess potent larvicidal as well as pupicidal activities [130–132]. Parthipan et al. [133] screened the mosquitocidal efficacy of lipopeptide type of biosurfactants produced by *B. subtilis* and *P. stutzeri* against *A. stephensi* mosquito. It was proposed that biosurfactant shows larvicidal efficacy as it can reduce the surface tension of water which create the unpleasant condition by reducing oxygen concentration at underwater.

18.3 Conclusions

Biosurfactants are amphiphilic in nature and can be isolated from various plants and microorganisms. Naturally occurring biosurfactants are more attractive than synthetic surfactants due to their high biodegradability, multifunctionality, low toxicity, and eco-friendliness. As a result, the last decade has shown tremendous outburst for the use of various naturally occurring biosurfactants in the medical field as well as in various industries such as cosmetic, food, petroleum, pharmaceutical, agricultural, textile, etc. Many naturally occurring biosurfactants possess a wide range of biological efficacies that include antimicrobial, antifungal, antiviral, antibacterial, antioxidant, anticancer, antithrombotic, antiinflamatory, antitumor, antibioflim, antiproliferative, ant-HIV, antimycoplasmal, antiadhesion, etc., activities. This chapter deals with various medicinal and therapeutic perspectives of naturally occurring biosurfactants.

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CHAPTER

Application of biosurfactants in the treatment of *Mycobacterium tuberculosis* infection

19

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19.1 Introduction

In the late 1960s, synthetic surfactants were replaced and the place was taken over by the surface-active compounds (SACs) of biological origin termed as "biosurfactants" or "bioemulsifiers" [1]. Arima et al. in 1968 purified and characterized the very first biosurfactants named "surfactin" [2] Later, Cooper and Goldenberg came up with several other biosurfactants which were produced by the two species of *Bacillus*, having different surface and emulsifying properties in the water-soluble substrates [3].

The biosurfactants are categorized into five major types, based on the extent of various studies performed across the globe that is, glycolipids, phospholipids, lipopeptides, polymeric biosurfactants, and particulate biosurfactants. To date, the data reveals that more than 250 biosurfactants have been patented so far.

Microbial biosurfactants or Biosurfactants are the chemical compounds synthesized on the surface of the microbial cells. Chemically these molecules are amphipathic in nature, i.e., having both hydrophobic and hydrophilic moieties, which makes the biosurfactant impart surface-active properties and aggregate between fluids having different polarities. Moreover, because of this property they act as good emulsifiers by forming micelles resulting in the degradation of contaminants/pollutants/pathogens [4].

The biosurfactants were called secondary metabolites, though they play a vital role in the rapid transport across the cell membrane, act as a biomedical agent or used in the host−microbe interactions. These classes of new-age surfactants are quite diverse in terms of structure and functions which make them a wonderful biodegradable and eco-friendly agent to be used in varied applicative fields [5].

Based on their growth on water-soluble or insoluble substrates [6,7] the biosurfactants are classified such as glycolipids, lipopeptides, phospholipids, polymeric, and particulate biosurfactants; which were produced by a vivid number of microorganisms. For instance polymeric biosurfactants such as emulsan, liposan, alasan, and lipomanan are water soluble surfactants. The best known among glycolipids are mainly rhamnolipids, trehalolipids, and sophorolipids produced by *Pseudomonas*, *Rhodococcus*, etc. [8]. Biosurfactants obtained from microorganisms like *Lactobacilli*, *Yeast*, *Candida*, etc., termed as "generally regarded as safe" (GRAS) microorganisms.

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Recently biosurfactants emerged as the most preferred surfactants due to their bioavailability, specificity, biodegradability, low toxicity, scale-up capacity, rapid production, and effectiveness over extreme temperature and pH which make them highly advantageous over the chemical and plant-based biosurfactants. They act as mediators, which increase the surface area of the hydrophobic substrates and regulate attachment and removal of microorganisms from surfaces by inducing a change in the cell membrane resulting in the adherence of biosurfactants with the pathogens [9].

Their high efficacy, antimicrobial, antibacterial, antiviral, adhesiveness, and immunomodulatory nature make them as better drug cargo systems, gene therapy, or used as medicinal implants for effective therapeutics. Biosurfactants offer great applicative roles not only in the biomedical fields but also in the field of cosmetics, agriculture, food additives, and pharmaceutics.

Few surfactants like lipopeptides and glycoprotein are of immense importance in cancer cell proliferation therapy. Also, biosurfactants play a major role in treating pulmonary disease tuberculosis (TB) caused by the acid-fast bacteria *M. tuberculosis*. This bacterium shows high virulence in the host and is surrounded by a lipid coat constituting trehalolipids. The surfactants are efficient in removing such pathogenic strain H37Rv of the *Mycobacterium* from the host immune system [10,11].

Therefore, in this chapter, we will discuss various biosurfactants, their classifications, nature, producing organisms, and how they are being used in biomedical.

19.2 Biosurfactants

Biosurfactants can be defined as the diverse group of green molecules synthesized on the microbial cell surface. These SACs are amphipathic, having both hydrophilic and hydrophobic domains that help them to fit into the fluid phase resulting in reduced surface area and surface tension between the interfaces [12].

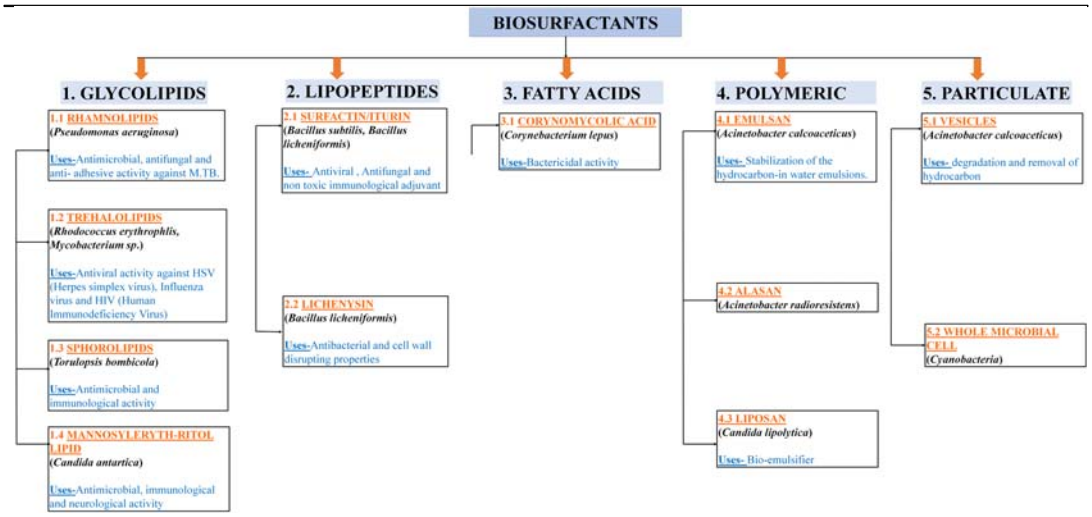
19.2.1 Classification

19.2.1.1 Microbial origin

Biosurfactants are divided into five categories which are discussed in Table 19.1 [13].

1. Glycolipids:
   Glycolipids, the most powerful surface-active properties, are by far the most common class of biosurfactants. The best known among the glycolipids are rhamnolipids, trehalose lipids, and sophorolipids. The rhamnolipids obtained from *Pseudomonas* sp., the trehalose lipids obtained from *Mycobacterium* sp., *Rhodococcus* sp., and the sophorolipids obtained from *Yeast*. These glycolipids show antimicrobial, antiadhesive, antiviral properties [14].
2. Lipopeptides:
   The biosurfactants with the highest potential in showing antibiotic and antimicrobial actions are mostly lipopeptides in nature. Antibacterial properties of *Bacillus subtilis* lipopeptide were identified by Singh and Cameotra [15]. Also, Arima et al. (1968) [2] found the first-ever biosurfactant named "Surfactin" which shows antiviral properties.

Table 19.1 Classification of biosurfactants, their producing organisms, and uses.



3. Fatty acids (FA)/Phospholipids:
   The hydrophilic and lipophilic balance (HLB) is specifically associated with the length of hydrocarbon chains in FA. They are produced during the growth of n-alkanes in bacteria and *Yeast*. Corynomycolic acid, a type of fatty acid surfactant, was extracted from *Corynebacterium lepus*. It is a collection of SACs with a different number of carbon atoms, that is, R′-CH(OH)-CH(R-COOH-R) [13].
4. Polymeric Biosurfactants:
   These biosurfactants are considered to be the best-studied biosurfactants including emulsan, Alasan, lipomannan, liposan, etc., mainly synthesized by *Acinetobacter calcoaceticus*, *A. radio resistance*, *Candida*, respectively. They are best used as an emulsifier, especially as a polysaccharide-protein complex.
5. Particulate Biosurfactants:

These are differentiated into two forms, that is, vesicles and whole microbial cells. Bacterium *Acinetobacter* sp. usually forms vesicles and Cyanobacteria from the whole microbial cells for the degradation and removal of the hydrocarbons.

19.2.1.2 Chemical nature

Rosenberg and Ron (1999) [16] differentiated biosurfactants into two major categories based on molecular masses and critical mass concentration (CMC), that is, low molecular mass (glycolipids, lipopeptides) which efficiently lowers the surface and interfacial tensions whereas high molecular mass (FA, polymeric and particulate) which are effective as an emulsion stabilizing agent (Fig. 19.1).

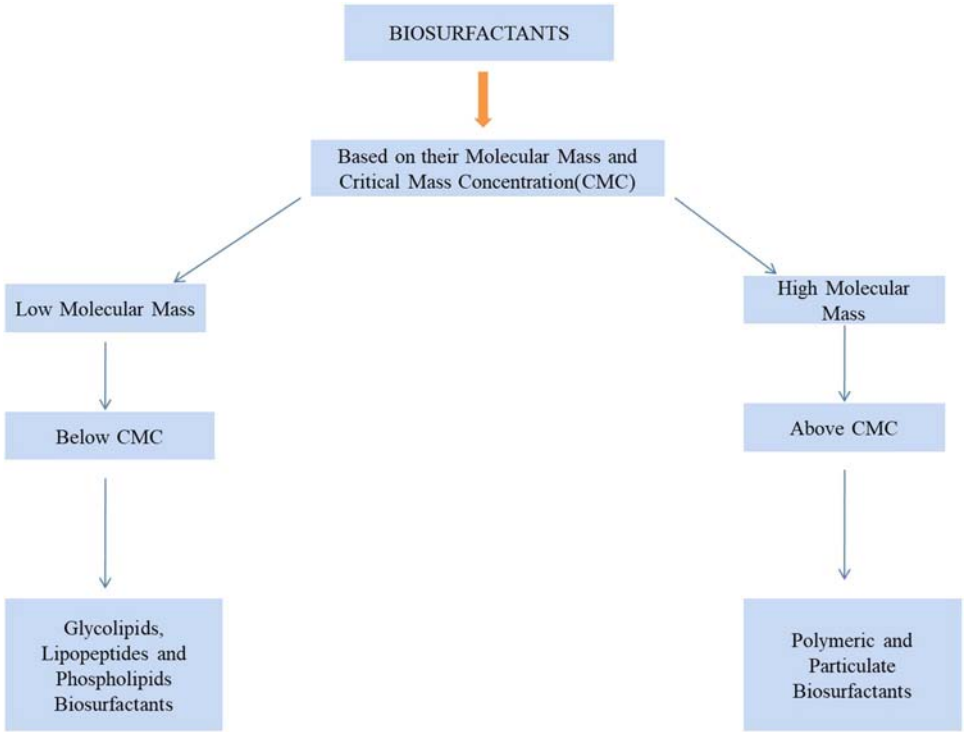


FIGURE 19.1

Classification of biosurfactants based on their molecular mass and critical mass concentration.

1. Glycolipids:

   The glycolipids are the conjugates of carbohydrates and fatty acids linked via either the ether or ester group. The various types are discussed:

   a. Rhamnolipids: Jarvis and Johnson (1949) [17] first formulated the production of the best-known glycolipids called rhamnolipids produced by *Pseudomonas aeruginosa*. They are composed of molecules partly of rhamnolipids linked via one or two molecules of beta-hydroxy decanoic acid by forming glycosidic bonds of one group and ester bonds in another.

   b. Trehaplolipids: They are majorly associated with *Rhodococcus sp*. and have disaccharide trehalose linked via C-6 and C-6′ to the mycolic acid. The mycolic acids are mainly correlated with the *Mycobacterium* sp., *Nocardia*, etc. The phospholipids differ in their configuration in varied organisms due to differences in their size and structure of mycolic acid, degree of unsaturation, and several carbon atoms [14,18].

   c. Sophorolipids: A long chain of hydroxy fatty acids linked to dimeric carbohydrates via glycosidic linkage forms the sophorolipids. Generally, they exist as a mixture of 6−9 different hydrophobic sophorolipids and macro lactones [19,20].

2. Lipopeptides:
   A lipid attached to a polypeptide chain, including a large number of cyclic lipopeptides, that is, gramicidin's, polymyxins is considered to be lipoprotenacious in nature. Surfactins and lichensyin are the lipopeptide biosurfactants and are discussed below:
   a. Surfactin: It is a cyclic lipopeptide, produced by *Bacillus subtilis*. It is composed of seven amino acid ring structures along with fatty acid chain-forming lactone linkages. Surfactins are of three different types, that is, Surfactin A, B, and C which are differentiated based on amino acid sequences.
   b. Lichensyin: They are quite similar in structural and physico-chemical properties when compared with surfactin. *B. licheniformis* produces lichensyin which exhibits excellent salt stability, temperature, and pH. These types of surfactants can lower the surface and interfacial tension of water to 27 and 0.36 mN/m, respectively [13].
3. Fatty acids (FA)/Phospholipids:
   In FA**,** the HLB is directly related to the length of hydrocarbon chains. They are produced during the growth of n-alkanes in bacteria and *Yeast*. Corynomycolic acid, a type of fatty acid surfactant, was extracted from *Corynebacterium lepus*. It is a group of SACs with varying number carbon atom, that is, R'-CH(OH)-CH(R-COOH-R) [13].
4. Polymeric:
   The biosurfactant with high molecular weight and having a backbone of ¾ sugar moieties with fatty acids form polymeric surfactants. The extracellular water-soluble emulsifier liposan is composed of 83% carbohydrates and 17% proteins; emulsan an extracellular acylated polymeric surfactant backbone is formed of unbranched polysaccharide with O/N-acyl fatty acid side chains [13].
5. Particulate:

They are of two types, i.e., vesicles and microbial whole cells. When *Acinetobacter* sp. were grown on hexadecane integrated into vesicles of approximately 20−50 mm diameter having a density of 1.158 g/cm^3 and composed of protein, phospholipids, lipopolysaccharides (LPS). Meanwhile, in whole microbial cells, the strong affinity of hydrocarbons acts as an interface between air/water and hydrocarbon-water systems [13].

19.3 Biosurfactant synthesis

19.3.1 Producers

The biosurfactant-producing organisms are usually the hydrocarbon degraders. A lot of biosurfactants producing microbes are found which produce different types of surfactants [21]. Among them, the producers are categorized mainly into two categories:

1. Bacterial biosurfactants: Biosurfactants producing microorganisms use a vivid range of organic compounds to compensate for the energy and carbon requirements for their growth. If the carbon source is available in the insoluble form the microorganisms diffuse into the cell by secreting biosurfactants, which emulsify the hydrocarbon in the growth medium. While several other microorganisms can change the cellular structure by producing nonionic or LPS

surfactants on the cell wall, for example, *Pseudomonas* sp. (rhamnolipids), *Torulopsis* sp. (sophorolipids), *Acinetobacter* sp. (surfactin) [22].

2. Fungal biosurfactants: As compared to bacterial biosurfactants, various fungi are known to generate biosurfactants likewise *Candida bombicola*, *Candida lipolytica*, *Aspergillus ustus*, etc., *C. lipolytica* and *C. bombicola* produce cell wall-bound LPS while growing on *n*-alkanes.

19.3.2 Physiology of production

The SAC of microbial origin uses energy and carbon sources for their growth and induces the production of biosurfactants having different surface activities and chemical structures. As we know that biosurfactants are amphipathic molecules, their synthesis involves two different synthetic pathways where one leads to a hydrophilic moiety and another the hydrophobic moiety. The hydrophobic fatty acid constituents comprised of a long-chain fatty acid and hydroxyl fatty acid are synthesized by a mutual pathway of lipid metabolism [22]. For the synthesis of polar moiety and cell metabolism microbes use hydrophilic substrates as a primary source; whereas for the production of the hydrophobic portion they use hydrophobic substrates. The production of biosurfactants usually occurs by four different routes (Sydatk and Wagner): (1) lipid and carbohydrates synthesis; (2) half carbohydrates synthesis while half lipid synthesis depends on the length of the carbon chain; (3) synthesis of half lipid and half carbon depend on the substrate used; (4) both carbohydrates and lipids [23].

Hence, the length of the *n*-alkanes chain used as a carbon source alters the production of surfactants. For instance, mannosylerythritol lipids (MEL) product of *C. antarctica* (yeast) does not grow in the media having *n*-alkanes of C10-C18; and the greatest yield obtained in media has C1-2-C18 carbon source. Glycolipids and lipopeptide type biosurfactants are produced by *P. aeruginosa* and *B. subtilis*.

Biosynthesis of biosurfactant offer variation in the kinetic parameter are grouped as growth-associated production (parallel relation between growth, use of substrates and biosurfactants production); production under growth-limiting condition (a prominent in biosurfactants concentration under the growth-limiting condition as a result of the low value of one or more components); production by resulting/immobilized cells (it's a type of biosurfactant in which there is no cell multiplication); and production with precursor supplementation (cell stop using the continuous use of carbohydrates on the source for biosurfactant production) [24]. A lot of raw material is used for the production of surfactants likewise vegetable oil, waste effluents, crude oil, molasses, starchy effluents, glycerol, animal fat, etc. But for the production of microbial surfactants we use microbes like *P. aeruginosa, B. subtilis, R. erythropolis, M. tuberculosis, Lactobacilli*, etc., should be selected and ensured to keep a proper balance to allow the growth of microbes and their releasing SACs [25].

Generally, if we look into the perspective of biosurfactant production firstly one needs to isolate bacteria and then allow it to grow under suitable conditions. Then only one can screen the biosurfactants produced by the microbe; after that, we can completely isolate them and purify them from the rest of the surroundings. Later on, the characterization of the purified biosurfactants is performed using thin-layer chromatography (TLC), Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), etc. [14,26,27].

Example:: Rhamnolipid biosynthesis pathway occurs in three parts, that is, hydrophilic part, hydrophobic part, and merging of the two parts for the synthesis of rhamnolipid.

Usually, biosurfactants don't undergo degradation eagerly because of their small size and simple structure. The recovery of biosurfactants mainly depends on the ionic charges, solubility of water, and intracellular/extracellular location. Via downstream processing, centrifugation, acid precipitation, foam fractionation, ion-exchange chromatography, and solvent extraction the biosurfactants can be recovered [28]. The biosurfactants after recovery become advantageous as their nature imparts low cost, high purity, fast recovery, and reusability (Fig. 19.2).

19.3.3 Factors affecting biosurfactant production

The structural complexities and emulsifying action of the biosurfactants depend on the producer strain but along with the other contributing factors affecting it sideways are discussed as follows [29]:

1. Carbon sources:
   It is the very important factor in biosurfactant production, which affects both the quantity and quality of biosurfactants and is quite influential in terms of the carbon substrates.

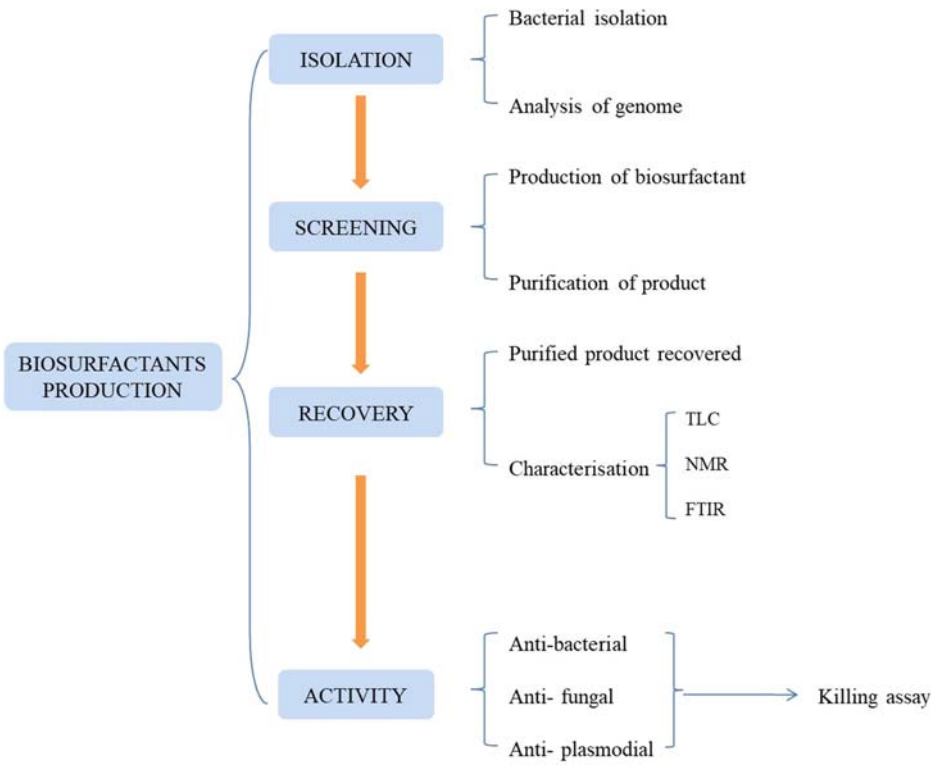


FIGURE 19.2

Production and recovery of biosurfactants.

The growth and production of biosurfactants vary from species to species but a few good substrates of carbon are vegetable oil, crude oil, glucose, glycerol, diesel, sucrose in *C. bombicola*, and *C. lipolytica* to produce a large number of sophorolipids [11].

2. Nitrogen sources:

   The next most preferable component for biosurfactants production as they play a major role in the growth of proteins, enzymes synthesis in a microbe. Various sources of nitrogen are used by *Yeast*, *P. aeruginosa*, *Arthrobacter* such as ammonium sulfate, ammonium nitrate, malt extracts, urea peptone. In the production of biosurfactants via the fermentative process, the C/N ratio affects the growth of the metabolites. Low nitrogen levels and a high C/N ratio favor the production of cell metabolites. In contrast, high nitrogen levels lower metabolite production [11].

3. Aeration and Agitation:

   The production of biosurfactants is mainly influenced by the facilitation of oxygen by passing from the gaseous phase to the aqueous phase for the proper physiological function of microbial biosurfactants, which is remarkably fulfilled by the aeration and agitation factors. The best-quantified value for the biosurfactant production is maintained when the dissolved oxygen saturation is 50% and the rate flow is 1 Vvm (volume of air/unit of medium/unit of time) for producing 45.5 g/L of biosurfactants.

4. Environmental factors:

   To obtain a better yield of biosurfactants in large quantities environmental factors play an extremely important role likewise pH, temperature, carbon, nitrogen, oxygen, salt concentration, etc. These factors either increase/decrease or inhibit the productivity of the biosurfactants. Not all microbes experience the same environmental factors instead, different conditions lead to production of varied types of biosurfactants. For instance, *P. aeruginosa* producing rhamnolipid grows in absence of oxygen, which effectively reduces the reactive oxygen stress on the bacteria [5,11,13,30].

19.4 Properties of biosurfactants

1. Antiadhesive agent:

   Rodriguez et al. found that the biosurfactants can inhibit the pathogenic microorganisms to adhere to the infection site. The pathogenic organisms start colonizing over the surface (i.e., biofilm) to cause infection but biosurfactants alter their hydrophobicity of the surface resulting in adherence over the surface. For instance, the colonization of *Streptococcus* on steel can be reduced by *S. thermophilus* released surfactant [13,31,32].

2. Low toxicity:

   Biosurfactants are usually measured as less or nontoxic because they cause little or no harm to the ecosystem; therefore, they are quite appropriate to use in food, pharmaceuticals, and cosmetics. It is suggested that chemical/synthetic anionic surfactants show LC50 (lethal concentration to 50% of test species) against *Photobacterium phosphoreum* which is ten times lower than rhamnolipids; meanwhile, biosurfactants show EC50 (effective concentration to decrease 50% of the test population) as in *P. aeruginosa*. Hence, it is concluded that

biosurfactants are nontoxic and nonmutagenic instead of synthetic surfactants which are toxic and mutagenic.

3. Surface and interface activity:

   Biosurfactants excellently hold the activity to decrease the surface tension of water up to 25 mN/M and the interfacial tension up to <1 mN/M of water /hexadecane. Microbes like *P. aeruginosa* and *B. subtilis* are some of the good examples leading to reduced surface and interface activity. Not only are they effective and efficient in reducing surface/interface activity; also, they are quite effective in lowering CMC.

4. Biodegradability:

   As compared to the synthetic surfactants, biosurfactants are easily degradable by natural processes by bacteria or fungi into more components that are basic, and because of that, they are the least concern to the environmental pollution, and therefore they are considered as "green-molecules" or "eco-friendly" compounds. Sophorolipids are one of the best-known biosurfactants with removal efficiency up to 90% in 30 minutes when treated using *Cochlodinium* controlled blooms of marine algae [33].

5. Biocompatibility and Digestibility:

   Biosurfactants offer the property of not damaging the bioactivity of the organism with which they interact. They are quite compatible, that is, they are easily tolerable by the living organisms and because of this, they allow them to be useful in cosmetics, the food industry, and pharmaceuticals.

19.5 Mycobacterium tuberculosis

In 1882, Robert Koch first discovered the species of the pathogenic bacterium, that is, *M. tuberculosis* (M.Tb.) causative agent of tuberculosis (TB), which belongs to the family Mycobacteriaceae. M.Tb. genome H37Rv consists of 4.4 million base pairs and contains approx. 4000 genes [15,34]. This bacterium is also called as "Tubercle bacilli" or "Koch's bacilli." It is a rod-shaped, nonmotile, nonspore-forming, facultative intracellular parasite and strictly an obligate aerobe in nature [35,36]. M.Tb. show slow growth, forms colorless colonies, and are impermeable to many stains and dyes as it weakly stains gram-positive and negative bacterium, only Zeihl–Nelson stain is used to identify the acid-fast stain bacteria [37].

19.5.1 Type and disease caused by *Mycobacterium tuberculosis*

The "Koch Bacilli" is a small-sized bacillus which is comprised of a *M. tuberculosis* complex (MTBC) evolved in Africa precisely in the Horn of Africa comprised of at least nine members, that is, *M. tuberculosis, M. sensu stricto, M. mungi, M. africanum, M. canetti, M. bovis, M. caprae, M. microti, M. pinnipedii, and M. orygis*. Among them, the TB causing species are *M. tuberculosis, M. africanum, M. bovis, M. microti, M. canetti; the* rest are not pathogenic for TB. The M.Tb. causes TB in humans, *M. bovis* in bovines, *M. microti* in murrains, *M. avium* in avian. The MTBC members exhibit different phenotypic characters and host ranges but they are genetically related to each other. The MTBC currently coevolved with humans and once compared that the M.Tb

phylogeny with the human's mitochondrial genome phylogeny and interpreted these as quite similar which reflect a stable association between host and strain [38].

TB usually based on their site of infection is categorized into two types; pulmonary and extra-pulmonary. Pulmonary site of infection usually causes primary disease if the infection with the tubercle bacilli who has not been previously infected or those who have previously been infected with secondary or postprimary or reinfection; (Fig. 19.3) [39].

In terms of illness and spread of infection on the pulmonary or extrapulmonary sites Tb is of three types (Fig. 19.4) [40]:

1. Active TB: An illness in which the TB bacteria rapidly replicate and invade different organs of the body. A person with active TB may spread TB to others by airborne contamination of the aerosol particle in the air.
2. Latent TB: It is a noninfectious and nonspreadable form of TB as the patient did not feel sick and is asymptomatic. The bacteria infect them but do not have remarkable TB disease. The only way to get its sign is a positive result of the tuberculin skin test.
3. Miliary TB: It is a rare form of TB that occurs when the infection finds its way into the bloodstream. It is a fatal form of TB as the bacteria rapidly spread all over the body in the form of small nodules and affect many organs.

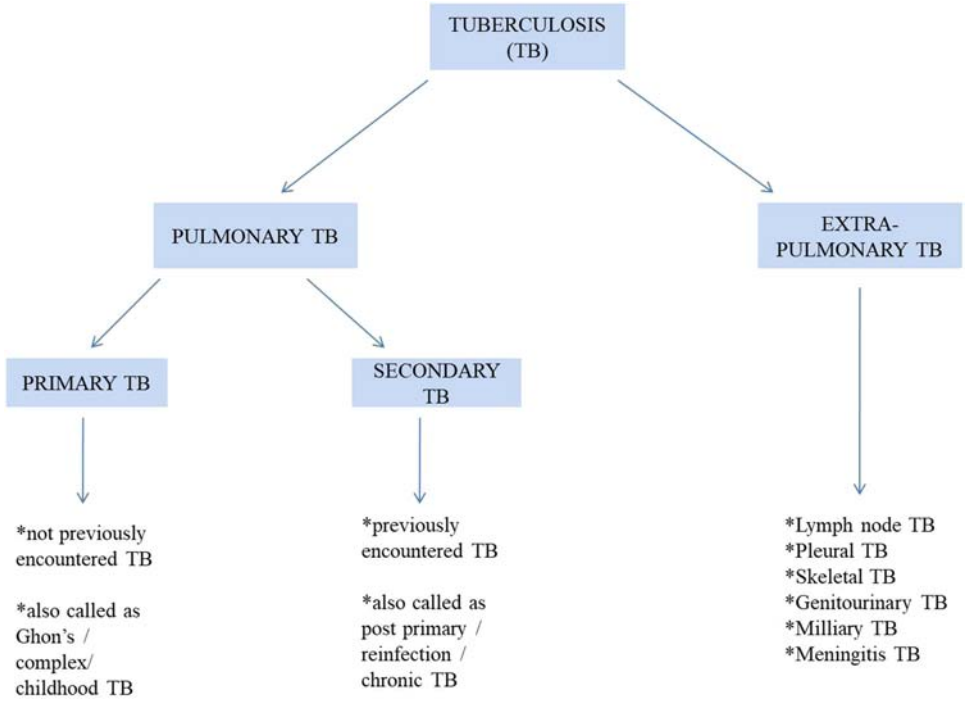


FIGURE 19.3

Type of tuberculosis based on site of infection.

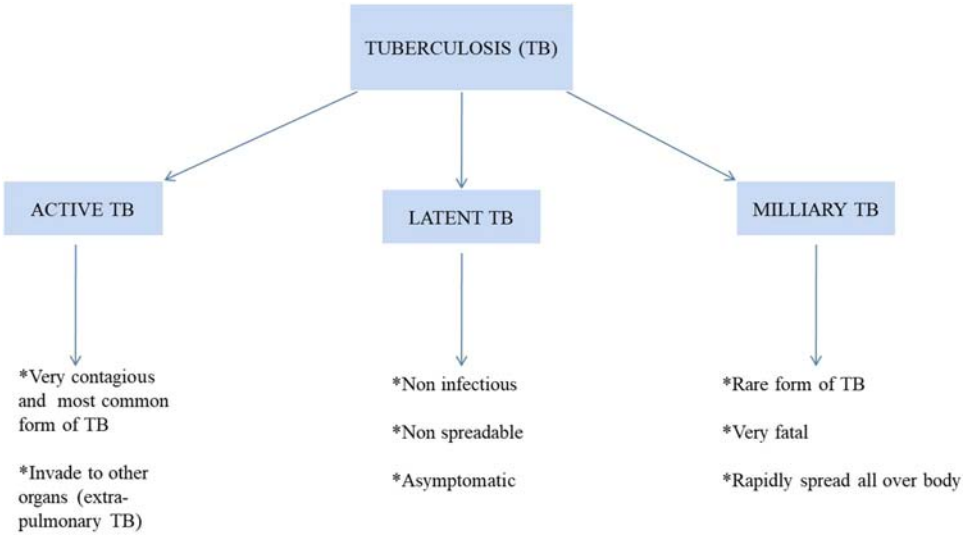


FIGURE 19.4

Types of tuberculosis based on illness and the spread of infection.

19.5.2 Pathogenesis

M.Tb. causing TB in the only known reservoir of humans, which spread like a communicable disease. The bacteria are transmitted when a person coughs, sneezes, speaks, sighs, etc., in the form of an aerosolized droplet infection which is transmitted in the air, and through the air, it is inhaled by the human. There are three routes to be exposed to the infection, that is: (1) inhalation of the contagious droplet having M.Tb. in the respiratory tract; (2) gastrointestinal route; (3) cutaneous route [11].

Initially, the aerosol droplet is secreted by the M.Tb. ($\sim 1-5$ mm in size) remain suspended in the air and act as a source of spreading the infection vigorously when a person inhales the infected air. Therefore, the cycle of TB begins with the inhalation of the M.Tb. associated droplets (a dose of $1-10$ bacilli is needed to cause the infection). The primary course of infection progresses through the respiratory tract to the lungs and then to the marginal alveoli, in which macrophages and dendritic cells (phagocytic immune cells) are engulfed. Other nonphagocytic cells also are infected in the alveoli likewise type 1, 2 pneumocytes, alveolar endothelial cells, etc. [41]. During the early phase of infection, the internalization of M.Tb. is done by the phagocytic immune cells, which replicates intracellularly resulting in an increase in infection to various pulmonary and extrapulmonary sites (lymph nodes, meninges, skeletal, bloodstream, etc.). The infection rate depends on numerous aspects like the closeness of contact, the cause of the bacilli, the load of the bacilli inhaled, and the immune potential of the host [42]. Thus, the hallmark of the infection starts when the monocytes of the nearby blood vessels from the beginning of granuloma when the host immune system wants to get rid of the infection (Fig. 19.5).

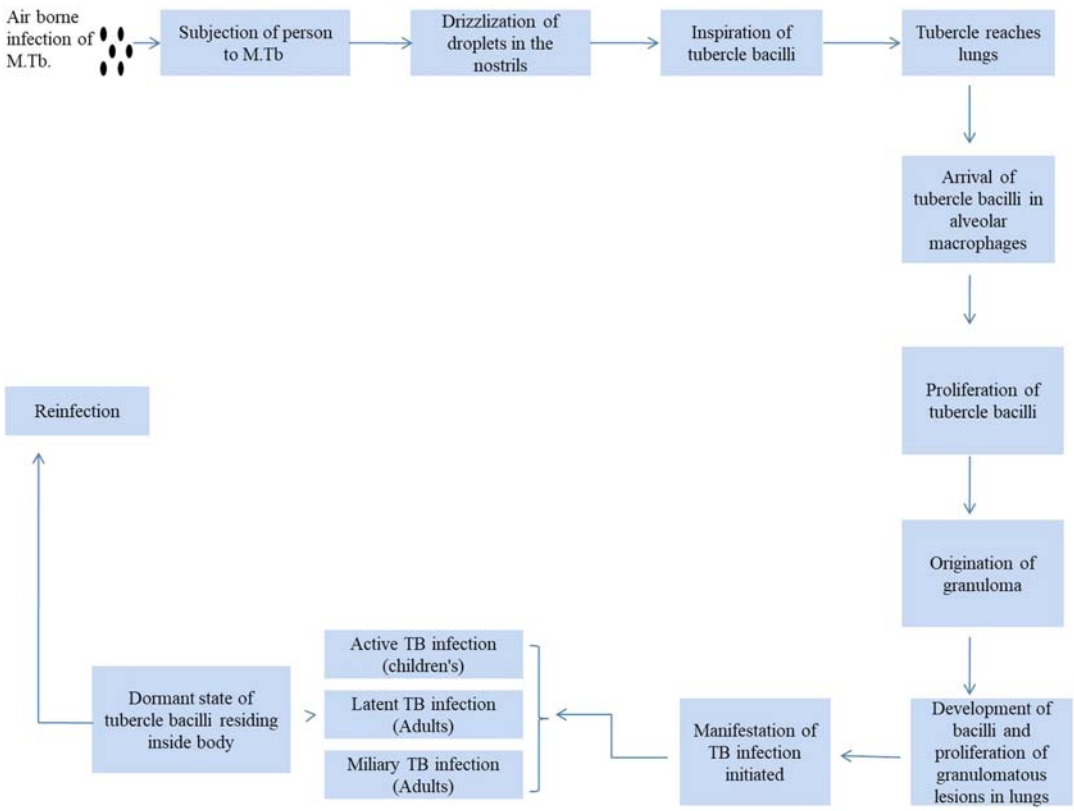


FIGURE 19.5

Pathogenesis of *Mycobacterium tuberculosis*.

19.5.3 Manifestation

The symptoms mainly depend on the fact whether the disease acts on pulmonary or extrapulmonary sites. Few common symptoms prevail during infection for instance bad cough which lasts for say three weeks or longer, pain in the chest, coughing up blood or sputum or mucus, weakness/fatigue, loss of appetite, chills/fever, sweating at night especially, intoxication or hemoptysis. However, in the case of latent Tb, the patient remains asymptomatic and can only be detected when examined by the test to confirm TB [43].

19.5.4 Diagnosis

Microbiological confirmation of TB is diagnosed by the presence of M.Tb. in the test. Usually, two tests are used to detect the bacteria in the host body, that is, tuberculin skin test (TST)/Mantoux test, and TB blood test (TBT). A positive TST or TBT tells that a person is infected, but it does not

clarify whether a person is suffering from latent TB or active TB type. Other such tests to demarcate the type are to be done likewise chest X-ray or a sample of sputum or phlegm examination verifies an latent tuberculosis infection (LTBI) [44].

19.6 Molecular mechanism of *Mycobacterium tuberculosis*

The molecular mechanism of M.Tb. includes various steps as described as follows:

(1) Entry of the M.Tb; (2) Immune reaction of the host to M.Tb; (3) Role of immune cell and cytokine in M.Tb; (4) Antigen presentation pathway and their modification by M.Tb. component; (5) Dampening of other macrophages functions by M.Tb. component; (6) Phagolysosome maturation and its inhibition by M.Tb. component; (7) Apoptosis and M.Tb inhibition of infected macrophages component; (8) Discharge of M.Tb. from phagosome/phagolysosome; (9) Perseverance and revival of LTBI; (10) Diagnosis of LTBI [45].

The infection of the tubercle bacilli starts when the phagocytosis of the bacteria via phagocytic antigen-presenting cell (APC) in the dendritic cells and alveolar macrophages. The specific pathogen recognition receptors (PRR's) recognize the pathogen-associated molecular patterns (PAMP's) initiating the host's innate immune response. The bacterium causing TB is internalized via three different types of receptors, i.e., complement receptor, mannose receptor, and scavenger receptor. The M.Tb. is recognized by the TLR which is present in host receptors or mannose receptors. Other receptors also recognize the pathogen likewise cholesterol receptor, surfactin protein A receptor (Sp-A) and Surfactin protein B, etc. These receptors are present in both the arms of the immune system (innate and adaptive immune system) and also on immune cells (macrophage, dendritic cell, B cell, and T cell) or nonimmune cells (epithelial or fibroblast cells).

The interaction of TLRs (TLR-2, TLR-4) initiates the cascade of events in the innate immune system. The proinflammatory and antimicrobial action starts when the pathogen interacts with the host. The cell envelope of M.Tb. encloses a thick waxy coating of lipid and polysaccharides along with high amounts of mycolic acid. Few of the potential ligands which interact with receptors of TLR are lipomannan (LM) and mannose-capped lipoarabinomannan (ManLAM), etc., which after interacting with TLR activates the nuclear transcription factor and produces cytokines (TNF-α, IL-1, IL-12). Later on, the continuous production of TLR-2 ligand lipoprotein by M.Tb. inhibits the MHC-II (cluster of differentiation, CD4 +) processing and expression on the alveolar macrophages which results in the niche formation of M.Tb. in the lungs and suppress the effectors T-cell [46].

After the entry of M.Tb. into the alveolar macrophages, the signal for infection initiated inflammatory action of cytokines and chemokines. The migration of immune cells (neutrophil, lymphocyte, monocyte) to the infection site is unable to destroy the bacteria properly due to which the bactericidal action causes macrophage necrosis preventing phagolysosome (phagosome−lysosome fusion) formation [47]. Thereafter, M.Tb. start multiplying and meanwhile, dendritic cells internalize the mature bacterium and migrate toward lymph nodes where they start accumulating on the macrophages, T cells (CD4 + and CD8 +) lead to the formation of granulomas at the infectious site.

Formation of granuloma tries to take off the bacilli from other parts of lung tissue just to decrease the spread of infection and activates the CD4 + T Cells which produce IFN-γ (interferon)

that kills the M.Tb. and halts the infection. But few of the bacteria resisting it escape the immune response and start living in a dormant state in the host avoiding its immune system.

A wide range of immune components is found to be effective against M.Tb. Besides dendritic cells and macrophages, the most important is the T cells (both CD4 + and CD8 +). CD4 + plays a major role along with CD8 + and natural kill produces IFN-gamma (key cytokine which imparts protection against immune response against M.Tb.) which are quite effective to halt the infection. Independent of IFN-γ; CD4 + is potentially defensive against the bacilli as it carries an important function to halt infection in the granuloma likewise apoptotic activity of infected alveolar (Fas/Fas ligands) or cytokine production or other immunoregulatory cytokines such as IL-10, 12, 15. So the inhibitory action of CD4 + and cytokine action of CD8 + are majorly responsible for the regulation of the infection. Whereas, their presence imparts the onset of latent TB infection which states that reactivation of TB is due to the depletion of CD8 + T cells [48]. Therefore, the MHC-I and MHC-II machinery of the immune system has the potential to fight against the M.Tb. and triggers apoptosis against them by destroying the cellular component of M.Tb. (i.e., ManLAM, Lipoproteins, etc.).

The M.Tb. receding into the phagosome after their entry into the macrophages and dendritic cells is accomplished by the protective immune response of CD4 + T cells representing the pathogen on the MHC-II complex. It is evident that the continued presentation of antigen results in the persistence of inhibiting the infection [49].

A series of fusion and fission events occur when the phagocytosis of bacilli by the alveolar macrophages in maturing the phagosomes enclose the pathogen forming a phagolysosome. The lower pH and vacuolar proton ATPase transport in the lysosomal formed acid- hydrolases work efficiently against the bacterium. The maturation of the phagolysosome is dependent on the calcium signaling cascade of events which begin with the phosphorylation intracellularly of the alveolar macrophage converting sphingosine and sphingosine 1-phosphate by sphingosine kinase forming calcium-calmodulin complex by the activation of PK-II. The PK-II converts phosphatidylinositol 3-kinase (PI-3P) into phosphatidylinositol-3-phosphate (PI-3P), forming a phagolysosome complex and inhibit the bactericidal action of the pathogen. But the M.Tb. has several strategies against the destruction by the phagolysosomal complex excluding the vH + -ATPase activity and reducing the acidification of the lysosome complex. Also, the ManLAM and lipomannan of the M.Tb. inhibit its formation and increases the pathogenicity of bacilli. If any bacilli encounter in the lysosomal compartment, it kills the bacteria by ubiquitin-mediated peptides and destroy it by autophagy [38].

Apoptotic vesicles formed by the host body in response to the defense against the infected macrophages enclosing the antigen are triggered by the CD8 + cells which are taken up by the dendritic cells as the phagolysosomal complex. The CD8 + T cells produce IFN- γ which are activated by the apoptotic vesicles and kills the M.Tb. infected cells intracellularly [50].

But several factors enable the pathogen enclosed in macrophages to skip the host-immune system via expressing the pro-apoptotic or antiapoptotic factors expressed in the bacterium. M.Tb. cell wall contains a ManLAM and a few secretory proteins (Rv3654c and Rv3655c) which inhibit the apoptotic pathway to avoid the host's immune system [51].

It's a known fact that M.Tb. finds its way to survive within the host by blocking the formation of phagolysosome and persist in the host. Another way to escape from the host immune system is done by the presence of the difference 1 (RD1) region in the genome of the M.Tb. which is the most important cause of virulence in the pathogen. The RD1 region forms a new protein secretion

system (ESX-1, that is, type VII secretion system) is involved in exporting M.Tb. to different proteins to transmit the infection. Along with ESAT system exporters (ESX-1, type IV secretion system) early secreted antigenic target (ESAT)-6 complex secreted by M.Tb. in the phagosome splits the bacilli and introduce itself into the lipid bilayer results in lysis and escape of the M.Tb. So, the formation and action of ESX-1 and ESAT-6 complex help in the enhancement of the infection and bactericidal escape from the immune system [52].

Due to the escapement and inability of the host immune system to eliminate the pathogen. The tubercle bacilli exhibit various strategies to stay invaded in the host by either staying in a state of dormancy because the dormant bacilli retain their ability to induce reactivation. Sometimes the cellular component of M.Tb. ManLAM persists and reactivates the infection by preventing bacterium from the microbicidal activity of macrophages or other immune cells or inhibiting the phagolysosomal formation. Later on, when the infection is at peak and the onset of symptoms starts appearing then one gets itself diagnosed by Tb tests (TST's/TBT's), chest X-rays, etc., to know whether they are positively diagnosed or not.

19.7 Therapeutics of *Mycobacterium tuberculosis*

Biosurfactants can act as promising molecules having diverse properties, structural novelty, versatility that is potentially useful for the applicative measures in biomedical approaches.

Due to their biocompatible surface activity, the biosurfactants interact with microbial cell membranes and can act as potential therapeutic agents against Tb or can act as a part of a drug delivery system. Likewise, lipopeptides, glycolipids distort the cell membrane and cause apoptosis of cells to inhibit cancer cell proliferation. This increased interest in biosurfactants and their applicative use on human/animal cells/cell lines make them effective and safe therapeutic agents.

19.7.1 Via drugs

Almost 90 years ago in 1802, the vaccination for tuberculosis came into action, the very known existing vaccine Bacillus Calmette Guerin (BCG) apart from the fact that it offers very limited protection against the disease [53]. Majorly the drugs for Tb have been categorized into two types, that is, first-line drugs which are used in the care of new patients with very little risk of having medication resistance, for example, Isoniazid, Rifampicin (Rifadin, Rimactane), Ethambutol (Myambutol) [54]. Among them, Isoniazid and rifampicin are the most powerful and potential first-line drugs. Second-line treatment drugs are only used for the treatment of drug-resistant TB, for example, pyrazinamide. In the case of drug-resistant TB, two criteria were their one is multidrug-resistant TB (MDR-TB) and the other is extensively drug-resistant TB (XDR-TB). MDR occurs when the bacteria causing it are at least resistant to isoniazid, rifampicin, and a few other MDR drugs like Fluoroquinolones, Para-aminosalicylic acids, Thioamides, Cycloserine, etc. In terms of XDR when the strain is resistant to at least rifampicin and isoniazid but also with Bedaquiline and Delamanid [55]. Drug-resistant TB is cured by the combinational medication of the drugs. In the case of active TB, one needs to take antibiotics for at least 6−9 months and the dosage, length of the treatment depends mainly on the age, drug-resistant or site of infection, etc. [56] (Fig. 19.6).

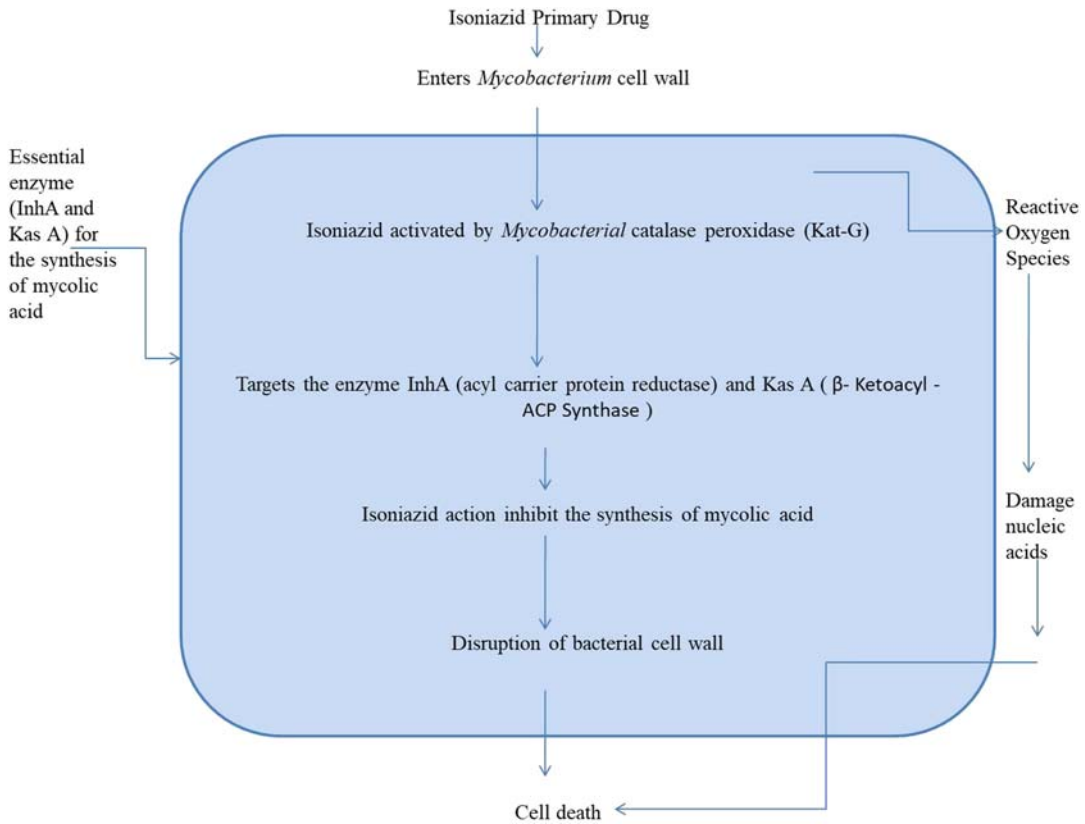


FIGURE 19.6

Mechanism of action of antitubercular drug (e.g., isoniazid-primary drug).

The M.Tb. of bacteria faces a lot of implications and stress environment in the host to make itself survive but the highly acidic and oxidative stress and deprivation, nutrients depletion make it hard for the bacteria to survive and enhance the infection. Moreover, along with all these stresses, the bacteria face genomic instability due to the DNA damaging stress which compromises and affects the bacteria's fitness. To maintain it for a longer period in the infected macrophage, the bacteria deal with the endogenous DNA-alkylating chemical spices and manage its genetic stability. A lot more of gene inactivation and genomic stability in the M.Tb is maintained in the DNA repair system of the multienzymatic type like nucleotide excision repair (NER), base excision repair (BER), or the protein accountable for the uninterrupted reversal of the DNA destruction. The otg gene in the Tb is secreted by protein in the M.Tb. used in the restoration of alkylated DNA, O6-methylated-guanine methyl-transferase which aptly reorganizes promutagenic O6-methylated-guanine found in alkyl-damaged DNA. Later on, when the OGT transferred damage of the O6 -alkyl group to modify it from guanine into cysteine helped in the promotion of degrading M.Tb biochemical pathogens. And these proteins in DNA metabolism are the most potent site for the

drugs to be targeted because these proteins are quite essential to perform the biochemical pathways of the bacteria to infect hosts. Meanwhile, the exposure to reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) of the host immune system is significant for the DNA damage of the M.Tb., halting the M.Tb. infection cycle. Another approach used nowadays efficiently is drug delivery via microbial biosurfactants. Due to their small size, low toxicity, high stability, biocompatibility, and amphiphilic nature, biosurfactants are easy to use against M.Tb. through diverse routes oral, nasal, intravenous, etc. For instance, glycolipids type biosurfactant Rhamnolipid secreted by *P. aeruginosa* is used against M.Tb. for their high potential antibacterial activity. Via liposomal complexes, they are being internalized in the host body along with the drug targeted (isoniazid) inside. Resulting in the activation process of killing *Mycobacterium* and making the host free from infection [14,57–59].

M.Tb. secretes the trehalose lipids type of biosurfactant which is an essential metabolite in the bacilli for maintaining cell wall synthesis and preventing it from desiccation, freezing, osmotic stress, etc., we can halt the infection by inhibiting the trehalose utilization pathway's (TUP) via first- or second-line drugs packaged into the lysosome forming auto phagolysosomes resulting into the inhibition of trehalose formation and degradation of the bacilli by inhibiting its cell wall formation [60].

19.7.2 Via biosurfactants

19.7.2.1 Antimicrobial activity

Therapeutic and probiotic agent biosurfactants are used in treating many diseases by playing roles as being antibacterial, antifungal, antiviral, and also antiadhesive agents, they act against pathogens, and hence their antimicrobial activity makes them highly useful for biomedical applications [60]. The unique properties of biosurfactants makes them most susceptible to health-related applications. Few of them are suitable for synthetic medicines, gene transfer, drug delivery, antiadhesive coating, etc. [61].

Lipopeptides show the best antimicrobial action among all the biosurfactants, that is, surfactin and iturin produced by *B. subtilis*. Several lipopeptides biosurfactants produced by marine organisms, *B. circulans* are quite effective against *Proteus vulgaris*, Methicillin-resistant *Staphylococcus aureus* (MRSA), and another multidrug-resistant (MDR) [53,62,63].

More than six rhamnolipids produced by *P. aeruginosa* exhibit antibacterial activity. Also, the antifungal activity of a glycolipid isolated from *Yeast* such as *P. flocculosa*, i.e., flocculosin shows activity against the pathogen causing human mycoses. MEL's produced by *C. antarctica* have surface activity along with the antimicrobial activity. MEL shows strong antibacterial activity against Gram-positive bacteria and shows a low response toward Gram-negative bacteria. Both MEL-A and MEL-B undergo chemical modification in their sugar moieties and provide potential antimicrobial activation [64]. The trehalose lipids of *Rhodococcus erythropolis* are effective in inhibiting HSV and influenza virus. The antibiotic effect of iturin is effective in suppressing HIV growth and has opened up new avenues of biomedical applications.

In the case of M.Tb., the most effective glycolipid to date is Rhamnolipids secreted by *P. aeruginosa* which shows excellent antibacterial activity against Mycobacterium. The effective approach of rhamnolipids in inhibiting the growth of the bacilli is either by affecting the membrane surface

properties, fluidity and permeability, gene regulation, enzyme production/activity, or degrading the cell membrane [65]. The mechanism of action of rhamnolipids against M.Tb. initiated when the surfactant interferes with the cytoplasmic membrane region and destroys its membrane permeability,. via increasing the membrane permeability by the formation of molecular aggregates in the membrane of the cell that results in the pore formation and membrane disruption which ultimately leads to cell death [66]. Sometimes, rhamnolipid instead of acting on the extracellular surfaces, act on the intracellular surfaces and bind on the LPS of the bacterium by altering the cells amphipathicity, where the LPS removal decreases the virulence of the bacterium strain H37Rv and increases the membrane degradation by solubilization and emulsification of the n-alkanes [67]. Another cyclic depsipeptide Massetolide A and Viscosin, derived from the two strains of *P. aeruginosa* from marine habitat is quite efficient in exhibiting in vitro antibacterial activity against M.Tb. and M. avian by reducing the rate of adhesion and deposition of the pathogen on solid surface or site of infection [68]. So, by degrading the cell wall of the bacilli we can halt the infection of TB in the host by the antimicrobial action of rhamnolipids mainly and also other types of microbial surfactants on the M.Tb. pathogenic strain H37Rv (Fig. 19.7) [69].

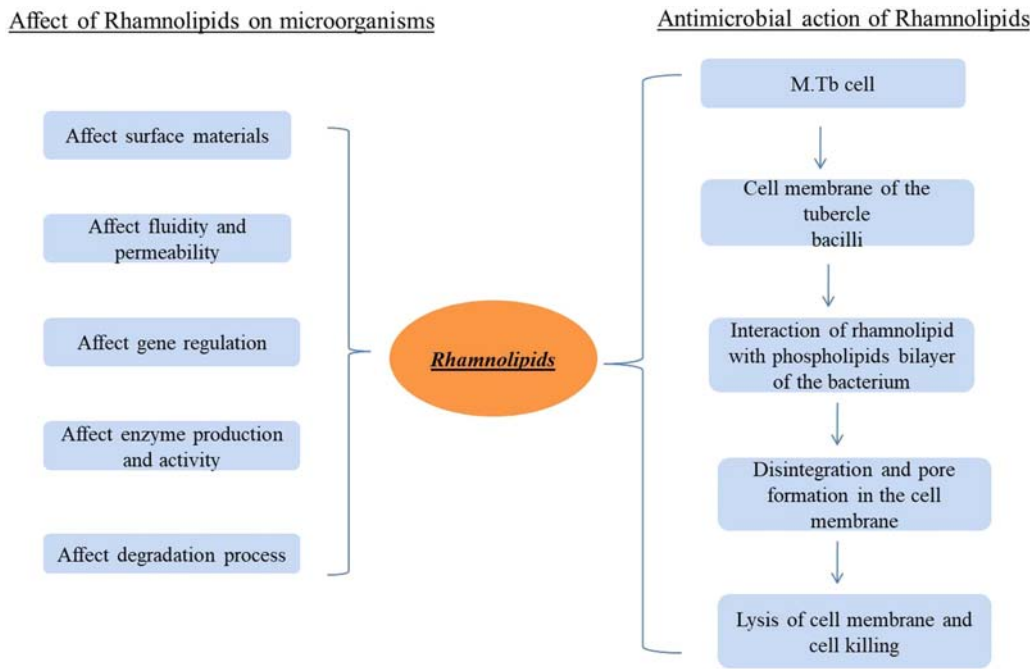


FIGURE 19.7

Affects and antimicrobial action of rhamnolipid against the pathogen.

19.7.2.2 Immunomodulatory actions

Biosurfactants show great potential in immunomodulatory actions. A large number of secondary metabolites producing organisms show an immune response toward the pathogen. This is due to the host cells receptor recognition ability, which acts as a sensor when pathogens encounter and recruit a signal cascade, thereby activating the immune response. Various immune cells such as neutrophils, dendritic cells (DC), macrophages, and toll-like receptors (TLR) are well-defined PRR's by sensing pathogenic derived components, i.e., PAMP's (Fig. 19.8)

Sophorolipids are the most potent modulators of immune response resulting in the decrease of IgE production by down resulting in the pathogenic secretions. Whereas, glycolipids modulate both humoral and cell-mediated immunity. Rhamnolipids and trehaplolipids are bacterial exotoxin and are known to act as soldier molecules in the immunomodulatory actions [70]. Effect of lipopeptide, that is, Surfactin acts mainly on the inhibition of costimulatory molecules like CD40, CD54, CD80, etc. Therefore, Surfactin is used in immunosuppressive activity in treating hypersensitivity-related immune disorders [71–73].

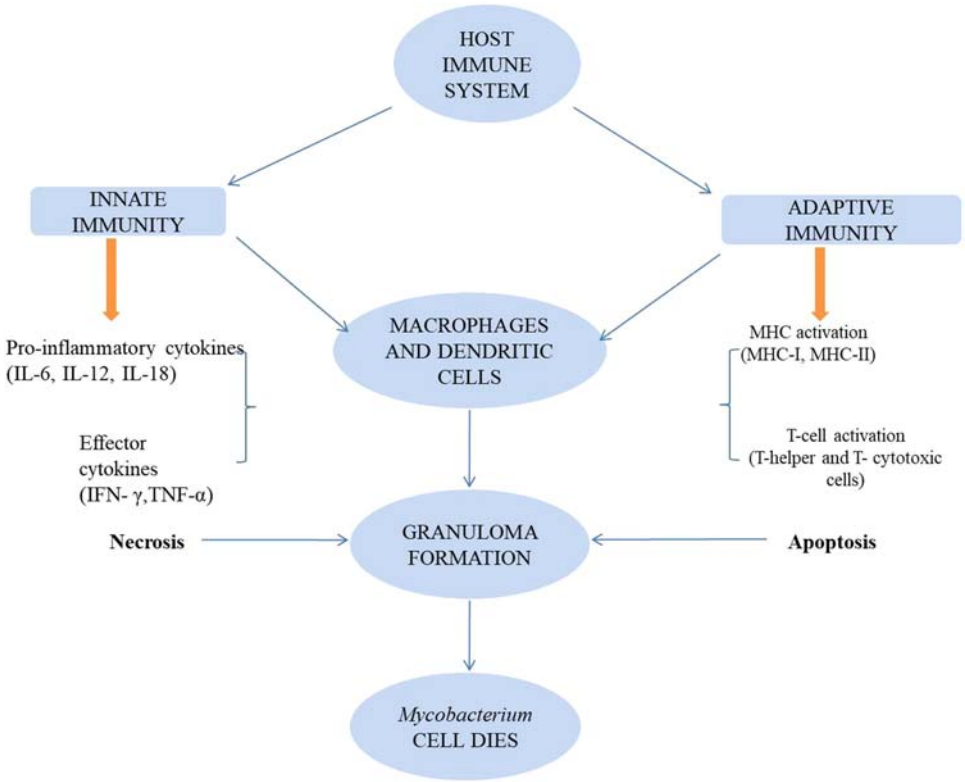


FIGURE 19.8

Host immune system action against *Mycobacterium tuberculosis*.

Specifically in the case of treating M.Tb., glycolipid biosurfactants are the most potent and impart both types of immunity (humoral and cell-mediated immune system). Particularly among glycolipids, rhamnolipids an exotoxin that shows immunomodulatory actions against M.Tb. and acts as a soldier against the pathogenic strain H37Rv. Normally they destroy the pathogen with antimicrobial activity by forming biofilm and causing cell wall disintegration. However, in terms of immunomodulatory effects they either chemotactically produce neutrophils or increase the production of ROS which help to secrete TNF-α, interleukins (IL), interferons (IFN), etc., resulting in the lysis of infected macrophages or decreasing the pathogens peptide expression [18].

Surfactin a biosurfactant belongs to the family of collectins, which is divided into three types such as Surfactin A, B, C. Among them, Surfactin A and B are the most abundant present in the human lungs and is an important mediator in innate immunity. Surfactin-A facilitates the increased opsonization against M.Tb. and increases the macrophages mediated defense in inducing the production of cytokines. Therefore, Surfactin-A in humans perform antimycobacterial response against M.Tb. Another approach to killing the bacilli is via the receptor and ligand binding present in the host and pathogenic strain respectively. Likewise, binding of ManLAM (a ligand of M.Tb.) and mannose receptor (host) induces the antiinflammatory action and inhibits the *Mycobacterium* species. Several other receptors and ligand bindings are presented in Table 19.2 [73–76].

19.8 Future prospective

Providers of various types of microbial surfactants have led to a varied choice of applications in the biomedical sciences. They are potent green molecules and regarded as immunomodulatory molecules, due to their antibacterial, antifungal, and antiviral properties. Biosurfactants offer a great possibility of replacing synthetic chemicals with microbial surfactants and thus enhance the eco-friendly values. Despite their immense usefulness, they are still limited in use; either because of their large manufacture and abstraction cost or due to the lack of knowledge. Furthermore, due to

Table 19.2 Uptake of *Mycobacterium tuberculosis* in phagocytic cells via different receptors and ligand interactions.

Ligands of M.Tb.	Receptor	Actions
1. ManLAM	Mannose receptor	Antiinflammatory, inhibit Mycobacterium delivery to lysosomes.
2. LAM	Complement receptor 3	Mediates the opsonic and nonopsonic uptake of M.Tb.
3. ManLAM, Lipomannan	Surfactant protein A	Aggregation of M.Tb. and internalization by macrophages.
4. ManLAM	Surfactant protein B	Aggregation of M.Tb. and delaying of internalization of macrophages.
5. ManLAM, PIM's	Mannose-binding lectin	Protection from M.Tb. at a low level.
6. Host IgG	FC receptor	Direct the M.Tb. to the lysosomes.

their lowtoxicity, great quality of biodegradability and biocompatibility, antiadhesiveness, and ability to reduce surface and interface activity; in the near future they can be regarded as most promising and emerging molecules in having a variety of opportunities in the field of biomedical approaches and health-related areas. Nevertheless, these microbe-originating molecules need to be explored and validated to meet the needs in the future for the effective therapeutics and biomedical areas.

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CHAPTER

Biosurfactants role in nanotechnology for anticancer treatment

20

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20.1 Introduction

Biosurfactants (BSs) are derived naturally from microorganisms (bacteria/yeast). The term surfactant point toward a critical property, that is, "surface-active." The surfactants can act as detergents, emulsifiers, or foaming agents. The primary purpose of the BSs is to act as secondary metabolites that are necessary for the survival of microorganisms. Secondary metabolites may function to acquire nutrition, develop interaction between the host and microbe, or act as biocide agents [1,2]. They are amphipathic with distinct chemical structures, which vary with different sets of microorganisms, for instance, glycoproteins, glycolipids, and lipopeptides. These molecules enhance membrane permeability by modulating the structure physically or by manipulating the proteins that make up the membrane. The molecular reorganizations, such as hydrophilic and hydrophobic interactions, lower the surface tension leading to an increase in the surface area, thus enhancing the bioavailability. BSs have exceptional properties due to which they are commonly used in various fields, including agriculture and food industries, and other biomedical areas. The principle features possessed by BSs include antiadhesive nature, antiviral, antimicrobial, low toxicity, specificity, effectiveness, biocompatibility, and temperature and pH tolerance [3,4].

The role of BSs has recently emerged as propitious molecules due to their properties and their versatile nature has led to their utilization in biomedical applications such as in clot targeting. A significant role has been highlighted in prosthetics, where they act as antiadhesive coatings. Further, some exciting results indicate the involvement of BSs as antitumor agents that ceased the cancer cell progression [5]. Earlier in 1999, BSs (glycolipids) showed growth arrest in melanoma cells [3,6]. Recently, various promising studies show success in cancer treatments. Lipoproteins and glycolipids have emerged as broad-spectrum BSs for cancer treatment. For instance, the microbial lipopeptide derived from *Bacillus subtilis* was used in targeting B cap-37 breast cancer cell lines. The result depicted a selective cytotoxic role in apoptosis induction and in targeting cancer cell progression [1,7]. Treatment in liver cancer (cell line: HepG2) through Surfactin derived from *Bacillus natto* led to ROS generation and calcium accumulation, thus leading to apoptosis in the cancer cells [1,8].

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The role of BioEG BSs was explored by Duarte in 2014; the breast cancer cell linesT47D and MDA-MB-231 were used in the study, and the BS was proved to halt the "G1-phase" of the cell cycle and reduce the cancer cell viability [1,9]. Recently, it is reported that glycolipid BSs can have a potential role in drug delivery and in enhancing the efficiency of liposome-DNA interaction, to pave its way into the cell. Modified leptosome through BS Mannosylerythritol lipid-A (MEL-A) were seen inhibiting cancer cell progression by promoting apoptosis [3,10].

The structural linkage of BSs to various drugs can enhance cancer treatment. These amphipathic molecules possess both hydrophobic and hydrophilic moieties that provide distinct degrees of polarity. The hydrocarbon tail is often hydrophobic (apolar) in nature, whereas the head is hydrophilic (polar) and can be ionic or nonionic [3,11]. The primary function of surfactants is to increase the solubility of polar molecules and also lower the tension generated at the oil/water interface. The point at which the lowest and stable state of the surface and interfacial tension is reached corresponds to Critical Micelle Concentration (CMC) [1,12]. Micelles are amphipathic molecules that arrange into spherical forms when in a liquid medium. They comprise polar head groups facing the water, whereas tails are nonpolar and are embedded far from water. When hydrophobic tails interact with each other, the water is released from nonpolar ends, thus increasing the entropy, and CMC is the concentration at which the organized molecular entities, micelles are formed [3,4]. Lately, the BSs are being linked to nanoparticles (NPS) to provide efficient cancer therapy to patients. Nanobiotechnology is a newly emerging field that describes the active interactions amongst microbes and nanotechnology. It was observed that BSs could lead to the enhancement of nanoparticle synthesis and stabilization [13,14]. The NPS upon fresh synthesis are highly prone to aggregate into larger structures that depict their instability, which leads to a loss in their characteristics and activity. Therefore, to obtain a confined nanoparticle geometry, the micelles aid as "nanoreactors" [1,15]. BSs can be adsorbed on the surface of metal NPS to stabilize the hybrid, and its antiadhesive nature prevents aggregation. The main purpose of the surface modifications through the surfactant is to reduce the cytotoxicity of NPS. Further the size and shape of NPS are also modulated to tune the particle [16]. For efficient delivery of the drugs, the variant geometries of NPS and monodispersity are necessary. Some microemulsion (micelle formation) techniques using the principle of the oil-water interface are used to modify NPS. Glycolipid biosurfactants can be employed as bioemulsifiers in silver NPS [17]. The particle for efficient delivery can be mended by reverse microemulsion; the literature reported that the glycolipid (biosurfactant) was employed to synthesize NPS. By utilizing rhamnolipid as BSs for the synthesis of nanorods and altering the pH, the particle size can be tuned accordingly. That is, size is directly proportional to the pH. Therefore, microemulsion techniques show promising results for nanoparticle synthesis and organization.

20.2 Types of biosurfactants

20.2.1 Glycolipids

The most common form of BSs is glycolipids with a molecular mass between 628 and 826 Da. They are composed of carbohydrates such as (monosaccharides, oligosaccharides) and fatty acids. It forms ester linkages that connect glycosidic to a lipid moiety. The typical examples include

trehalose lipids derived from *Mycobacterium*, rhamnolipids from *Pseudomonas*, polyols, sophorolipids from yeast, cellobiose lipids, and mannosylerythritol (MEL) from *Candida sp.* The biological activities of glycolipids such as MEL, polyol lipids, trehalose, and sepharose lipids show cell differentiation despite cell proliferation in leukemia cell line HL60 [18].

They possess low toxicity and are surface-active, antimicrobial in nature, and thus show promising results in pharmaceutical industries [19]. Critical micellar concentration (CMC) remains between 20 and 366 mg/L.

Rhamnolipid is a crystalline acid that comprises either one or two units of the α-L-rhamnose unit, which are connected to 3-hydroxyalkanoyl-3-hydroxyalkanoate fatty acid through *O*-glycosidic bonds. Obtained through *Pseudomonas aeruginosa* can be classified into monorhamnolipd and rhamnolipids, different microorganisms produce either mono or di rhamnolipids [20]. It has many unique properties, due to which it is utilized as a natural emulsifier and can be used in detergent and laundry products [21].

Sophorolipids are naturally derived lipids that generate small-sized (nm) micelles of different geometries. Synthesized from *Candida bombicola* and comprise two parts, a head group, and a tail region. The head is the carbohydrate moiety (sophorose), and the tail is made up of 16−18 carbons. They are made up of disaccharide sophorose, which is connected to 17-hydroxylic acid through glycosidic linkage. They exist in either acidic or lactonic form, derived from yeast, and possess antimicrobial, antifungal properties which allow them to be used in germicidal mixtures [22] for the inhibition of algal blooms. Further, the anticancer role has also been observed in the promyelocytic leukemia cell line (HL60) due to the inhibition of protein kinase C [18]. It can also induce apoptosis; the cytotoxic effect was seen in the case of liver cancer (H7402 cells) [23].

Mannosylerythritol lipids comprise mannose and erythritol (4-*O*-β-D-mannopyranosyl-meso-erythritol) that are connected to fatty acids of variant hydrocarbon chains [24]. They are synthesized from *Candida antartica* and are antimicrobial, immunological can lead to cell differentiation in promyelocytic leukemia (HL60 cells) [18]. The use of MEL has also been implicated in growth arrest, apoptosis; when mouse melanoma B16 cells were exposed to it, a halt at the G1 stage of the cell cycle and DNA fragmentation was recorded [25]. They also induce an increased activity of the enzyme acetylcholinesterase (AchE) in neuronal PC12 cells resulting in cellular differentiation and partial apoptosis.

Trehalose lipids are derived from *Mycobacterium* and related bacteria. They can be used widely as their role has been highlighted in decreasing surface tension. It comprises two glucose subunits connected via α, α-1, 1-glycosidic linkage. Glycolipids that contain variant trehalose are known to be produced by *Nocardia, Rhodococcus*, and *Gordonia*. The most acknowledged glycolipid amongst this class is trehalose 6, 6′- dimycolate, connected via an ester linkage to C6 of glucose. Trehalose has antimicrobial and antiadhesive properties; moreover, cellular toxicity has also been investigated, and a decreased cell survival is noticed in a time and dose-dependent manner [26].

20.2.2 Lipoproteins/Lipopeptides

Lipoproteins are made up of proteins and lipids; these are high molecular weight compounds. Microbial surface-active compounds are isolated from *Bacillus* and *Pseudomonas* species and comprise of 7−10 amino acid residues; these hydrophilic peptides are linked to hydrophobic fatty acid moieties [27]. The BS also lowers the surface tension from 72 to 20 mN/m which is the extent of

free energy invested in stabilizing a bulky molecule, and in turn determines the effectiveness of the BS [28]. Lipopeptides act as antibiotics, antitumors, enzyme inhibitors, and antiviral agents [29]. The surfactin, iturin, and fengycin of *B. subtilis* are amongst the most prevalent and utilized lipopeptides.

The strain *B. subtilis* produces *iturin-A* BS. The lipopeptide character in this varies according to different microbial strain. They are derived from Iturin-A, an antifungal and antimicrobial agent which works by forming vesicles and disordering the plasma membrane, thus increasing the molecular permeability of the plasma membrane [30].

Surfactin comprises a standard peptide loop of seven amino acids linked to the carboxyl and hydroxyl group on fatty acid chains and is isolated from the Gram-positive microorganism *B. subtilis*. The surfactin displays a lower surface tension of 72−27 mN/m compared to the other BSs; moreover, it has a lower CMC (23 mg/L) [31]. Surfactin is an antiinflammatory, antiadhesive, antimicrobial, and antimycoplasmal agent [32].

Fengycin is lipopeptide BS that comprises 10 amino acids and a lipid connected to the N-terminal of the fragment. They are different from other BSs as they include two different types of amino acids, ornithine and allo-threonine. It exhibits robust antifungal activity and obstructs the progression of a varied variety of pathogens, especially filamentous fungi [33]. Other lipopeptides include viscosin, amphisin, and putisolvin. Viscosin, an another interesting lipopeptide is a cyclic molecule with antimicrobial potential. It is derived from *Pseudomonas sp.* Its role has also been highlighted in cancer treatment; results depict the antimetastatic role in targeting prostate cancer [34]. Moreover, some isoforms of fengycin and surfactins extracted from *Bacillus circulans* showed toxicity against cancerous cells [35].

Amphisin is also isolated from *Pseudomonas* species DSS73, just like viscosin, and comprises of a β-hydroxydecanoyl fatty acid connected to a sequence (D-Leu-D-Asp-D-aThr-D-Leu-D-Leu-D-Ser-L-Leu-D-Gln-L-Leu-L-Ile-L-Asp) that will form a cyclic lactone ring on the C-terminal domain. It comes in the class of surface-active molecules, which possess less toxicity index and with a CMC range of 0.075 mmol/L in the water at pH 7.0. Amphisin comprises both BS and antifungal properties; due to such characteristics, it's used as a multifunctional biomolecule [4] (Fig. 20.1).

20.2.3 Phospholipids

Phospholipids are the major constituents of the microbial cell membranes; these are derived from several yeasts and bacteria. BSs are produced when bacteria dwell on the surface of n-alkanes via a process of microbial oxidation [37]. The structure comprises a phosphate group linked via 5−12 carbon atoms to fatty acid chains. The fatty acids aid in lowering the surface tension up to 21 mN/m, and the CMC varies from 58.9 to 0.007 mM [38]. There are different types of phospholipids, like phosphatidylethanolamines (PEs), phosphatidylglycerols, and phosphatidylcholine. They are produced from *Aspergillus sp.*, *Thiobacillus thiooxidans*, and *Acinetobacter sp.*, the BS produced from *Acinetobacter*, that is, phosphatidylethanolamines can form microemulsions of alkanes in water [39]. PEs isolated from *Rhodococcus erythropolis* can reduce the interfacial tension (<1 mN/m) in between hexadecane and water and a CMC ranging from 30 mg/L [40].

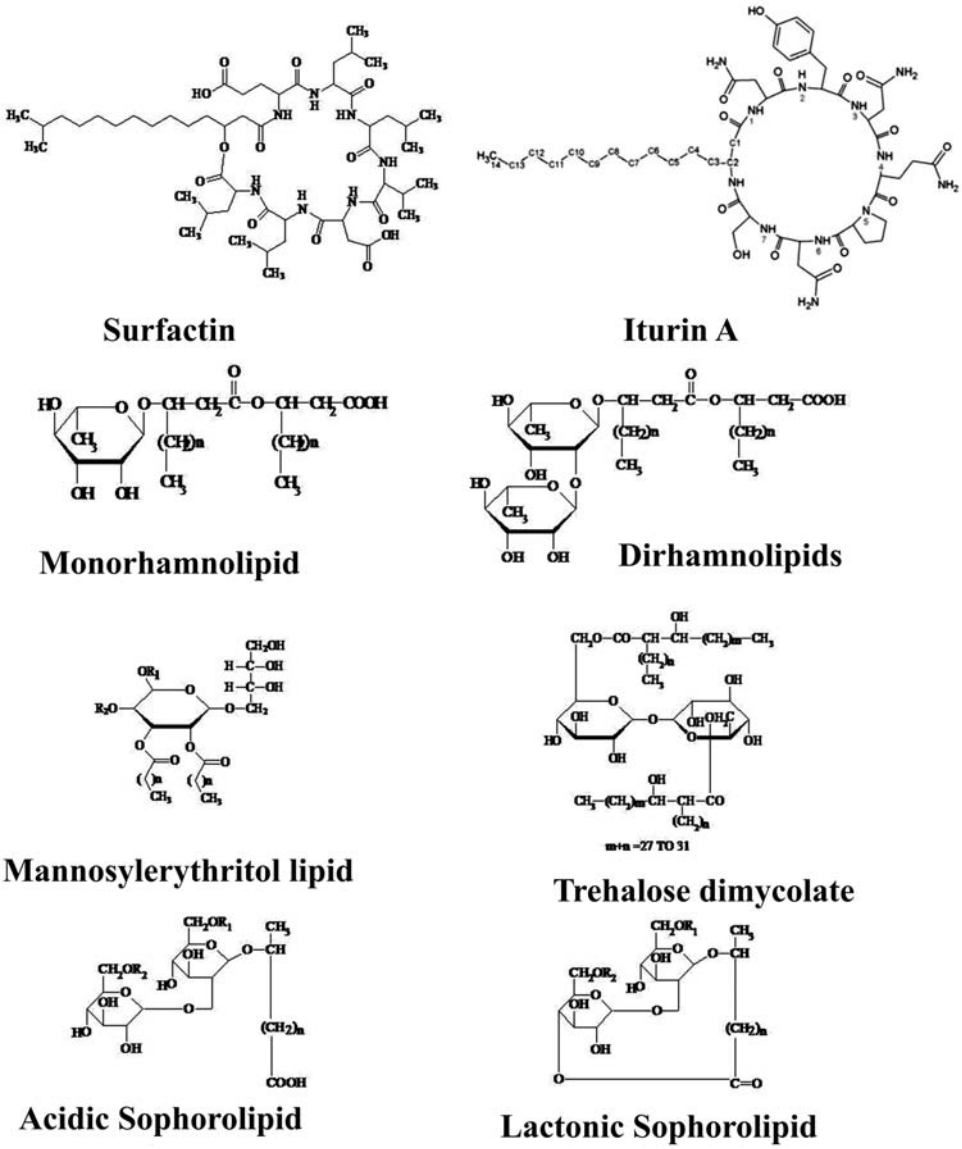

FIGURE 20.1

Chemical structures of Surfactin, Iturin A, Rhamnolipids, Mannosylerythritol lipids, Trehalose lipids, acidic, and Sophorolipids.

Reprint with permission from L. Fracchia, M. Cavallo, M.G. Martinotti, I.M. Banat, Biosurfactants and bioemulsifiers biomedical and related applications—present status and future potentials, Biomed. Sci. Eng. Technol. 14 (2012) 326–335 [36].

20.2.4 Polymerics

Polymeric BSs possess complex compositions, heteropolysaccharides with a molecular mass greater than and equal to 1000 Da with an ability to lower the water surface tension from 72 to 390 mN/m [41]. The widely explored polymeric BSs includes emulsan, liposan, alsan, and other polysaccharide-protein complexes. The heteropolysaccharide consists of repeated *N*-acetyl-D-galactosamine trisaccharides, *N*-acetyl galactos amineuronic acid, and unknown *N*-acetyl amino sugar and polysaccharide, connected via o-ester, to the fatty acid chain. Different types of microorganisms produce different polymeric BSs, for example; most common biopolymer is emulsan (Fig. 20.2 (B)) which is produced by *A. calcoaceticus* RAG-1, and is regarded as a polyanionic heteropolysaccharide [42]. It is a powerful emulsifying agent used at low concentrations (0.001%–0.001%) to emulsify hydrocarbons in water. Another polymeric BS is also derived from *Acinetobacter radioresistens* KA-53 and is an alanine containing heteropolysaccharide BS. Liposan is isolated via *Candida lipolytica* the primary structure is made up of carbohydrates, and the rest is protein. The mannoprotein is isolated from *Saccharomyces cerevisiae* and *Candida tropicalis*; it is a potent emulsifier made up of mannose and protein [43].

20.2.5 Particulate biosurfactants

Particulate BSs are divided into two parts, the extracellular vesicles and the whole microbial cell. Extracellular vesicles of the membrane divide the hydrocarbons to form a microemulsion that enhances the uptake of alkane by the microbial cells. The vesicles of proteins, phospholipids, and lipopolysaccharides lie within the range of 20–50 nm [44]. Synthesized from *Acinetobacter sp.* strain HO1-N and *P. marginalis*, these BSs help in the microorganism in alkane uptake. Another type is the whole-cell microbial cells, comprise of hydrocarbon-degrading and nonhydrocarbon degrading species of microorganisms. The microbial cell is regarded as a surfactant; the hydrocarbon-degrading property is concerned with surface components like M protein, lipoteichoic acid (*Streptococci*), protein A (*Staphylococcus aureus*), and layer A (*Aeromonas salmonicida*), etc. [45,46] (Fig. 20.2).

20.3 Biosurfactants as surface modifiers

The BSs possess various properties as described above; the major ones include low toxicity, bioavailability, and high biodegradability, effectiveness at extreme conditions of pH, temperature, and salinity. The ability to reduce the interfacial tension and thus form stabilized emulsions (oil/water) all such advantages lead to the utilization of BSs with nanotechnology [47]. A field that has gained remarkable evolution in recent decades is nanotechnology; different NPS can be grouped into inorganic, organic, and metallic NPS [48–50]. The properties necessary for nanoparticle formation include a large surface area that allows different surface coordinates like a drug, DNA, peptide, etc., for performing functions such as biosensing, drug delivery, imaging, etc. The size as well as the shape matters, the size is important as the migration, and radial drift of NPS toward blood vessels depends on the size (Fig. 20.3). The smaller the size, the more is the diffusion rate [51]. The shape enhances the quality of deposition, for example, spherical-shaped particles tend to remain in

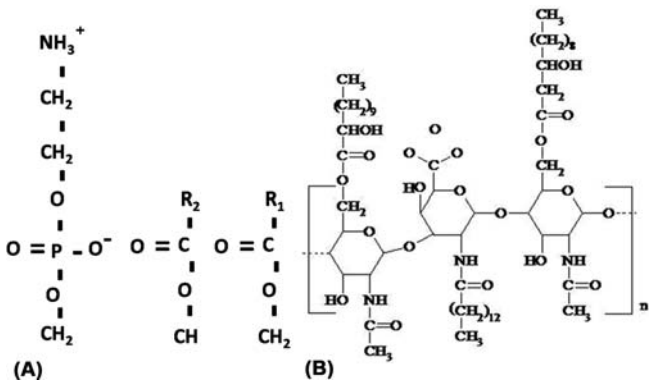

FIGURE 20.2

(A) Phosphatidylethanolamine: R1 and R2 are hydrocarbon chains of fatty acids, (B) Polymeric biosurfactant-emulsan.

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the middle of the blood vessel, and various forces drive the rod-shaped NPS toward the vessel periphery. Nanorods exhibit sevenfold more buildup as compared to nanospheres [52]. Therefore, the efficiency of the NPS can be modulated depending upon the modifications induced during the synthesis; similarly, the surface of NPS can also be modified to achieve outstanding performance. The NPS although provide a broad gamut of applications, but at the same time, can act as toxic contaminants that behave differently in the body. Several problems can be raised at the time of the introduction of NPS to the human system. One similar issue is the loss of an active state of the protein on encountering NPS. Therefore, surface modifications of NPS area much needed, as the tailoring of NPS with BSs provides a solution for all these issues [53]. NPS can be divided into two types inorganic and organic. Inorganic or metallic NPS consist of gold, silver, iron oxide, carbon nanotubes (CNTs), quantum dots, etc., and organic NPS includes chitosan, liposomes, dextran, and poly (lactic-*co*-glycolic acid) (PLGA) [54].

20.3.1 Inorganic nanoparticles

Metallic nanoparticles (MNPs), due to their small size and unique properties, are widely employed in biomedical areas. They are coated by a layer of adsorbent, that is, surfactant to make them hydrophilic so water-soluble. The MNPs include Iron oxide (Fe_2O_3), silver (Ag), gold (Au), and zinc oxide, etc.

20.3.1.1 Iron oxide nanoparticles

Iron oxide nanoparticles (IONPs) possess superparamagnetic properties and therefore are the most preferred molecules for in vivo applications. The comprehensive utilization in biomedical industries makes its toxicity study necessary; a detailed analysis of its pharmacokinetic and biodistribution

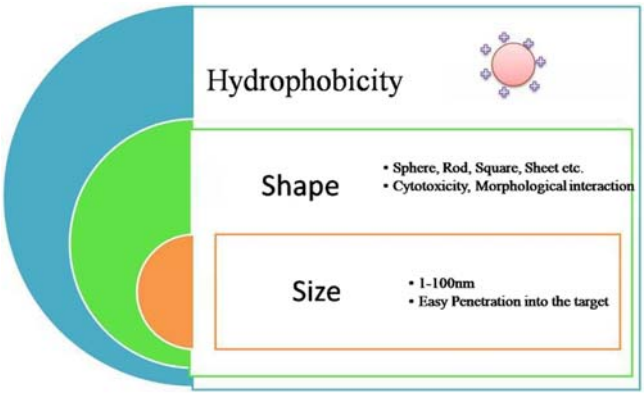

FIGURE 20.3

Size and shape, surface and interior properties of nanoparticles that enhance their use in cancer therapy.

depicts high chemical reactivity. Various in vitro and in vivo studies show the formation of free radicals and oxidative injury. Loading the cells with IONPs leads to Fenton reaction, the magnetite NPs generate free radicals, Voinov in 2011 has reported the release of hydroxyl radicals (OH) as a result of catalytic reactions [55], these catalytic reactions take place at the surface of Fe_2O_3. Further, the uncoated residues tend to aggregate and minimize the surface energy as a result of the large body-to-volume ratio. Therefore, they need to be functionalized by surface ligands, coatings because they are toxic and can be cleared out before even reaching the target. The BSs are therefore being used along with NPS to achieve efficient delivery, enhance the retention time in the body [16].

In a study, a positively charged iron nanoparticle possessing a size of approximately 26 nm is modified with four different BS surfactin, rhamnolipid, polyethylene glycol (PEG), and dextran. To confirm the deposition of the coatings, fourier transforms infrared spectroscopy, and thermogravimetric analysis were performed. The protective coating leads to an increased hydrodynamic size, and biocompatibility due to PEG and dextran, whereas surfactin and rhamnolipid were proved to be highly cytotoxic [56]. Recent research represents the production of sophorolipid-coated monodisperse IONPs. Due to the acidic nature of sophorolipids, they are exploited as surface stabilizing agents. The sophorolipid-nitrodopamide (SL-NDA) was produced by replacing the -COOH group of sophorolipids with nitrodopamine. The resulting iron NPS obtained were stabilized with a single layer of the BS SL-NDA and showed extraordinary colloidal stability even under extreme conditions of high salt and protein content. Further, no cytotoxicity was reported; therefore, it could be considered stable for biomedical use [57]. The IONPs are explored in other industries as well, for instance, in wastewater management. In a similar study, the IONPs were functionalized with Rhamnolipid (RL) (RL@IONPs), glycoprotein isolated from *P. aeruginosa* ATCC 9027. The RL@IONPs resulted in the low toxicity and highly selective characteristics of IONPs [58]. The NPS functionalized with BSs are also used in cancer treatment; in recent research, the use of a glycoside (stevioside) as a surface modifier was exploited, and the results obtained showed the efficiency of cell to stimulate cell death in rat C6 glioma cells [16].

20.3.1.2 Silver nanoparticles

Silver nanoparticles (AgNPs) possess unique morphology, stability, and geometries; they are widely employed in sensing devices, protective coverings, coatings, diagnosis, treatment of diseases [59]. AgNPs display unusual antimicrobial activities and are therefore used as coatings for cerebrospinal fluid drainage [60], medical devices, bone cement, wound dressing, etc. They can be synthesized via physical (sonication, ball milling, high temperature and pressure, radiation) and chemical (condensation, sol−gel technique, and biochemical methods) methods. To make these NPS compatible, stabilizers are required [61].

These NPS are therefore used along with different BSs and protective coatings that can enhance the rate of uptake and reduce toxicity. Nowadays, plant extracts/microorganisms are being used to generate low-cost formulations. A BS extracted from *P. aeruginosa* formulated in 2.5% vegetable oil, steep corn liquor and distilled water was used to functionalize AgNPs silver nanoparticles (1.13 nm). The result proposed that AgNPs can be formed in reverse micelles through UV-visible spectra analysis; thus, providing a low-cost, nontoxic, and biodegradable nanoformulation [62]. A glycolipid (Rhamnolipid) BS was isolated from *P. aeruginosa* BS-161R was functionalized with AgNPs. The characterization of NPS through UV-Visible spectra, Transmission Electron Microscopy (TEM), X-ray spectroscopy revealed spherical shaped, 15.1 nm-sized nanoparticles. The resultant AgNP exhibited antimicrobial and antibiotic activity against Gram-positive and Gram-negative pathogens [17]. For targeting antibiotic-resistant bacterial classes, a facile strategy was utilized employing silver and iron oxide NPS coated with rhamnolipids. The properties such as the antiadhesive and antibacterial nature of the BS were exploited, and the resultant silver and iron oxide NPS were able to target the biofilm formation by two strains *P. aeruginosa* and *S. aureus*. The NPS obtained were about the size of 35 and 48 nm, respectively, the generation of reactive oxygen species leads to antimicrobial activity. This suggested the probable use of these rhamnolipid-coated NPS in antibacterial coatings and wound dressings [63].

20.3.1.3 Gold nanoparticles

Gold nanoparticles (AuNPs) have recently acquired attention because of their potential roles in antibacterial, antimicrobial, antioxidant, and antitumor therapy. Like other NPS, they also need to be biocompatible due to the hazardous and toxic nature of actual NPS. Therefore, they are used along with BSs, which act as reducing and stabilizing agents in nanoparticle synthesis. Mannosylerythritol lipid (MEL), glycolipid BS derived from *U. mayelis* was functionalized with AuNPs as a reducing and capping agent. The results depict the antibacterial, antimicrobial, antioxidant, and anticancerous role in the biomedical industry [64]. Lipopeptide BSs were used in a study to stabilize the AuNPs; the surfactin was produced by *B. subtilis* and recovered from culture supernatant through foam fractionation; the chloroaurate solution (yellow) was introduced to observe the visible color changes. On the formation of AuNPs, the yellow-colored solution was converted into a purple-colored solution indicating a change in metal oxidation state. Further, a decrease in the mean particle size was observed on increasing pH value, and stabilized nanoparticles were obtained at 7−9 pH at room temperature [65].

20.3.1.4 Similarly, zinc oxide nanoparticles

Similarly, zinc oxide nanoparticles (ZONs) are also inorganic NPS, and zinc is considered an essential nutrient in existing organisms. Scientists have discovered its probable part as an antibacterial

and antimicrobial agent [66,67]. Conventionally, ZONs were produced using physical and chemical processes, but these methods were expensive, toxic, and required high energy. Recently, green processes like the use of microorganisms, bacteria, yeast, and fungi are being utilized for the synthesis of ZONs. Despite having all the nutrition values and antimicrobial qualities, ZON might still cause toxicity in the body. Therefore, they are functionalized with BSs that modulate their characteristics and avoid toxicity. In a study, a Glycolipid (sophorolipid) was utilized to stabilize the ZONs. The formation of ZON was characterized using UV-Visible, X-ray diffraction, Scanning Electron Microscope, and Fourier transform infrared spectroscopy. The result depicted the antimicrobial role of the sophorolipid coated NPS and the role of BS as a bio stabilizer and a novel functionalization agent [68].

The two major properties of ZONs include antimicrobial and antiadhesive nature. Bacterial biofilms containing homogenous or heterogeneous microorganisms are formed on biomedical devices such as catheters, breast implants, cardiovascular implants, contact lenses, dental implants, intrauterine devices, and prosthetic joints. The biofilm formation leads to the development of various infections; therefore, an antimicrobial agent is necessary for targeting biofilm formation [69]. The ZONs coated with polymeric BSs are observed to be less toxic as a result of their low solubility. The antibacterial nature of the BS that is coated on the nanoparticle can vary with the size and shape of the nanoparticle. The biofilm developed by *E. coli* and *S. aureus* was targeted using PEG-coated ZONs. The antimicrobial activity was found to be inversely related to the size and directly to its concentration [70].

20.3.1.5 Carbon nanotubes

CNTs are heterogeneous nanomaterials that are in demand these days because of their extraordinary properties. Fields like electronics, drug delivery, nanocomposites, targeted cellular imaging [71, 72], mapping and distribution [73, 74] employ CNTs. CNTs comprise round graphene sheets packed in a cylindrical form. They are of two type's single-walled CNTs (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). They possess good emulsifying activity, but such NPS showed low toxicity, and therefore, these are modulated through BSs. Thus, the ecotoxicity effects of BS-coated CNTs were analyzed in a *Daphnia* model. A mixture of BSs (fengycin and surfactin) was isolated from *B. subtilis*; the observed formulation did not cause any acute toxicity in the model until 30 mg/L [75].

20.3.2 Organic nanoparticles

Organic NPS form an essential part of food industries, cosmetics, and biomedical applications. In nature, a wide variety of examples exist, such as lipid, protein aggregates, emulsions, and many complex systems. The pharmaceutical composites include liposomes, polymers, dendrimers, etc. The major characteristic of the organic NPS is that they form a three-dimensional orientation with the organic molecules they comprise of. The ability to self-assemble and requirement of the zwitterion is the main constituents of NPS; moreover, the ability to encapsulate other organic compounds is essential for the functionalization and fabrication of organic NPS. They can be synthesized by two approaches, "top-down" and "bottom-up." The top-down technique is complex and involves microfluidics, lithography, etc. Here we will be considering "bottom-up" strategies, which involve

synthesis through precipitation and condensation; some examples of the organic NPS include dendrimers, vesicles, liposomes, polymeric NPS, and capsules.

20.3.2.1 Dendrimers

Dendrimers are extremely branched organic polymers employed in the biomedical industry in repairing tissues, antiviral, anticancer treatments, gene delivery, and as antiinflammatory agents [76, 77]. The specific characteristics of dendrimers are their exclusive domains: the multivalent surface, inner core, and inner shells that surround the core of the nanoparticle, which can be tailored for achieving specific activities. Novel core−shell nanocapsules made up of lipid and valine (stearic acid) conjugate were prepared; the whole idea was based on modification through BS. The drug loading capacity was enhanced, and hydrophilic drug methotrexate (MTX) was loaded. The in vitro discharge of the drug was checked, and biphasic release was recorded after an initial release of the drug that was followed by the sustainable release of the drug, increased cellular uptake of methotrexate, and decrease in IC50 value were additional advantages provided by BS coated NPS. The molecules were also biocompatible, further increased apoptosis, and enhancement of lysosomal membrane uptake was also noticed in the study [78].

20.4 Role of biosurfactants in cancer therapy

Cancer is the second-highest reason for mortality and morbidity across the world, as reported by WHO [75]. Cancer is the consequence of unrestrained cell growth; it can be regarded as a "genomic disease" [79]. Conventional therapies used in the treatment of cancer include anticancer drugs, radiotherapy, chemotherapy, and surgery. However, these treatments have their disadvantages; after a while, the patient starts developing resistance against these therapies. Chemotherapy has various harsh side effects as a result of a lack of specific targeting, and this is the reason for developing resistance as well. Despite targeting particular cells, chemotherapy tends to destroy healthy cells and tissue in the vicinity, too, which leads to the development of cancer [80]. Similarly, radiotherapy is another option, but various disadvantages are associated with it; the present therapies are less specific, and there is an urgent need to figure out more ways to cure cancer. Therefore, many different techniques are being used to target cancers these days; nanotechnology is a field that has shown promising results in targeting cancer. Despite various improvements in the field of nanotechnology and biomedical advancements, cancer remains the most challenging disease to treat.

Nanotechnology is a compilation of various fields such as physics, medicine, electricity, chemistry, and biological pathways [81]. Through the use of NPS, the pharmacokinetic properties of a drug can be modified, thus increasing the specificity of a deficiency of the treatment, which ultimately results in lowering the resistance. Other factors that are considered in developing treatment using nanotechnology include reducing the toxicity and side effects of a drug, enhancing specificity, solubility half-life, drug release, diagnosis of the disease, and hybridization of anticancerous medications to enhance the specificity. The NPS range from 10 to 400 nm in size, depending on the surface properties; various cures, enhancers, and surfactants are utilized to reduce the toxicity caused due to the NPS. To generate biocompatible NPS, various stabilizers are used, such as BSs.

BSs such as lipoproteins, glycolipids, polymers, and other surfactants are used in stabilizing the NPS, and BSs themselves are also used in cancer therapy. Recently, BSs have been explored, and their broad-spectrum role is recorded in cancer treatment.

Until now, some studies based on the use of BSs targeting various cancers have been reported. The cytotoxic nature of BSs against cancer cells has been recorded in various studies [82] (Fig. 20.4).

20.4.1 Breast cancer

The prominent reason for cancer mortality amongst women is breast cancer. All the conventional therapies are considered, but none has proved to be effective in targeting breast cancer; Doxorubicin (DOX), an anticancer drug, is widely employed in targeting breast cancer [83]. The failure of the drug to treat the disease leads to multidrug resistance (MDR). Recently, in a study, the BS (surfactin) derived from *B. subtilis* was used as a nanoparticle for targeted *drug delivery*. There are reports about the role of surfactin in inducing apoptosis through the Janus-kinase-mediated caspase pathway and inhibiting the proliferation of breast cancer cell lines (MCF-7) [84]. The study involved loading the anticancer drug DOX through the solvent-emulsion method to surfactin (DOX@SUR). The resistant cell lines used were MCF-7/ADR; the results showed higher cytotoxicity of the BS coated nanoparticle toward the resistant breast cancer cell lines.

Moreover, this increases the retention time of the drug, resulting in specific and more robust tumor inhibition with fewer side effects [85]. Surfactin interacts with cell membranes, as the cancer cells comprise a membrane protein called phosphatidylcholine that plays a crucial role in stabilizing the section. The part of surfactin is to target phosphatidylcholine via getting induced into the lipid bilayers of the membrane, building channels in the cell membrane, thus disrupting the cell membrane. Moreover, it can pierce freely with phospholipids at minimum concentrations, leading to the formation of micelles. At medium concentrations, the ion-conducting pores can form in the

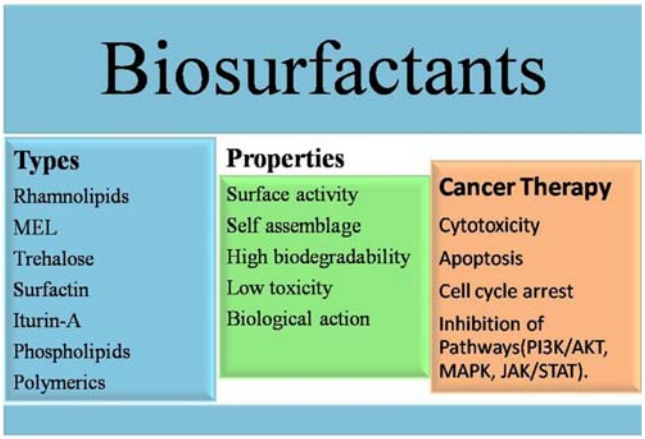

FIGURE 20.4

Anticancer effects of biosurfactants in nanoparticles modifications.

membrane, and at maximum concentrations, surfactin shows detergent-like effects, which leads to membrane disruption.

The techniques used to identify the disease can also be manipulated for efficient performance. The diagnosis and early detection of cancer is an essential factor in the treatment of cancer, and this increases the chances to curb the spread of the disease. Chemotherapeutic drugs and graphene quantum dots nanoformulation can enhance the ability of the drug to target cancer cells [86,87]. A study suggested the use of BS conjugated NPS could be used as a theranostic tool for treatment; the BS was derived from *Candida parapsilosis*. The bioconjugate was formed using nanomaterials graphene quantum dots (GQDs), three types of formulations were GQDs only, GQDs with BS, and folic acid (FA) plus BS conjugated with GQDs. The nanoformulation was not as toxic as the MTT assay depicted ninety percent cell viability at 1 mg/mL. The results displayed a 50% cell death when targeted via BS-GQDs conjugate within a span of 24 hours; further confocal laser scanning microscopy confirmed the increased specificity of the FA- conjugated formulation. This study concluded that FA-BS conjugated GQDs show a high amount of drug uptake; thus, these can be employed as theranostic tools for detecting and treating cancer patients [88].

Organic nanoformulations, like lipopeptides, were tested for potential cytotoxic effects on breast cancer cell lines (B cap-37). Surfactin-like lipopeptide (BS) utilized in the study was extracted from *B. subtilis*, which led to a decrease in cell survival with an IC50 of 29 ± 2.4 μM at 24 hours. Apoptosis occurred as a result of membrane disruption due to a decline in the concentration of unsaturated fatty acids [7].

20.4.2 Lung cancer

Lung cancer is the leading cause of mortality, with an approximate survival rate of five years. It can be classified into two types nonsmall-cell lung carcinoma (NSCLC) and small-cell lung carcinoma (SCLC) [89]. "Lung and bronchus cancers would be responsible for more than 234,030 new cases that represent about 14% of all new cancer cases detected around the world," according to the American Cancer Society in 2018 [90]. Existing treatment strategies for lung cancer involve combination therapy of chemotherapeutic drugs, chemotherapy, surgery, and radiation therapy intensely dependent on the extent of malignancy and stage at the time of diagnosis but often involve a combination of surgery, chemotherapy, and/or radiation therapy. Nanoparticle-based systems are utilized in treating lung cancers, too; the nanoformulations can cross biological hurdles and effectively and efficiently deliver drugs to the site. Nanoparticle-based studies have shown roles in analysis, imaging, screening/diagnosis, and treatment of benign and metastatic tumors. Further, NPS functionalized with BSs are used these days. The antitumor activity of a lipopeptide BS, fengycin was analyzed through in vitro and in vivo analysis in lung cancer cell lines (95D). Flow cytometry and laser confocal microscopy were used to assess the reactive oxygen species, mitochondrial membrane disruption, and ion uptake that occurs as a result of the treatment with fengycin. A reduction in cell proliferation was achieved in cancerous cell lines, and it also led to the inhibition of cyclin D- cyclin-dependent kinase 4 (CDK4) complex, which resulted in a cell-cycle arrest at the G0/G1 phase. Fengycin reports an increase in the levels of apoptotic related genes such as caspases, cytochrome c, and Bax, thus suggesting that it can act as a potential treatment to target cancerous cells [91].

Similarly, other BSs possess properties like antimicrobial, antibiofilm, and antitumor. A new BS belonging to a class of glycolipoprotein was derived from *Acinetobacter indicus* to check the

cytotoxic activity against the lung cancer cell line (A549). The BS displayed low toxicity, and the cell viability was seen decreasing with the increasing concentration of BS. The most optimal concentration at which the G1 phase of the cell cycle was inhibited is 200 μg/mL [92].

20.4.3 Colon cancer

The studies were performed regarding the use of glycolipid BSs in treating colon cancer. A study depicted the inhibition of the proliferation of colon cancer cells (LoVo) with an IC50 value of 26 μM at 48 hours. This antiproliferative effect was induced by the surfactin that occurred as a result of DNA fragmentation, morphological alterations, and modifications in the regulatory proteins of the cell cycle. There were amendments in the apoptotic pathways, for example, an increase in the levels of Fas receptors/ ligands, p53, p21, and a decrease in the cell proliferative Cyclin-CDK complex (E-2). Surfactin led to the seizure of the cell cycle by inhibiting the G0/G1 phase and enhanced apoptotic cell death [93].

Another study showed that refined BSs like Surfactin targeted colon cancer cell lines (HCT-15 and HT-29). It was isolated from a marine *B. circulans* and comprised an IC50 of 77 and 116 μM ($>$ 24 hours). This depicted the specificity and selectivity of the BS, as a higher concentration (482 μM) led to a decline in 50% cell viability (mouse fibroblasts) [35].

Similarly, a BS derived from *Pseudomonas sp*. was employed in a study targeting colon cancer (SW480) due to its anticancer and physiochemical potential. The physiochemical properties include solubility, foaming index, and to assess the toxicity of the BS, an MTT assay was performed. The histopathological staining with acridine orange-ethidium bromide, dichlorofluorescein diacetate, and a wound-healing assay was performed to enquire about the anti-tumorigenic potential of the BS (ENO14BS). A decrease in the cell viability was recorded at 250 μg/mL; the maximum concentration was after 24 and 48 hours. The physicochemical properties accessed included the oil holding capacity, water activity, foaming ability, and water solubility index for BS was 4.36 ± 0.05 g/g, 0.32 ± 0.03, $52.28\% \pm 0.10\%$, and $80.86\% \pm 0.20\%$ correspondingly. Therefore, ENO14BS can be used as a nontoxic BS that can be used in targeting colon cancer [94]. All these properties of the BSs can be used along with the NPS to enhance their effects.

20.4.4 Brain tumor

Brain tumor diagnosis and treatment is a crucial challenge, particularly malignant brain tumor types like glioblastoma (short survival span for patients below one year) [95]. The blood-brain barrier is played a crucial role in reducing the therapeutic effect of chemotherapy and other available drugs. The blood-brain barrier, formed by the endothelial cells of the brain capillaries paired with tight junctions, prevents 98% of all small-molecule drugs and 100% of large molecule pharmaceuticals entering the brain [96,97]. Surface modification of NPS could be a promising tool to achieve targeted delivery in the brain. Polybutylcyanoacrylate (PBCA) polysorbate-80-coated NPS was widely proposed to deliver of drugs to the animal brain and showed great potential for therapeutic applications [98]. Kreuter et al. evidenced that BBB impermeable peptide could be delivered to the brain using poly (butyl cyanoacrylate) as a carrier. To increase the penetrability of NPS to the BBB, several drug NPS, including Temozolomide. Gemcitabine, DOX, and loperamide have been coated with a surfactant such as polysorbate 80 [98–100], poloxamer 188, and Tween 80 [101].

Liposomes and other lipid-based NPS have also been reported to have the property of high uptake by BBB in the in vitro and in vivo models [102].

20.4.5 Leukemia

Lipopeptides are a type of BS; in a study, crude oil lipopeptides (CLPs) derived from *B. subtilis natto* T-2 was employed with surfactin against human K562 leukemia cells. At lower concentrations, CLPs were unable to inhibit the viability of normal lung fibroblast (HLF) cells. Based on the dose and time, CLP-mediated inhibition led to a decline in the growth of K562 cells and displayed an IC50 value between 10 and 20 μM for 24−48 hours treatment period. The antiproliferative activity of surfactin was used in targeting cell cycle progression. It is also known for the initiation of apoptosis due to an increased number of cell death on encountering surfactin. Various modifications were recorded in the morphology of the cells, and the levels of proteins like caspase-3, cleaved PARP, p21waf1/cip1, and p27kip1were also enriched, while the cyclin necessary to pass the G1 phase, that is, cyclin-D1, was reduced, thus leading to cell-cycle arrest [103] (Fig. 20.5).

20.5 Future perspective

Lately, several new technologies have been developed for targeting cancer and other disorders. The introduction of nanotechnology into the biomedical field has already shown promising results in various diseases. Due to their efficiency in targeting the particular disease with minimum side effects and vicinity damage, further, the NPS show high intracellular uptake than other

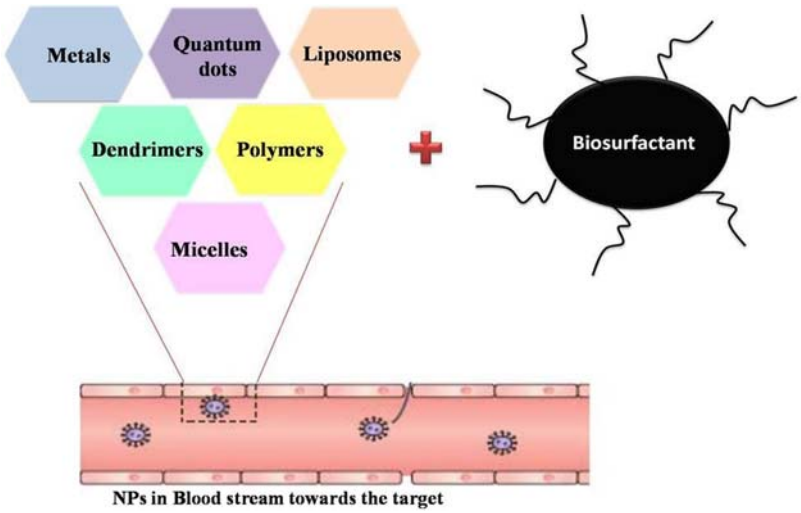

FIGURE 20.5

Nanoparticles functionalized with biosurfactants.

conventional therapies. But sometimes, the NPS themselves are not stable to target a particular disease; therefore, to enhance their efficiency and potential, they are functionalized with high efficiency-providing materials like BSs.

Biological organisms like bacteria, fungi, actinomycetes, and algae are known for the production of BSs and their use in nanotechnology. The primary role of BSs in the preparation of nanoformulations is to reduce toxicity make them stable and more biocompatible. The affluent diversity of microbes portrays them as efficient "bio-factories" for the synthesis of NPS; they play an essential role in biosynthesis and manipulate the shape, size, dispersion, and properties of NPS. Future research should be aimed toward the increased synthesis of functionalized NPS in every field, such as biomedical, industrial (chemical, electronics, etc.), and the food industry.

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CHAPTER

21 Application of low- and high-molecular-weight biosurfactants in medicine/biomedical/pharmaceutical industries

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21.1 Introduction

Surfactants are amphiphilic molecules comprising polar (hydrophilic) and nonpolar (hydrophobic) parts in which the hydrophilic part may be ionic whereas the hydrophobic part is the nonionic hydrocarbon chain. This amphipathic nature empowers them to concentrate at the interface and alter the conditions existing at the interfaces [1]. Hence, they are widely used for the applications such as cosmetics, food, agriculture, preparation of suspensions of polymer particles by heterogeneous nucleation, industrial, and pharmaceutical industries [1,2]. Based on origin, the surfactants are classified as synthetic or natural. Much usage of synthetic surfactants may cause environmental problems and this has initiated a thrust to search for an alternative [3]. Biosurfactants are surface-active amphiphilic molecules created in nature by plants, animals, and a variety of microorganisms such as nontoxic yeast, bacteria, and fungi [4,5]. Few examples of biosurfactants produced by microorganisms are depicted in Table 21.1 [6]. Biosurfactants constitute a wide range of surface-active molecules such as lipopeptides, fatty acids, glycolipids, phospholipids, and lipoproteins [7]. Various factors such as bioreactor characteristics, oxygen availability, pH of the medium, temperature, and the nutrient composition affect the composition and yield of biosurfactants [8]. Like synthetic surfactants, the biosurfactant can minimize the surface and interfacial tension and can lead to emulsions [7,8].

21.2 Classification of biosurfactants

Based on the molecular weight, biosurfactants are generally categorized into low- and high-molecular-weight surfactants (Fig. 21.1) [7].

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Table 21.1 Representative examples of biosurfactants and the microorganisms producing this class [6].

Glycolipids	Lipopeptides	Phospholipids	Polymeric surfactants
• *Acinetobacter calcoaceticus* • *Arthrobacter paraffineus* • *Candida antarctica* • *Pseudomonas sp.*	• *Acinetobacter* sp. • *Bacillus subtilis* • *Candida lipolytica* • *Pseudomonas fluorescens* • *Streptomyces* • *Thiobacillus thiooxidans*	• *Acinetobacter* sp. • *Aspergillus*	• *A. calcoaceticus* • *Bacillus stearothermophilus* • *Candida lipolytica* • *Candida utilis* • *Mycobacterium thermoautotrophium*

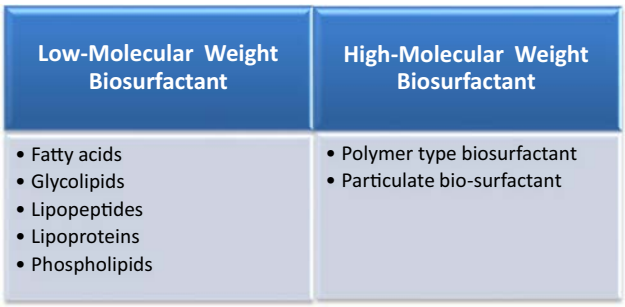


FIGURE 21.1

Classification of biosurfactants [7].

21.2.1 Low-molecular-weight biosurfactants

Low-molecular-weight biosurfactants are the biosurfactants such as glycolipids, fatty acids, lipopeptides, phospholipids, and lipoproteins that can reduce the surface and interfacial tension significantly. High-molecular weight biosurfactants are generally effective bioemulsifier comprising polyanionic lipopolysaccharides, membrane vesicles that are efficient emulsion stabilizers [5].

21.2.1.1 Glycolipids

Glycolipids are the most common biosurfactant comprising the carbohydrate moiety attached to long-chain aliphatic or hydroxy aliphatic acids by a glycosidic bond. A few examples of this category are rhamnolipids, trehalolipids, and sophorolipids (Fig. 21.2) [7]. Rhamnolipids consist of one or two rhamnose molecules attached to β-hydroxydecanoic acid. Generally, these rhamnolipids are produced by the *Pseudomonas* species. Sophorolipid comprises sophorose linked to the hydroxy fatty acids with a long hydrocarbon chain. These are generally generated by yeasts. For example, sophorolipid generated by *Torulopsis petrophilum* from vegetable oil and alkanes is reported in the literature [9].

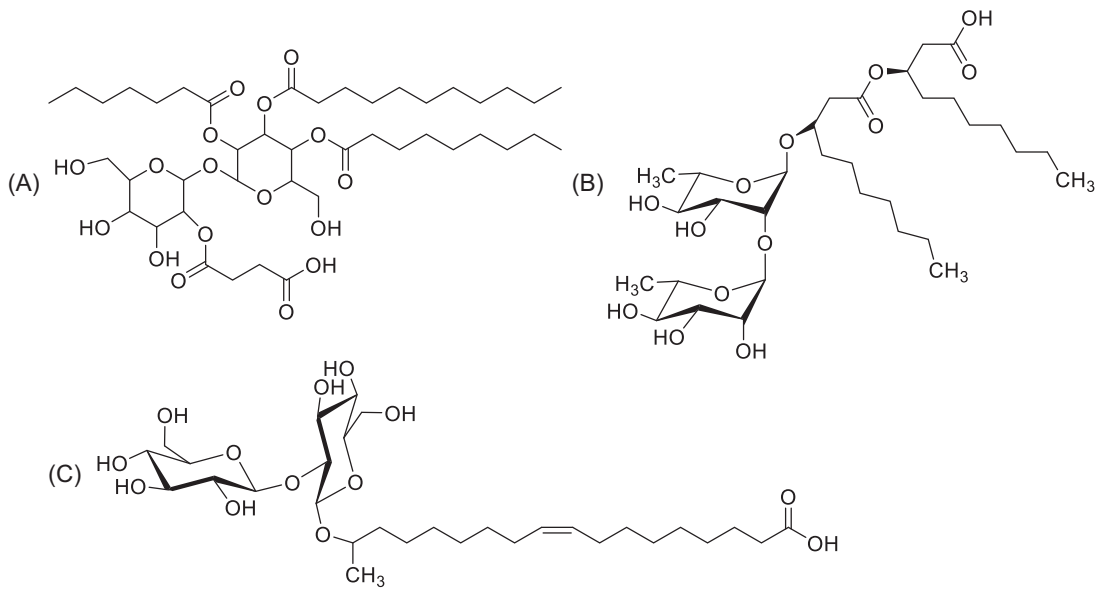


FIGURE 21.2

Representative examples of glycolipids: (a) rhamnolipids, (B) trehalose, and (C) sophorolipid.

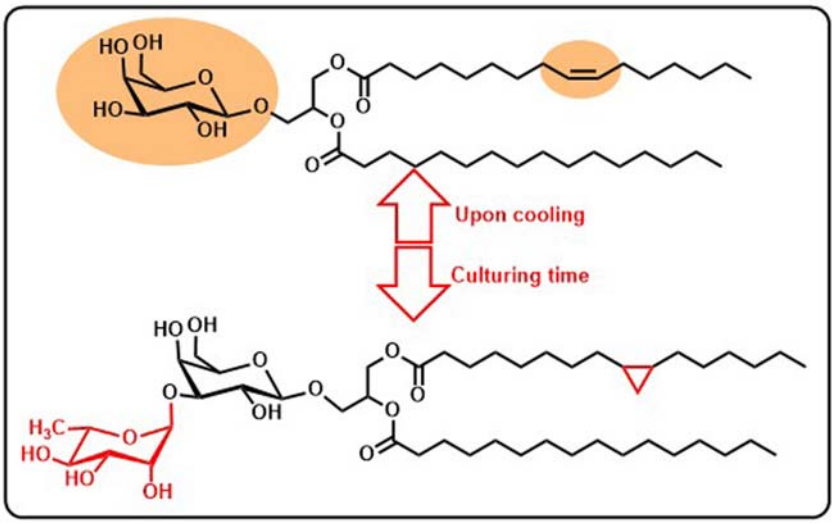


FIGURE 21.3

Biosynthesis of rhamnosylgalactosyldicylglyceride [10].

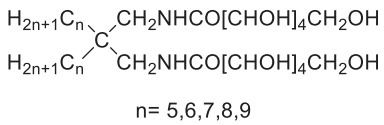


FIGURE 21.4

Molecular structure of glucamide surfactants [11].

Tamiaki and coworkers reported the biosynthesis of glycolipids of varying structure and composition depending on the culturing temperature and time. They reported the biosynthesis of rhamnosylgalactosyldicylglyceride comprising methylene-bridged palmitoleyl and palmitoyl group at 45°C and at 25°C monogalactosyldiacylglyceride with palmitoleyl and palmitoyl group using the photosynthetic bacteria, Chlorobaculum tepidum (Fig. 21.3) [10].

Eastoe and Rogueda have reported the synthesis and surfactant properties of a nonionic surfactant, fatty acid glucamides derived from glucose and fatty acid methyl esters in a two-step reaction involving reductive alkylation of glucose followed by acylation with fatty acids (Fig. 21.4) [11].

21.2.1.2 Lipopeptides

Lipopeptides consist of linear or cyclic peptides linked to lipid moiety and display thermal and pH stability. Surfactins, iturins, and fengycins are a few examples of lipopeptides (Fig. 21.5).

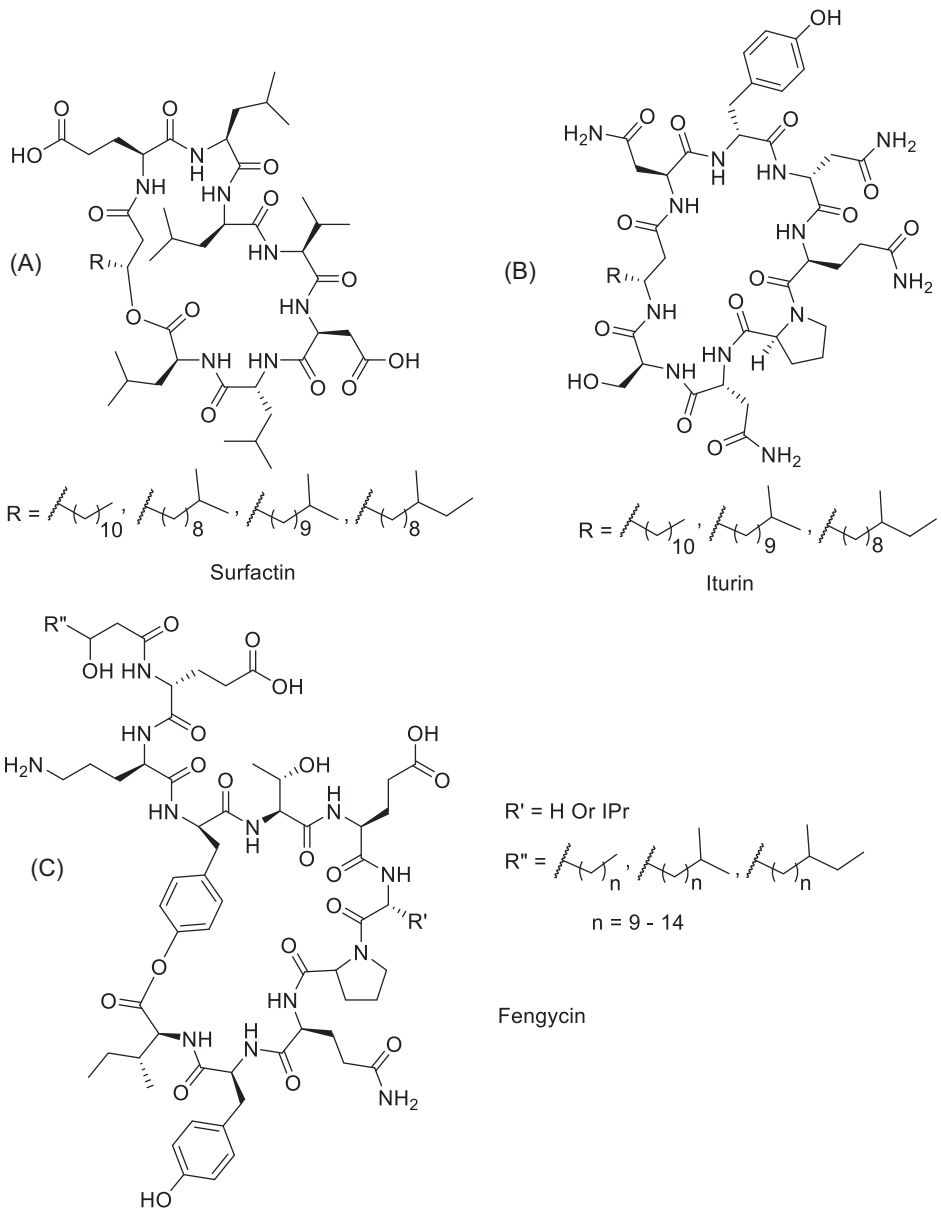


FIGURE 21.5

Representative examples of lipolipids [12]. Surfactins (A), iturins (B), and fengycins (C).

Surfactins and iturins are cyclic lipoheptapeptide whereas fengycin is lipodecapeptide. One of the most effective biosurfactants is surfactin capable of lowering the surface tension significantly [12]. A review of the literature reveals that lipopeptides exhibit antiviral, anticoagulant, antitumoral, antimycoplasma, antifungal, antiwrinkle, and emulsifying properties which enable them potentially suitable for pharmaceutical, cosmetics, food, and biotechnological applications [12].

21.2.1.3 Phospholipids, fatty acids, and neutral lipids

A variety of biosurfactants like phospholipids, fatty acids, or neutral lipids, which are suitable for biomedical applications, are created by the bacterial, fungal, and yeast growth on *n*-alkanes [6,13] The length of the hydrocarbon chain in the structure of biosurfactant determines the hydrophilic−lipophilic balance, responsible for self-assembly. A few examples of phospholipids are given in Fig. 21.6.

21.2.2 High-molecular-weight biosurfactants

High-molecular-weight polymeric biosurfactants include polysaccharide−protein complexes such as emulsan, lipomanan, liposan, and alasan and particulate biosurfactants, the extracellular membrane vesicles consisting of proteins, lipopolysaccharides, and phospholipids produced by microbial cells can function as both bioemulsifier and demulsifier [14]. An excellent emulsifying activity is reported for these polymeric biosurfactants in the literature [14,15]. Panilaitis demonstrated the structural modification of emulsan, the lipopolysaccharides produced by *Acinetobacter calcoaceticus* by varying the culture conditions can alter the emulsification behavior [16,17]. They have also shown that these polymers could activate the innate immune response and act as adjuvants for humoral immunity [16,17]. Few examples of bioemulsifier, their microbial origin, and physiological functions are given in Table 21.2 [18].

Compared to synthetic surfactants, the biosurfactants are advantageous because of their bioavailability, cost-effective production from inexpensive raw materials, biodegradability, no/less

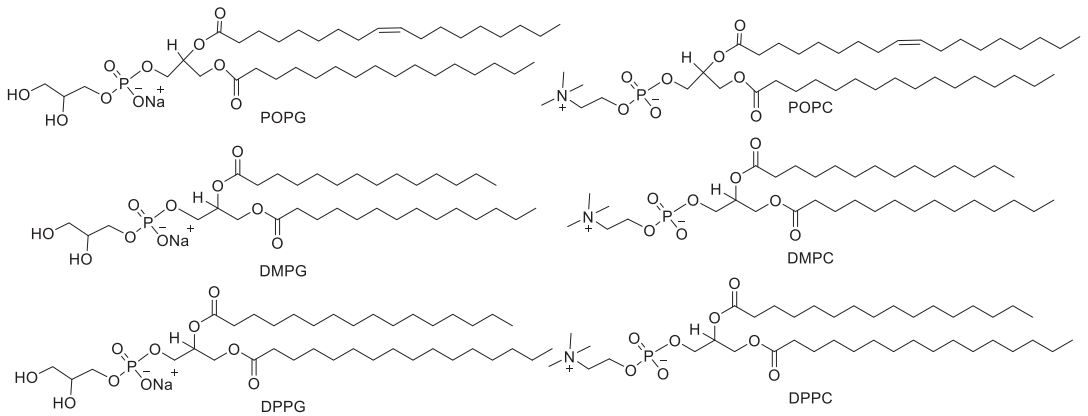


FIGURE 21.6

Representative examples of phospholipids.

Table 21.2 Few examples of high-molecular-weight bioemulsifier and their physiological role [18].

High-molecular-weight bioemulsifier	Microbial origin	Physiological roles
Emulsan (lipopolysaccharides or protein−polysaccharide complex)	*Acinetobacter sp.*	To enhance the bioavailability of poorly soluble substratesTo stabilize the oil−water emulsionsHelps to remove toxic metals
Alasan (alanine containing polysaccharide and proteins)	*Acinetobacter radioresistens*	Commonly employed in solubilization and emulsification activity
Mannoproteins (polysaccharide and proteins)	*Saccharomyces cerevisiae*	Helps to stabilize emulsion with hydrophobic substrates
Uronic acid bioemulsifiers (polysaccharides−proteins−uronic acids)	*Halomonas eurihalina*, *Klebsiella sp.*	Helps to stabilize emulsion with hydrophobic substrates and their detoxification

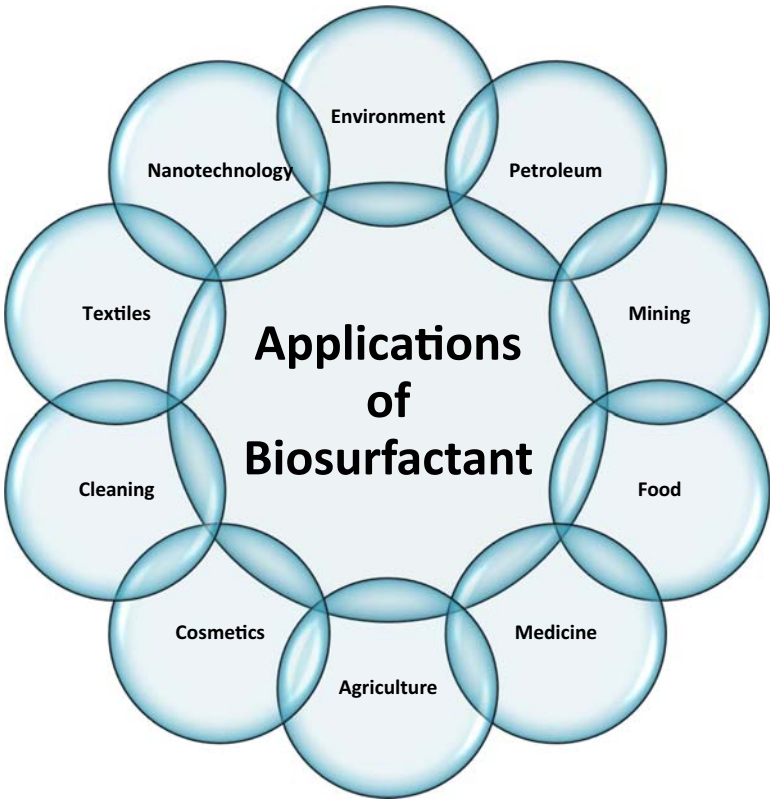


FIGURE 21.7

Applications of biosurfactants.

toxicity, tremendous surface activity with high biocompatibility, specificity, and better foaming property. These features facilitate the biosurfactants to emerge as a potential green alternative to synthetic surfactants and apt significantly for diverse industrial and medical applications such as emulsifier, detergent, cosmetics, lubricant, removal of pollutants, oil recovery, antimicrobial, antitumor, and antiinflammatory agents [19–21]. Fig. 21.7 summarizes the various applications of biosurfactants. In this chapter, various applications of low- and high-molecular-weight biosurfactants in medicine and pharmaceutical industries are discussed.

21.3 Applications of biosurfactant

21.3.1 Applications in the field of medicines

Owing to the structural diversity, amphipathic nature, low toxicity, cost-effective production feasibility, and better activity of microbial surfactants under conditions of extreme pH or temperature, they are expected to exhibit various physiological functions such as heavy metals binding, quorum sensing, bacterial pathogenesis, and prominent surface and emulsifying activities [4,22,23]. Gomaa produced the lipopeptide using the *Bacillus licheniformis strain M104* grown on whey and showed that this lipopeptide exhibited time and concentration-dependent antimicrobial activity against *Staphylococcus aureus ATCC25928* [24]. The addition of the biosurfactant (48 μg/mL) to *S. aureus* medium was found to decrease 53.06% of the acid-soluble phosphorous, 90.47% of total lipid, 53.43% of proteins, 83.29% of RNA, and 48.50% of DNA for an incubation period of 12 h suggesting its suitability for potential applications in medical fields [24]. Supramolecular glycolipids were synthesized by connecting the naphthyl glucosamine (electron donor) and alkyl viologen (electron acceptor) RV8 inside the macrocyclic host molecule cucurbituril (Fig. 21.8) [25]. They have also established the redox responsiveness of the glycolipids and their ability to interact with Concanavalin A (ConA) through the recognition of lectin specifically suggesting its suitability for medical applications [25]. Generally, the presence of a flexible hydrophobic siloxane group in silicone surfactants minimizes the surface tension because of their capability to adopt various orientations. Hence many research works were carried out to produce the biosurfactants by exploiting the advantages of both hydrophilic sugars and hydrophobic siloxanes [26]. In this context, Zhang et al. reported the synthesis of gluconamide-based trisiloxane surfactant using environmentally friendly materials, trisiloxane and gluconolactone, and demonstrated the vesicle formation and its application in drug delivery (Fig. 21.9) [26].

The synthesis of new linear sugar-based cationic surfactants 2-(alkyldimethylammonio)ethyl gluconamide bromides was reported [27]. Examining the interactions of the cationic surfactant with DNA and model lipid membrane suggested that these surfactants could act as potential gene delivery agents for gene therapy applications (Fig. 21.10) [27].

The interaction of natural rhamnolipids with plant and fungi biomimetic plasma membrane models was explored by Monnier and coworkers at the molecular scale [28]. Their studies revealed that the rhamnolipids fit into the plasma membrane models without affecting the lipid dynamics significantly [28]. Further, they observed that the rhamnolipids increased the membrane fluidity which depends on the nature of phytosterols. They have also shown that the interaction of rhamnolipid with sterols influences the destabilization of the plant plasma membrane [28]. The ability of

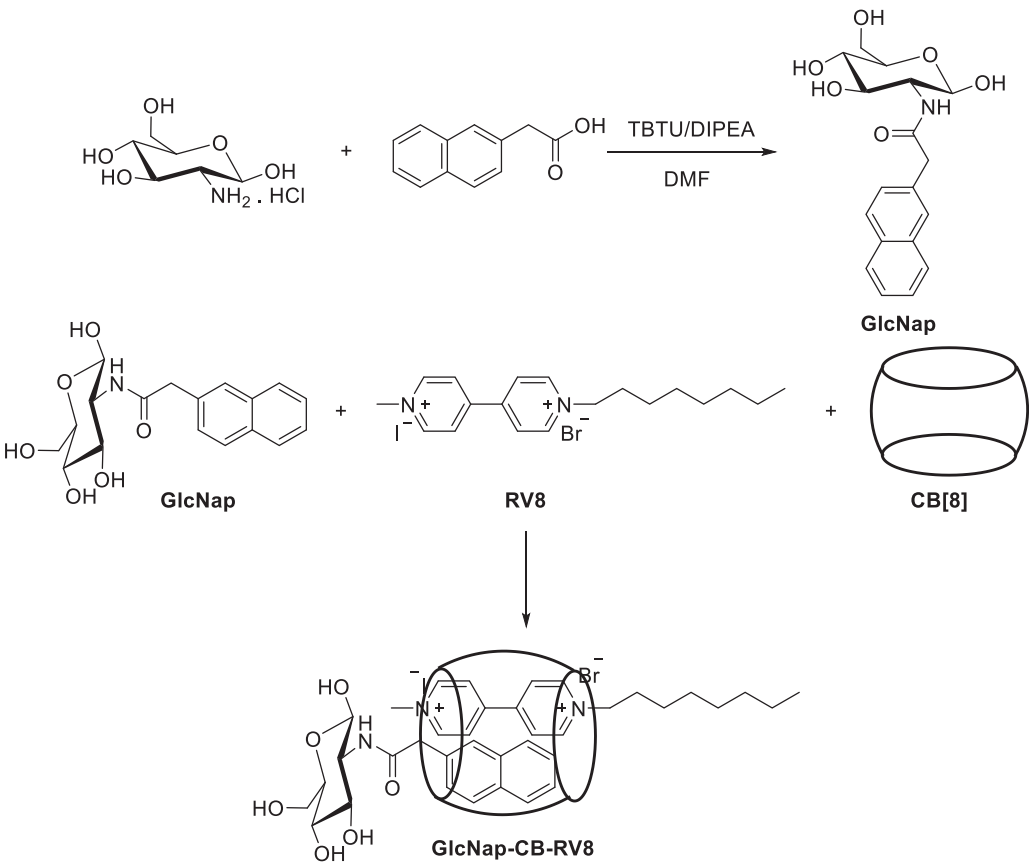


FIGURE 21.8

Synthesis of supramolecular glycolipids [25].

the lipopeptide from *Bacillus subtilis*, fengycin to induce ion-permeable pores resulting in the formation of ion channels of feeble cation selectivity was demonstrated by using the lipid membrane model mimicking the composition of target fungal cell membranes [29].

To overcome the inefficient delivery of drug to treat brain diseases, Heldman and coworkers have developed the cationic vesicles using the bolalipids GLH-19 and GLH-20 and investigated the efficiency of these bola vesicles in drug encapsulation and the subsequent decapsulation at a controlled rate to deliver the drug moiety by encapsulating analgesic peptides [30]. Fig. 21.11 depicts the structure of GLH-19 and GLH-20. Their studies proved the capability of bola vesicles to deliver the analgesic peptides [30].

Uzan et al. constructed the drug delivery system using amino alcohol–based bis-(amino alcohol)oxalamides derived from various amino alcohols such as leucenol, isoleucinol, valinol, phenylglycinol, phenylalaninol, and 2-amino-1-butanol (Fig. 21.12) [31]. Through the gelation studies, they observed that these gelators could gel biocompatible fatty acid esters (FAEs) and illustrated

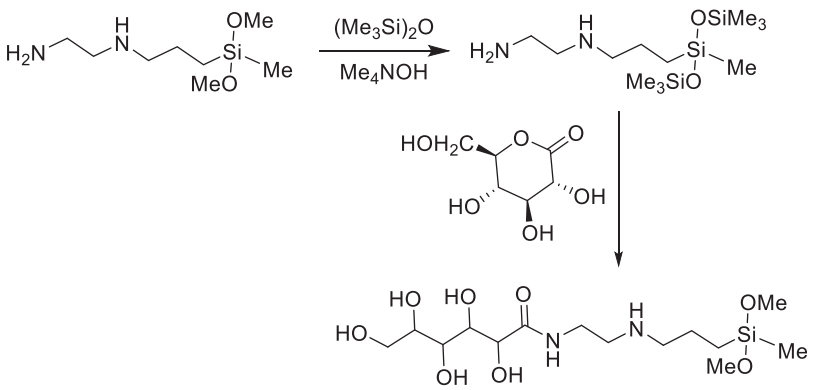


FIGURE 21.9

Synthesis of the gluconamide-based trisiloxane surfactant [26].

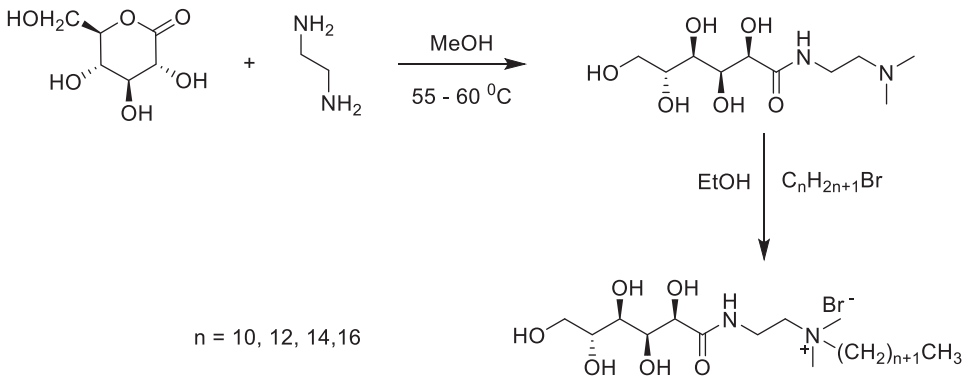


FIGURE 21.10

Synthetic route for gluconamide cationic surfactants [27].

the possibility of using them as a carrier to encapsulate the nonsteroidal antiinflammatory drug moiety and deliver it for dermal and topical applications [31].

A pharmacokinetic study using methotrexate (MTX)-loaded core−shell nanocapsule derived from the biosurfactant, stearic acid−valine conjugate revealed that the nanocapsule could be used to deliver the hydrophilic drug at a controlled rate with significant hemocompatibility and minimizing tumor [32]. Though the paclitaxel (PTX) is an approved drug for tumors because of its anticancer activity, its usage is limited by poor aqueous solubility and membrane permeability. To improve the sustained release and therapeutic efficacy, core−shell nanocapsules using lipid-/surfactant (conventional or biosurfactant) are employed. In this context, Wang and Ho fabricated the arginine−glycine−aspartic acid (RGD) peptide-functionalized core−shell nanocapsule via a double self-assembly procedure. In this the PTX is encapsulated within the hydrophobic poly(lactic-*co*-

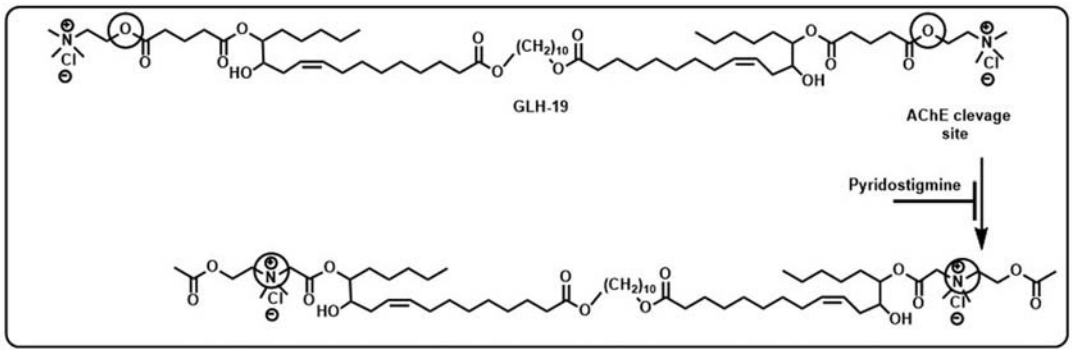
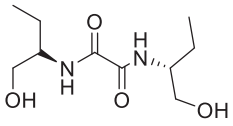
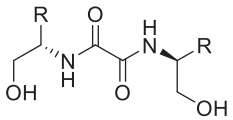


FIGURE 21.11

Structures of the bolalipids GLH-19 and GLH-20 [30].



(R,R)-6



(S,S)-1 R=iso-butyl

(S,S)-2 R= Sec-butyl

(S,S)-3 R= iso-propyl

(S,S)-4 R= benzyl

(S,S)-5 R=phenyl

FIGURE 21.12

Structures of various bis(amino alcohol)oxalamide-based gelators [31].

glycolic acid) (PLGA) core formed with the aid of lecithin and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)2000] conjugates which self-assemble around the PLGA core in the aqueous phase. This is surrounded by another self-assembled lipid layer that can act as a depot to load the vascular disrupting agent, combretastatin CA4 (CA4), and for the sustained release of PTX from the polymeric core [33] (Fig. 21.13).

Similarly, to enhance the therapeutic potential of PTX, Jain and coworkers have developed the core–shell nanocapsules in which the layer of stearic acid–valine conjugate (biosurfactant) surrounds the core loaded with PTX (~19%) (Fig. 21.14) [34]. With the intravenous administration of this PTX-loaded nanocapsule, a sustained release pattern of the drug in a controlled manner was observed [34]. The microscopic studies revealed that the nanocapsule is spherical-shaped core–shell nanocapsules comprising PTX-loaded core surrounded by biosurfactant layer and the in vitro biological characterization using PTX-loaded core–shell type nanocapsule exposed the significant increase in the cellular uptake in MCF-7 cell line in case of PTX released from core–shell

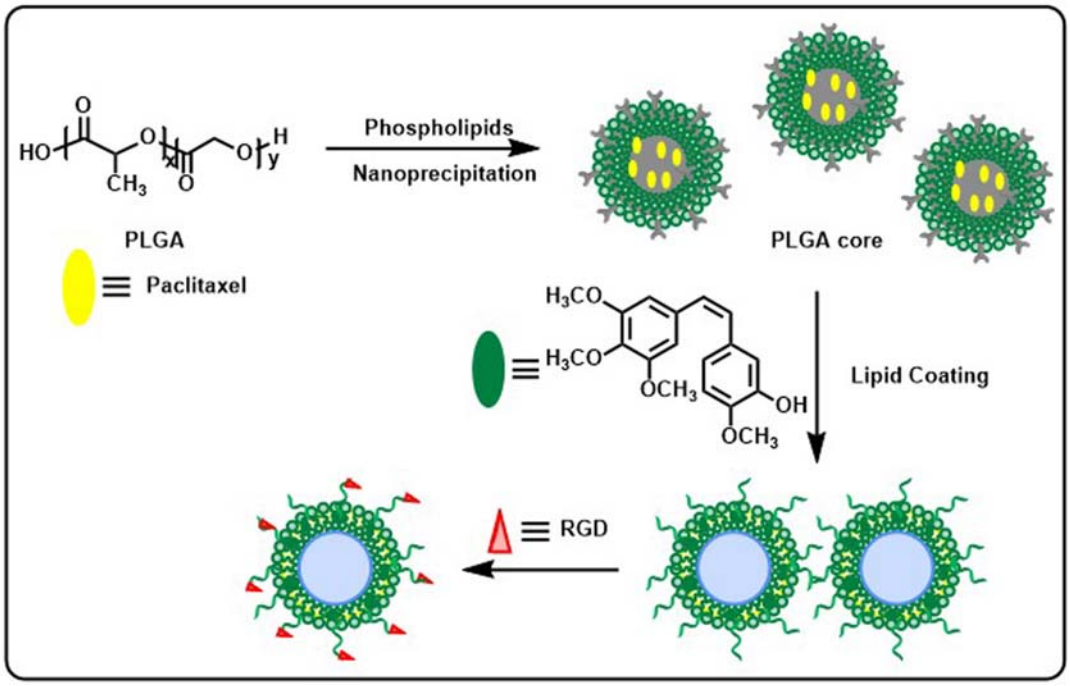


FIGURE 21.13

Schematic representation of nanocapsule preparation [33].

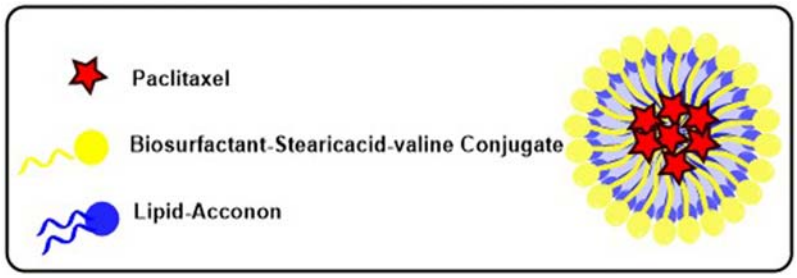


FIGURE 21.14

Pictorial representation of core–shell nanocapsule loaded with paclitaxel [34].

nanocapsule compared to free PTX [34]. Hemocompatibility and enhanced cellular uptake using PTX-loaded nanocapsules revealed that the use of this core–shell nanocapsule with high PTX loading can reduce the toxicity, taper the frequency of dosing, and improve the standards of PTX therapy to treat cancer [34].

Owing to the potential applicability of soft materials in the field of tissue engineering and regenerative medicine, the design, and synthesis of amphiphiles for the construction of injectable hydrogel-based scaffolds with intrinsic biological and mechanical characteristics are considered to be crucial exclusively, for in vivo applications [35–37]. In this context, Ramin et al. synthesized bolaamphiphiles containing urea and amide functionalities from commercial thymidine (Fig. 21.15) [36]. They demonstrated the potential of these amphiphiles in constructing injectable hydrogels with biological properties like decreased inflammatory, reduced fibrosis, slow degradation in vivo, etc. [36]. On comparing with most natural polymers, these gels displayed excellent mechanical properties such as fast gelation, thermoreversibility, thixotropic nature, high elastic moduli, and limited shrinkage compared [36]. They have also shown that the soft material derived from urea-based bolaamphiphile inhibits recognition by macrophages, fibrous deposition, and exhibits long-term stability after in vivo injection [36].

Compared to low-molecular-weight surfactants, the use of polymeric micelles as pharmaceutical carriers can produce stable micelles in vivo with high loading capacity. The unique physical and chemical properties of nanomaterials have increased the interest in developing nanoparticles using natural biopolymer and biosurfactants without employing complicated synthetic protocols and toxic materials. In this context, Marangon reported the formation of the antimicrobial nanoparticles using the biosurfactant, rhamnolipid, and the natural biopolymer, chitosan [38]. These nanoparticles were found to have increased positive surface charge with enhanced stability and excellent antimicrobial activity toward *Staphylococcus* strains both in planktonic and biofilms [38]. Polymeric micelles produced by the self-assembly of ionically modified chitosan by interaction with oleic acid were developed by Dellera et al. to deliver the antiinfective drug, silver sulfadiazine in wound healing applications [39].

To transport and deliver the hydrophobic drugs effectively, microemulsion drug delivery systems (MDDS) using biosurfactants were developed. Generally, MDDS comprises lipids, surfactants, cosurfactants, solvents and are formulated to enable diverse routes of drug delivery such as intravenous, topical, nasal, oral, and ocular. The factors such as insufficient solubility in lipid constituents, minimum drug-loading capacity, and the presence of large amounts of surfactants cause

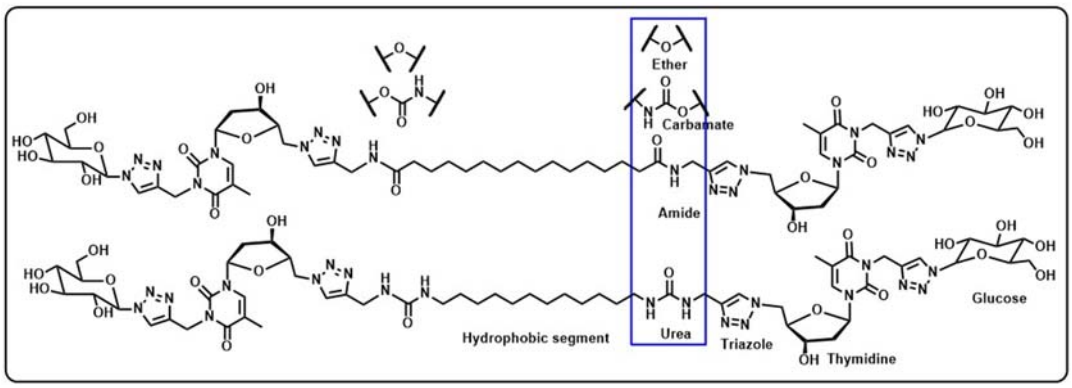


FIGURE 21.15

Structures of urea-based bolaamphiphiles [36].

gastrointestinal irritation to affect the formulation of MDDS. Compared to synthetic surfactants, the biosurfactants are less toxic with safety characteristics. Hence they are widely used for the applications such as wound healing, gene delivery, antimicrobial, anticancer, nanoparticle synthesis [40].

Globally, the rising bacterial infections and their treatment create a serious crisis for public health. Though antibiotics can be used, their treatment is facing increased risk due to the rising antimicrobial resistance caused by the factors such as the use of inapt antibiotics, excess use of prevailing broad-spectrum antibiotics, inclusion of antimicrobics in food, and household products. As the majority of bacterial infections are accompanied by biofilm formation, much work has been carried out to disrupt the biofilm. Owing to the inability of the bacteria to develop genetic resistance toward the antimicrobial peptides, a series of natural polymyxin-based synthetic cationic cyclic lipopeptides were synthesized by Grau-Campistany et al. [41]. They studied the interaction of sp-85, an analog consisting of a C12 fatty acid at the N-terminus and two arginine residues (Fig. 21.16), and showed that the antibacterial activity and selectivity is due to the destruction of the cytoplasmic membrane of bacteria [41].

Gudiña and coworkers examined various *Lactobacillus* strains in DeMan, Rogosa, and Sharpe (MRS) medium under aerobic conditions at 37°C for producing biosurfactants and reported the formation of biosurfactant by *Lactobacillus agilis* CGUG31450 [42]. Through fourier transform infrared spectroscopy (FTIR) characterization, the biosurfactant was identified as a glycoprotein that displayed greater antiadhesive activity against *S. aureus* and antimicrobial activity against *S. agalactiae, S. aureus*, and *Pseudomonas aeruginosa* compared to that produced by other lactobacilli [42]. Further, they observed that the production of biosurfactant increased to about 11 times on using alternative culture medium cheese whey [42]. The sophrolipids were isolated from yeast strains *Candida albicans* SC5314, *Candida glabrata* CBS138, and their formation was confirmed using FTIR characterization [43]. These sophorolipids were found to exhibit antibacterial properties with the generation of reactive oxygen species in *B. subtilis* and *Escherichia coli* [43].

In 2017 Lalitha et al. have synthesized amphiphilic molecules derived from the cheap renewable resources cardanol, a phenolic compound isolated from cashew fruits to form an efficient antimicrobial thin-film coating material (Fig. 21.17) [44].

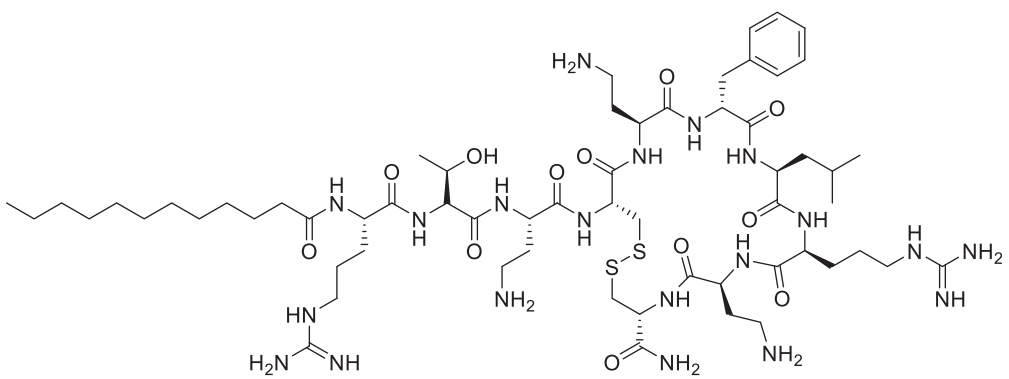


FIGURE 21.16

Structure of the lipopeptide sp-85 [41].

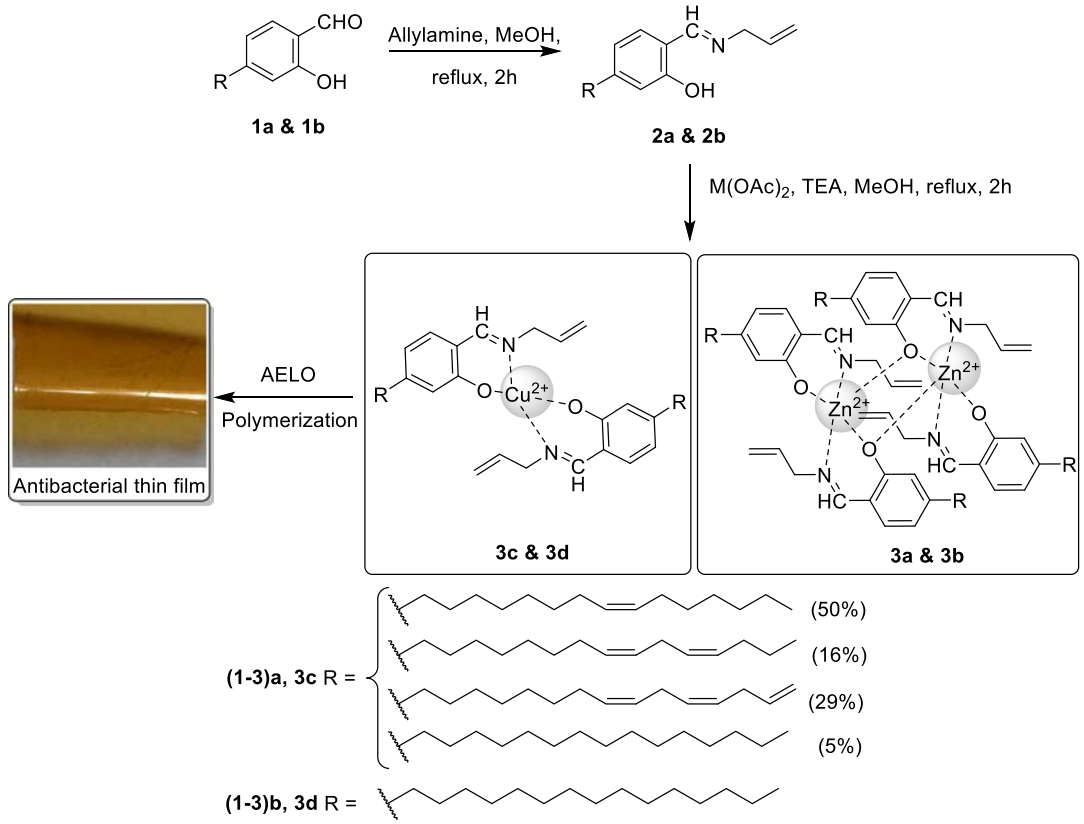


FIGURE 21.17

Synthesis of thin-film antimicrobial thin-film coating materials [44].

The antimicrobial and biofilm inhibitory behavior of synthesized compounds and thin films were investigated against various human pathogenic bacterial strains. The assembled thin-film coated catheter tube completely inhibits the biofilm formation of uropathogenic *E. coli*. Thus the developed thin-film coating material holds promise to be used as metal-enabled, nonleachable coating materials for public bacterial threats, and food and biomedical applications. In particular, this material can be potentially used for developing urinary catheter tubes with antibacterial properties [44].

In this context, Prasad et al. have synthesized the glycolipids of varying alkyl chain length using renewable feedstocks such as cardanol derivatives, monosaccharides like glucose and galactose by using simple synthetic protocols, in good yield (Fig. 21.18) and demonstrated that the self-assembled materials were effective in disrupting the pathogenic biofilms [45].

Among the numerous proposed alternative pioneering approaches for treating infections due to drug-resistant microbial strains, the nanoplex formed from transcription factor decoys (TFD), the short oligonucleotides, and the bola amphiphilic cationic vehicle, 1,1′-(dodecane-1,12-diyl)-bis-(9-amino-1,2,3,4-tetrahydroacridinium) (chloride or iodide), 12-bis-THA (Fig. 21.19) was shown to be

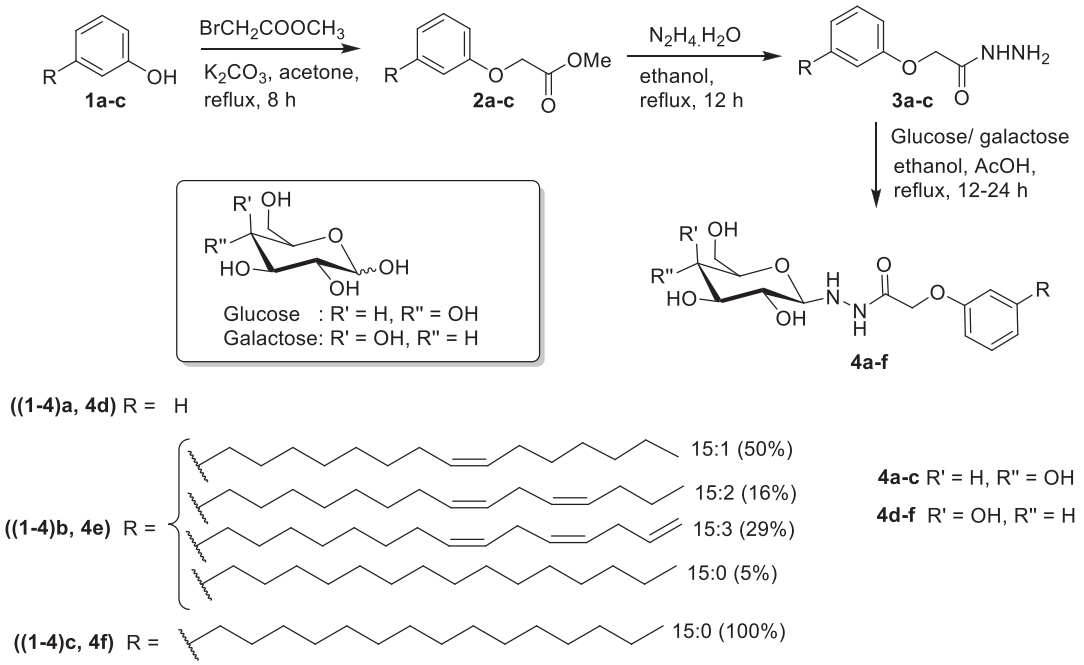


FIGURE 21.18

Synthesis of glycolipids [45].

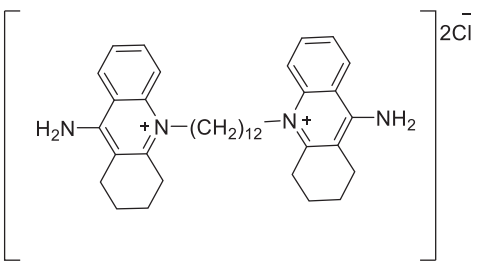


FIGURE 21.19

Molecular structure of 12-bis-THA [46].

effective in treating these drug-resistant infections [46]. To enhance the stability of this nanoplex, the liposomal formulation was created the liposomal formulations by decorating the surface of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and POPC/1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) with 12-bis-THA and investigation of the liposomal carrier containing fluorescently labeled TFD was found to deliver the TFD to the *E. coli* cytoplasm efficiently (Fig. 21.20) [46].

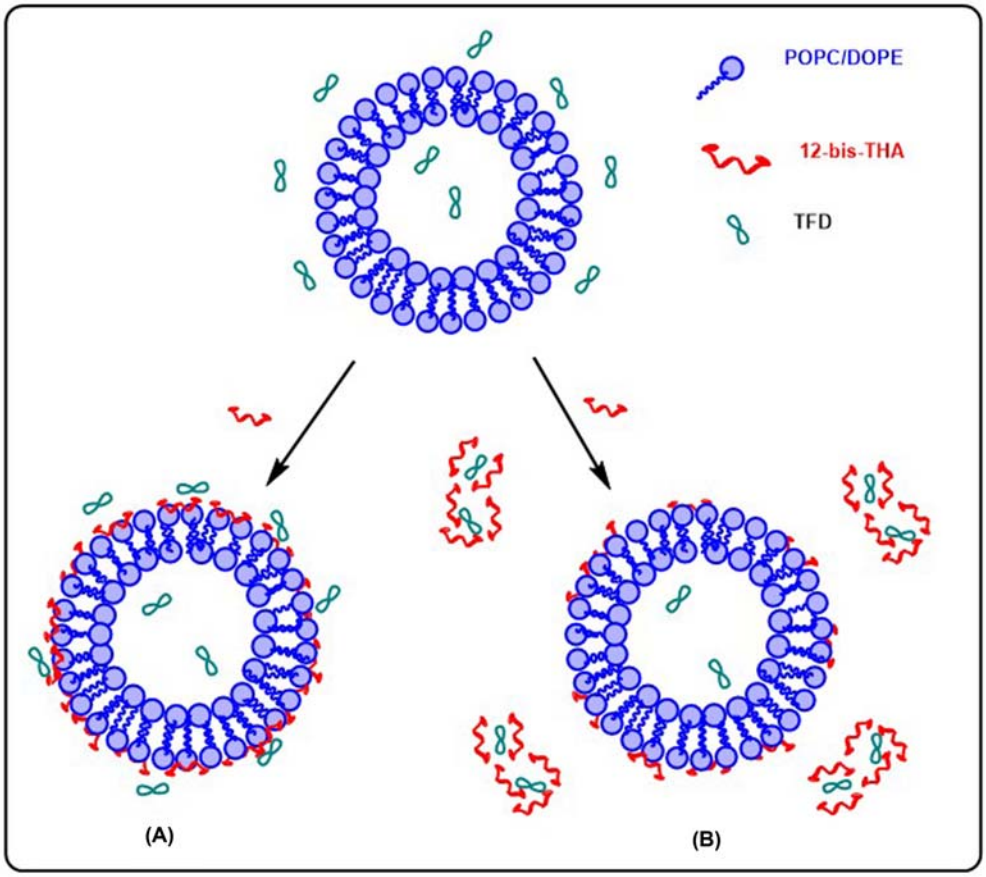


FIGURE 21.20

Pictorial representation showing (A) the adsorption of 12-bis-THA onto the lipid bilayer and the interaction with TFD, (B) nanoplex formation from 12-bis-THA with TFD. *TFD*, Transcription factor decoys.

21.3.2 Other applications

Owing to the high binding affinity of microbial surfactants toward heavy metal ions, they can be used to remove the pollutants [47–49]. Torrens et al. have revealed the rhamnolipid-facilitated removal of soil-bound cadmium and evaluated its efficiency in removing the soil-bound cadmium [49]. Their studies have revealed that the metal ion removal was influenced by the interaction with the metal [49]. Similarly, Noghabi and coworkers have investigated the potential of rhamnolipid to remove cadmium, copper, and zinc from wastewater [50]. For this purpose, they have produced rhamnolipids (Fig. 21.21) and demonstrated the possibility of using these rhamnolipid as ion collectors to remove cadmium, copper, and zinc in wastewater treatment. On comparing the competitive binding ability, the order of selectivity was found to be $Cd^{2+} > Zn^{2+} > Cu^{2+}$ [50].

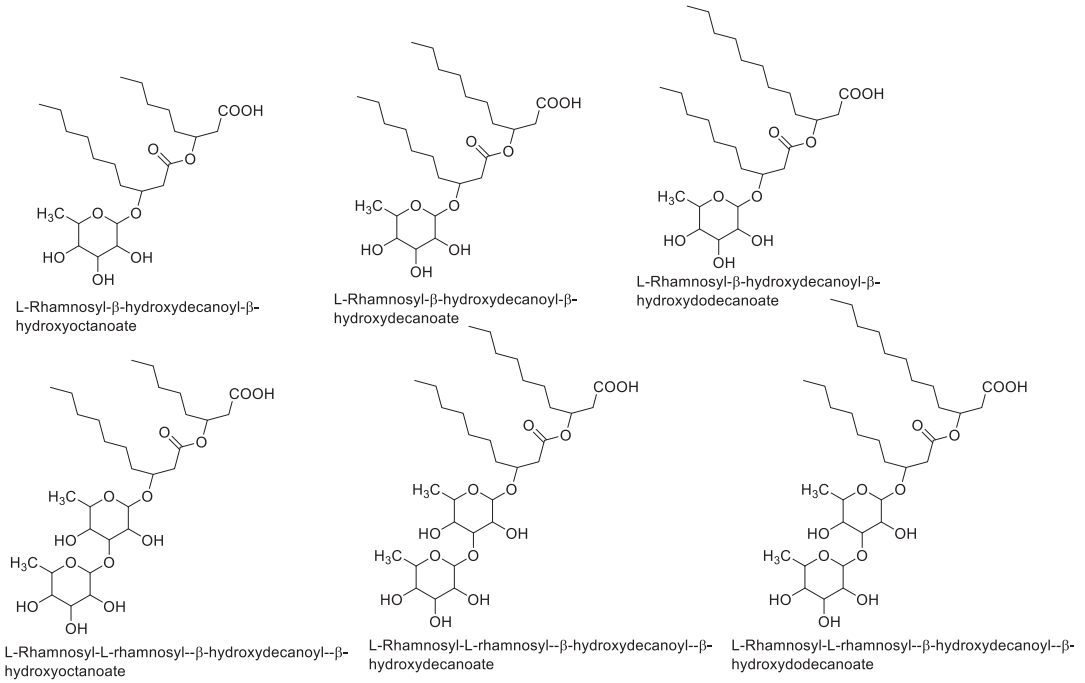


FIGURE 21.21

Representative examples of rhamnolipids for the removal of metal from wastewater [50].

As rhamnolipids exhibit properties such as wetting, foaming, solubilizing, emulsifying antimicrobial, and antiadhesive properties, they can also be used to increase solubility, biodegradation of pollutants like hydrocarbons, oil, and dye removal [48,51–53].

A cost-effective fed-batch cultivation strategy was developed by Samak et al. to enhance the production of rhamnolipid and sophorolipid using *P. aeruginosa* and *Starmella bombicola* [54]. On comparing this cultivation strategy with the normal condition, the production of rhamnolipid and sophorolipid was increased by 1.4- and 1.96-fold, respectively [54]. Fig. 21.22 shows a schematic representation of binding of biosurfactant with the synthetic surfactant, t-octylphenoxypolyethoxyethanol. Examining the binding efficiency of these biosurfactants with t-octylphenoxypolyethoxyethanol showed that they reduced interfacial tension and enhanced the oil recovery process [54].

Nomura and coworkers synthesized the gelators based on L-phenylalanine derivatives of varying alkyl chain length using naturally available raw materials [55]. They demonstrated the potential suitability of these nontoxic, biodegradable gelators in oil recovery applications by removing several oils selectively and efficiently from the oil–water mixture via gelation [55] (Fig. 21.23).

Chemical surfactants are employed in many current cosmetics for efficient dirt removal from skin and hair, lather enhancer in shampoos, skin and hair conditioner, moisturizer, and wetting agents because of their amphiphilic character, absorption, surface tension reduction, and other

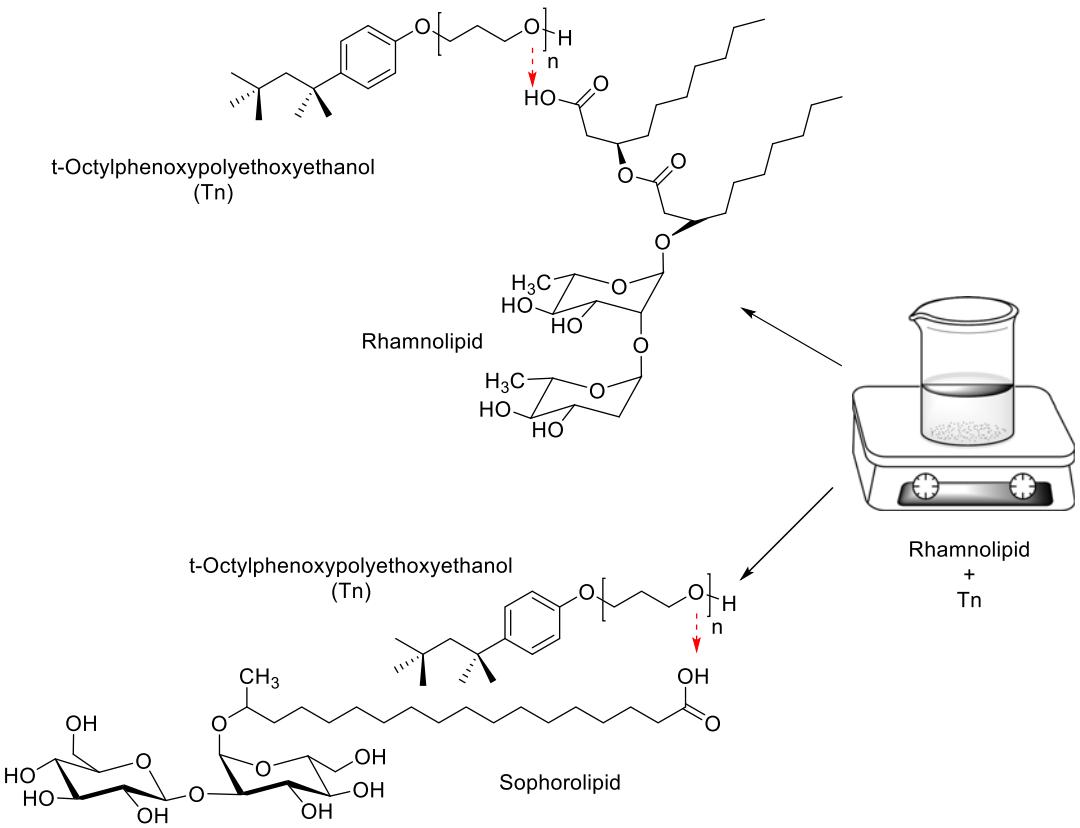


FIGURE 21.22

Schematic representation of binding of biosurfactant with the synthetic surfactant [54].

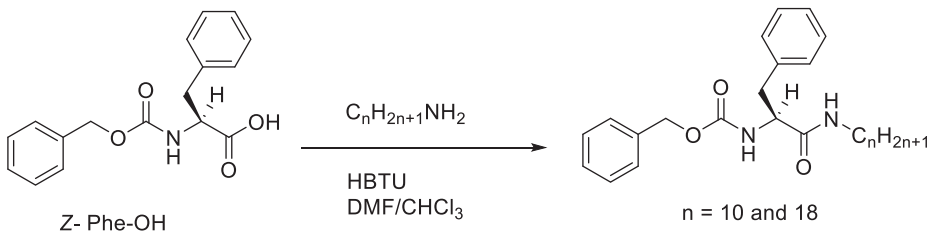


FIGURE 21.23

Synthesis of gelators based on L-phenylalanine derivatives [55].

unique characteristics [56]. Though the chemical surfactants are commonly used in cosmetics formulations, the prolonged use of cosmetic and personal care products consisting of the chemical surfactants in the formulation could have negative effects such as allergic reactions, skin irritations,

and alterations in the skin microbiome on the human skin due to the interaction of chemical surfactants with the epidermal layer of the skin. This creates the need for an alternative [56] In this regard, Adu et al. have discussed the classifications, the antimicrobial efficacy, cytotoxicity, skin moisturizing potential of the biosurfactants, and their interactions with skin cells that make them suitable for the usage in cosmetics and personal skincare pharmaceutical formulations [56]. Owing to the poor solubility of the active ingredient, zinc pyrithione in both oil and water, a large number of surfactants are used in antidandruff formulations. In this context, Moldes used the biosurfactant extract from the corn stream to produce biocompatible antidandruff formulations that are stable even after 30 days [57].

21.4 Conclusion

Biosurfactants are the surface-active molecules of natural origin such as lipopeptides, fatty acids, glycolipids, phospholipids, and lipoproteins. Enhanced biodegradability, low toxicity, and the retainable surface-active properties of the biosurfactants even at extreme pH and saline conditions compared to the synthetic surfactants have increased the interest in developing large-scale production of biosurfactants, In this chapter, we have discussed the classification of biosurfactants and the synthesis of few biosurfactants that are capable of reducing surface and interfacial tension. Various factors such as oxygen availability, pH of the medium, temperature, nutrient composition, and bioreactor properties alter the composition and yield of biosurfactants. The inherent properties of the biosurfactant such as wetting, foaming, solubilizing, emulsifying antimicrobial, antiadhesive properties provide the potential to be used for applications in the field of medicine, agriculture, pollutant removal, cosmetics, oil recovery, food and pharmaceutical industries.

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CHAPTER

Biosurfactants for pharmacological interventions in cancer therapy 22

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22.1 Introduction

Cancer, characterized by uncontrolled cell division, is a group of deadly noncommunicable diseases and was the reason behind the death of 9.6 million people during the year 2018 [1]. The cancer treatment mainly relies on chemotherapy and interestingly, sixty percentage of currently marketed anticancer drugs are originated from nature [2,3]. Many factors limit the direct extraction of anticancer entities from plant sources and hence researchers have come up with strategies to engineer microbes to produce the compounds of interest. Microorganisms are widely engineered for enhanced production of various potential bioactive including enzymes [4,5], antibiotics [6,7], drugs [8–10], and biosurfactants [11–13]. The advancements in microbial biotechnology have enhanced the identification and use of bacterial metabolites as cancer therapy agents [14].

Biosurfactants are one of such microbial metabolites whose production is arbitrated by a quorum sensing system to enhance survival in unfavorable environments [15]. These amphiphilic exopolymers include low to high molecular mass compounds (glycolipids, phospholipids, carbopeptides, lipopeptides, neutral lipids, and fatty acids), and exhibit various biological properties like antimicrobial, antiviral, antiinflammatory, and anticancer effects [15–17]. The biosurfactants are preferred over synthetic surfactants due to their reduced toxic effect, higher efficacy as well as biodegradability, and these properties make them suitable for medical applications [18].

In particular as anticancer agents, the biosurfactants are reported to inhibit various stages of cancer progression [19–21]. Being so important, it is relevant to understand the biosurfactants in detail based on their types, sources, and mechanism by which it induces a biological activity. In this chapter, we will explain the types and sources of biosurfactants and their anticancer efficacy against various cancers with the mechanism of action.

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22.2 Types and sources of biosurfactants

The molar mass of biosurfactants generally ranges from 500 to 1500 Da and they can be generally classified as lipopeptides and lipoproteins, fatty acids, phospholipids, and neutral lipids, glycolipids, polymeric biosurfactants, and particulate biosurfactants.

22.2.1 Lipopeptides and lipoproteins

The *Bacillus* species produce extracellular secretions containing lipopeptides having peptide chains connected to fatty acids [22]. Surfactins, fengycins, kurstakins, and iturins are the main class of lipopeptides [23]. Several such biosurfactants have shown antimicrobial action against various bacteria, algae, fungi, and viruses. Iturin which is produced by *B. subtilis* has both antifungal [24] and antibacterial property [25], and can be stored for six months at −18°C [26]. Among the reported lipopeptides, surfactin, iturin, and fengycin are well-known biosurfactants.

Surfactin is a cyclic lipopeptide which is one of the most powerful biosurfactants, produced by *B. subtilis*, contains a 7 amino acid ring structure bound to a fatty acid chain through lactone [27]. The surfactin decreases the surface tension and interfacial tension of water. The surfactin is also found to inactivate herpes and retrovirus.

Lichenysin is produced by *B. licheniformis* was found to be stable under intense salt pH and temperature conditions. It also decreases the interfacial and surface tension of water [28].

Fatty acids, phospholipids, and neutral lipid biosurfactants are produced in large quantities by several bacteria and yeasts while cultured on n-alkanes. *Acinetobacter* species are known to produce microemulsions of phosphatidyl ethanolamine rich vesicles in water that have immense applications in the medical field [29]. Premature infants show difficulties in respiration due to the deficiency of phospholipid-protein complex [30]. The genes responsible for the surfactants like this can also be used for fermentative production.

Glycolipids are characterized by the linkage of carbohydrate molecules with long-chain aliphatic acids or hydroxyaliphatic acids by an ester group. Glycolipids consist of rhamnolipids, trehalolipids, and sophorolipids [31]. In rhamnolipids, rhamanose molecules are attached to hydroxydecanoic acid. *Pseudomonas aeruginosa* usually produce these class of rhamnolipids [32]. The anticancer activity of dirhamnolipids synthesized by *P. aeruginosa* M14808 against lung and breast cancer cell lines was well documented [33]. Trehalolipids are produced by strains of *Nocardia, Rhodococcus, Corynebacterium*, *Arthrobacter*, and *Mycobacterium*. These chemical moieties are found to decrease the surface and interfacial tension in culture broth [34]. Sophorolipids are mainly produced by strains of *Candida* genus [35,36] and here sophorose is linked to hydroxyl fatty acid through glycosidic linkage. *Candida bombicola* is a widely using strain for synthesizing sophorolipids [37]. These sophorolipids especially lactone type of sophorolipid wide applications in various fields [38].

Polymeric biosurfactants comprise of lipomanan, emulsan, liposan, alasan, polysaccharide-protein conjugates. *Candida lipolytica* produces liposan which has applications in industries due to their emulsifying nature and are active even at low concentrations [39−41].

Particulate biosurfactants The alkane utilization by microbes is facilitated by the microemulsions formed by extracellular particulate biosurfactants that form membrane vesicles. These vesicles made up of phospholipids, lipopolysaccharides, and proteins, enhance the growth rate of the organisms [39,42,43]. Table 22.1 summarizes the microbial sources from which biosurfactants are produced.

Table 22.1 Sources of biosurfactants.

Organisms	Biosurfactants	References
Bacteria		
Pseudomonas fluorescens, Leuconostoc mesenteroides	Viscosin	[44]
Bacillus cereus	Iturin A	[45]
Bacillus thuringiensis	Lipopeptide	[46]
Bacillus subtilis	Iturin A, Fengycin, Surfactin A	[47]
Bacillus amyloliquefaciens	Fengycin	[48]
Bacillus atrophaeus	Lipopeptide	[49]
Bacillus licheniformis	Lipopeptide	[50]
P. fluorescens, Debaryomyces polymorphus	Carbohydrate–lipid	[51]
Lactobacillus fermentum	Diglycosyl diglycerides	[52]
Streptomyces griseoplanus	Carbohydrate–lipid–protein	[53]
Serratia marcescens	Serrawettin	[54]
Pseudomonas sp.	Fatty acid, mono- and diglycerides	[55]
Rhodococcus erythropolis, Arthrobacter sp.	Trehalose lipids	[56]
Pseudomonas sp. *Thiobacillus thiooxidans*	Ornithine lipids	[57]
Rhodotorula glutinis, R. graminis	Polyol lipids	[58]
Lactobacillus sp.	Glycolipids, glycoproteins, glycolipoproteins	[59]
E. coli, B. cereus	Xylolipid	[60]
Fungi		
Ustilago maydis	Glycolipids	[61]
Candida ishiwadae	Glycolipids	[62]
Candida bombicola	Sophorolipids	[63]
Aspergillus ustus	Glycolipoprotein	[64]
Aspergillus sp. MSF3	Glycolipoprotein	[65]
Torulopsis bombicola	Sophorolipids	[66]
Trichosporon ashii	Sophorolipid	[67]
Candida lipolytica	Protein–lipidpolysaccharide complex	[68]
Rhodotorula sp.	Carbohydrate–lipid–protein	[69]
Rhodotorula babjevae	Sophorolipid	[70]

22.3 Raw materials used for biosurfactant production

Even though the interest over microbial biosurfactants is mounting daily, it usually fails to compete with synthetic surfactant molecules in an economically feasible manner. However, the microbes producing biosurfactants have an immense role in hydrocarbon degradation [71,72] and are extensively used for recuperation of oil and bioremediation purposes [72,73].

Our major concern is to minimize the impact on the environment and hence precedence is given to recover, recycle and reuse sources [74]. The following section describes the usage of residual products for synthesizing biosurfactants using microbes.

C. lipolytica UCP 0988 was found to successfully utilize animal fat for the production of glycolipid [75]. Lactic whey is rich in lactose, proteins, organic acids, and vitamins and is reported to be a good source for the microbial production of biosurfactants [76]. *Pseudomonas* strains can produce rhamnolipids from the olive oil mill effluent [77]. Biosurfactants can be produced from olive and sunflower oils as substrates. *P. aeruginosa* and *C. lipolytica* are reported to produce a biosurfactant like rhamnolipids using canola soybean and corn oil [68,78].

Molasses was used as a substrate by *B. subtilis* for the production of biosurfactants in a minimal medium supplemented with molasses as the carbon source produces biosurfactants [79,80]. The chemical refining of oil seeds results in the formation of oil cake residues [81]. Oil cake residues formed during the processing of olive, sunflower, and soybean oils have been reported to produce various biosurfactants such as rhamnolipids, biodispersan, and emulsan [82,83]. Starchy Substrates such as cassava wastewater and potato starch [84] have been utilized for the production of biosurfactants using *B. subtilis* strains.

22.4 Biosurfactant with potent anticancer activity against different cancers with mechanism

Biosurfactants have an important role in the diagnosis and treatment of various diseases including cancer. Even though reports are stating the anticancer potential of biosurfactants, they are not exploited to the fullest. More novel biosurfactants with better anticancer potential have to be identified and they have to be directed for clinical evaluations without delay. The anticancer potential of some biosurfactants is discussed in this section.

22.4.1 Breast cancer

Surfactin, a glycoprotein from *Lactobacillus paracasei*, was found to inhibit the proliferation of T47D and MDA-MB-231 breast cancer cell lines, by arresting cell division at G1 phase [85]. A similar trend was observed when MCF7 breast cancer cells were treated with surfactin from *B. subtilis* [86]. Moreover, Cao et al. [87] showed that surfactin purified from *B. natto TK1* induced ROS/JNK-mediated mitochondrial/caspase apoptosis in MCF7 cell lines. Surfactin treatment increases ROS production and phosphorylates JNK resulting in depolarization of mitochondrial membrane affecting its permeability. Surfactin isolated from *B. natto TK1* strains prevents the propagation of MCF7 cells in a dose-dependent and time-dependent way. The cytotoxicity was tumor-selective as

surfactin treatment induced a similar underlying mechanism of ROS-mediated apoptosis. The lipopeptides from *B. subtilis* HSO121 induce apoptosis and affect the fatty acid distribution on Bcap-37 breast cancer cell lines [88].

22.4.2 Colon cancer

The study conducted by Kim et al. [89] showed that surfactin treatment on LoVo (colon cancer cells) induces apoptosis and arrests the cell cycle progression. The surfactins from *B. circulans* were able to inhibit the growth of HCT15 and HT29 colon cancer cell lines [90]. All these studies demonstrated that anticancer activity of surfactin is by apoptotic effects and G_0/G_1 phase cell cycle arrest. In addition to this, biosurfactant treatment suppresses the phosphorylation levels of P13K/Akt signaling.

22.4.3 Leukemia

Enhanced differentiation was observed when pro-myelocytic and myelogenous leukemia cell lines were treated with mannosyl erythritol lipid [91]. Succinoyl trehalose lipid (STL-1) from *Rhodococcus erythropolis* enhances growth inhibition and promotes differentiation in human monocytoid leukemic cell line U937 [92]. Another study proved that the effects of STL 3 on HL 60 cell depends on the structure of the hydrophobic moiety of STL 3 [93]. Reports have proven that the cytotoxic effect of surfactin molecule is implemented by preventing cell division, especially in G_1 stage, and inducing apoptosis in cancer cells mainly by altering the expression of apoptotic proteins [94].

22.5 Biosurfactant-nanoconjugates for cancer treatment

When we consider the demerits and disadvantages of other conventional prognostic and treatment methods of cancer, the search and development of new diagnostic and treatment systems are carried out to target cancer cells specifically and make them respond to chemotherapy [95]. Conventional methods in diagnosis like X-rays, tomography, or mammography require mutagenic agents in carcinogenic cells. In such cases, the utilization of harmful substances and X-rays within the designation of cancer is additionally joined to the causes of cancer. The failure of conventional anticancer treatments is mainly due to chemoresistance [96], myelosuppression [97], cytotoxicity [98], nonspecificity [99], the low solubility of drugs [100] which make them unable to enter through the biological membranes. Here the use of biosurfactant-nanoconjugates becomes relevant to improve the potency of drug delivery system in tumors.

Indeed, biosurfactants are one of the important microbial metabolites that confers significant biological potential [19]. The usage of nanoparticles for the diagnosis and treatment of cancer was found to be very successful, recently. The nanoformulations of biosurfactants increase the drug load, bioavailability, circulation period, specific targeting of cancer cells, and enhanced the release of drug [101], and so many nanoformulations have been developed so far with these benefits [102,103]. The anticancer efficacy of drug delivery compounds has been analyzed by various researchers using in vitro and in vivo methods [104–106]. Due to the smaller size, the nanoformulations permeate and accumulate in the target location, and the better retention property is utilized to identify and treat cancer (Table 22.2).

Table 22.2 Biosurfactants with anticancer potential.

Cancer type	Biosurfactant	Origin	Mechanism	References
Breast	Surfactin	*Bacillus subtilis*	Cell cycle arrest at G1 phase	[85]
	Glycoprotein	*Lactobacillus paracasei*		
	Lipopeptide	*Bacillus safensis*	Growth inhibition	[107]
	Surfactin	*Bacillus subtilis*	ROS-mediated cell cycle arrest and apoptosis	[87,108,109]
	Sophorolipids	*Starmerella bombicola*	Inhibits growth and cell migration and increase intracellular ROS	[110]
	Surfactin	*Bacillus subtilis*	Inhibits cell invasion by inactivating MMP-9	[20]
	Surfactin	*Bacillus subtilis*	Growth inhibition; Inhibits invasion, migration and colony formation	[20,86]
	Biosurfactant	*Leuconostoc mesenteroides* ssp. *cremoris*	Growth inhibition	[111]
	Itruin A	*Bacillus megaterium*	Inactivate MAPK and Akt kinase, induce apotosis	[16]
	Surfactin nanoparticles with doxorubicin	–	Overcome drug resistance	[112]
	Folic acid-biosurfactant-based graphene quantum dot conjugate	*Candida parapsilosis*	Enhanced drug intake and inhibits growth	[113]
Colon	Lipopeptide	*Bacillus circulans*	Growth inhibition	[90]
	Surfactin	*Bacillus subtilis*	Cell cycle arrest	[89]
	Biosurfactant - ENO14BS	*Pseudomonas aeruginosa* ENO-14	Induce apoptosis and prevents cell migration	[114]
	Lipopeptide-rakicidin A	*Micromonospora*	Cytotoxic growth inhibition	[115]
	Lipopeptide-Apratoxins F and G	*Lyngbya bouillonii*	Tumor suppression in mice model	[116]
Lung	Cyclic lipopeptides apratoxin A sulfoxide and apratoxin H	*Moorea producens*	Cytotoxic growth inhibition	[117]
	Fengycin	–	ROS-induced apoptosis	[118]
	Glycoliporotein	*Acinetobacter indicus*	Growth inhibition, cell cycle arrest	[119]
Leukemia	Iturin	*Bacillus subtilis*	Induction of apoptosis and paraptosis	[120]
	Cyclic lipopeptide	*Bacillus subtilis*	Induction of apoptosis	[94]
Esophageal cancer	Sophorolipids	*Wickerhamiella domercqiae*	Growth inhibition	[121]

Table 22.2 Biosurfactants with anticancer potential. ***Continued***

Cancer type	Biosurfactant	Origin	Mechanism	References
Pancreas	Sophorolipids	–	Growth inhibition	[122]
Cervix	Sophorolipids	*Starmerella bombicola*	Induction of apoptosis	[123]
	Sophorolipids	*Candida bombicola*	Arrest cell cycle, mitochondrial membrane depolarization, apoptosis	[124]
Liver	Sophorolipids	–	Apoptosis induction	[125]

22.6 Biosurfactant-nanoconjugates in diagnosis

One of the most used diagnoses of cancer is the distinct pictorial representation. For example, Computerized axial tomography is one of the widespread diagnostic methodologies which measure the radio-density of the fabric in its imaging method. This method can result in a significant impact on health. A nonionizing methodology that uses variance sputtering and lightweight wave absorption for dissimilar kinds of tissue is named optical coherence pictorial (OCT) representation. Here, the gold nanoparticles play a versatile role within the OCT methodology. Gold nanoparticles can even be utilized in dissimilar sizes and shapes for pictorial representation in beneficial ways [126]. Radiation is another area where these nano drugs have a dynamic role. Gold features a higher absorption than alternative counterparts and which is very necessary for radiation. Kannan and Katti report that cancer emission with a bigger concentration of gold nanoparticles will be found directly from cells for these neoplasm cells and cell fragments square measure necessary for the action of diseases. Even a lesser concentration of gold nanoparticles makes blood quicker than iodine [127].

22.7 Biosurfactant-nanoconjugates in treatment

The use of liposomes in nanoscale measure is one of the earliest applications to deliver chemotherapy payloads to the tumor [128]. The nanoformulations are successfully engineered to cover them so that they can escape the warriors and guards of the immune coordination. For example, liposomal formulations of doxorubicin developed in an aqueous phase, apart from using cremophore that usually triggers hyperimmune reflexes, are used for the treatment of refractory ovarian cancer, Kaposi sarcoma, and breast cancer [129].

One of the main concepts that liposomes stay within the blood for an extended time and area unit composed passively from neoplasm cells is the enhanced permeation and retention (EPR) effect. The EPR results are found to be effective to resolve treatment-related toxicity issues by taking lower and perennial doses of liposomal medication. According to Sengupta and Sasisekhanan, reflexive targeting in the course of the EPR increases the release of more drugs to the tumor location. The surface of nanoformulations is anchored with hydrophilic polyethylene glycol (PEG)

which delays the clearance from the bloodstream [130], and helps to accumulate in the target site [131].

In addition to this, the liposomal medication with the reticuloendothelial system (RES) builds it attainable to interrupt the immunologic recognition too. Furthermore, the restricted lymphatic leakage slows down the removal from the tumors. According to Devalapally et al. [132] PEG-linked liposomes have an additional general half-life indicating a big decrease in nonspecific uptake, and a similar effect is observed with surfactin nanoformulation also [133]. The increased EPR property can be attributed to PEG, as they draw water which protects the formulations from RES [134] and accumulates in the tumor.

The surface of nanocarriers was compartmentalized to exhibit target selectivity in cancer treatment. During angiogenesis in cancer cells, there is a characteristic expression of certain markers to enhance the uptake of nutrients [135]. The biosurfactants can be engineered with specific ligands specific to these markers that finally improve the target specificity. The targeting of receptors using ligands along with the consequence of EPR helps the accumulation of biosurfactants in cancer tissue and thereby facilitating the diagnosis and treatment.

The blend of polymeric prodrug and enzyme are being used for cancer treatment, and this technique is known as polymer-directed enzyme prodrug treatment. The multivalent side chains of these blend molecules enhance the drug loading capacity to the tumor tissue. PEG-campothecin and N-(2-hydroxypropyl) methacrylamide (HPMA) −paclitaxel/-platinate are such conjugates currently in the clinical trial. The HPMA-TNP470 complex is found to be efficient in preventing angiogenesis [136].

New strategies are adopted to make complex molecules of chemotherapeutic drugs and biodegradable macromolecules (such as polylacticcoglycolide, polylactide, polycaprolactone, chitosan, albumin, alginate) which can be converted to nanoparticles [137,138]. The nanoformulations are customized to control the discharge of attached biosurfactant molecules, that significantly reduce toxicity and increase the anticancer activity [133]. The drug is protected against degradation activities by the polymer network provided by enzymes found in the body [137]. The discharge is regulated either by enzymatic degradation, hydrolysis, or diffusion.

22.8 Conclusion and future perspectives

The microbial biosurfactants have many advantages over synthetic surfactant molecules. The amphiphilic nature helps them to impart many biological activities including anticancer activity. They can be used for cancer treatment and also for the delivery of cancer drugs. The surface-active potential, facilitate the interaction of these biosurfactants with cancer cell membranes and alters many downstream pathways, hindering the growth, arresting cell cycle, inducing apoptosis and preventing invasion. Researchers are continuously identifying novel biosurfactants and biosurfactant-producing strains from different sources and environments. The identification of biocompatible biosurfactants be capable of acting specifically on cancer cells will facilitate cancer treatment. For better results, the gaps in the exact mechanism of action of these molecules should be elucidated scientifically. More data have to generate to expand the use of biosurfactants for drug delivery. The available data showed that the combination of drug and biosurfactant or biosurfactant and nanoparticles enhances the specificity, uptake, and activity, and also overcomes the drug resistance. Hence

more research have to be done to identify novel biosurfactants and strains, new strategies to enhance production, understand the mechanism of action, and finally to confirm the safety of using these biomolecules. Addressing these concerns will promote the usage of biosurfactants for the treatment of various cancers.

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CHAPTER

Biosurfactants in respiratory viruses and the Coronavirus disease 2019 pandemic

23

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23.1 Introduction

The growing importance and the harsh reality of emerging and re-emerging viral infections have literally begun to dawn on us. The increasing globalization, that is, worldwide travel and trade amenities, widespread deforestation and urbanization, exposure to more and more diseases, their vectors and reservoirs, colossal population expansion, poor public healthcare systems, nosocomial infections and mutations in existing microbes, and increased drug resistance are the predisposing factors making us fall prey to these emerging infectious diseases [1,2]. Their threat of bringing life to a standstill has been now witnessed globally, with major bearings on health care delivery systems, agriculture, industry, politics, economies, and trade [3,4]. Therefore to meet these emerging health care needs, applications of existing research and new research are fortified. One possible solution could be biosurfactants (BSs). Their multifunctional roles in medical and allied fields are it as an antimicrobial, antiadhesive, antibiofilm, anticancer, and antioxidant agent may benefit us in these desperate times [5–7].

23.2 A quick overview of biosurfactants

23.2.1 Definition

To understand BSs, one must grasp the concept of surfactants. The term "surfactant" came from "*surface–active agent*" which are amphiphilic compounds having nonpolar hydrophobic

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hydrocarbon chains and polar hydrophilic tail [8]. When such compounds are produced from prokaryotic and eukaryotic organisms, they are called as BSs [7,8].

These are largely secondary metabolites of microbes with vital roles its metabolism, structure, and development. The last two decades have witnessed their extensive use in industry and other environmental applications. This has resulted in replacement of the synthetic surfactants to a great extent. The structure of BS is depicted in Fig. 23.1.

23.2.2 Types of biosurfactants

The classification of surfactants is mainly based on the polarity of its head end, that is, nonionic, anionic, cationic and zwitter ionic which means uncharged, negatively charged, positively charged and having both charges, respectively [8]. BS are classified based on their hydrophobic component into five major types [6,9,10] as shown in Table 23.1.

23.2.3 Advantages of biosurfactants

The shift from synthetic surfactants to green BSs was mainly facilitated by its good biodegradability hence its eco-friendliness and less toxicity, easier conditions required for its production and the better functionality of these surfactants [11].

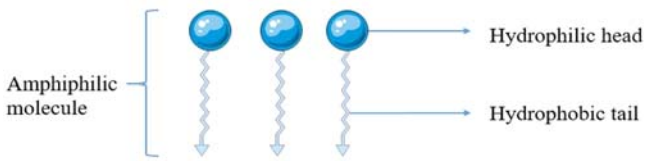

FIGURE 23.1

Biosurfactant structure having an amphiphilic nature.

Table 23.1 Classification of biosurfactants.

Sl. no.	Types of biosurfactants	Further details or sub classification
1.	Glycolipid type	Extracellularly produced by microbes They are further classified into sophorose lipids (SLs), mannosylerythritol lipids (MELs), cellobiose lipids (CLs), rhamnose lipids (rhamnolipids, RLs), and trehalose lipids (TLs)
2.	Fatty acid type	Phospholipids and neutral lipids with growth on n-alkanes
3.	Lipopeptide type	Fengycin, surfactin (cyclic LP further divided into A, B, and C based on differences in their amino acid distribution) and iturin families of *Bacillus* sp., viscosin from *Pseudomonas* sp. Decapeptides (Tyrocidine, gramicidins) and lipopeptide antibiotics like polymyxins
4.	Polymer type	Emulsan, liposan, mannoprotein, and other polysaccharide−protein complexes. Few of this list may be part of other types like glycolipid or lipopeptides too
5.	Particulate type	Microemulsion which is needed in alkane uptake by microbial cells

23.2.4 Production and application

23.2.4.1 Sources of biosurfactant production and screening

Generally, the microbes may either openly secrete BSs extracellularly or are part of their cell membrane. The common microbes which are used in the production of BSs are listed below [10,12–14].

23.2.4.1.1 Bacteria

Pseudomonas aeruginosa, P. fluorescens, P. putida, P. chlororaphis, Bacillus subtilis, B. licheniformis, B. pumilis, B. polymyxa, Serratia marcescens, S. rubidaea, Nocardia sp., *Mycobacterium* sp., *Corynebacterium* sp., *Renibacterium salmoninarum, Rhodococcus* sp., *Acinetobacter* sp., *Lactobacillus* sp., *Tsukamurella* sp., and *Arthrobacter* sp.

23.2.4.1.2 Yeasts

Candida bombicola, C. apicola, C. antartica, C. batistae, C. lipolytica, C. ishiwadae, Kurtzmanomyces sp., *Trichosporon asahii, Rhodotorula glutinis, R. graminis*, and *Ustilago maydis*.

23.2.4.1.3 Filamentous fungi

Aspergillus ustus, Tolypocladium inflatum, few species of *Penicillium*, *Trichoderma*, and *Fusarium*.

It all begins with a screening of BS production capacity. The various assays include the hemolytic activity, checking the surface tension reduction, performing a blue agar plate or CTAB agar plate method, drop collapsing test, oil displacement test emulsification index, or a hydrocarbon overlay agar method, etc.

Following the biosynthesis of BSs, they need to be purified through various extraction protocols and characterized. The characterization of BSs using thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR), electrospray ionization-mass spectroscopy (ESI-Ms) for direct identification of the BS. Once all this is over it is ready to be used for a particular application. An overview of the process is shown in Fig. 23.2.

23.2.4.2 Factors involved in its production

The strain involved in the bioprocess is one of the critical factors for the production of BSs. However, other factors like the optimum conditions under which the strain has been cultured like the nutritive medium, used with appropriation carbon and nitrogen substrates, salt concentrations, cations, pH, temperature, and whether it is well agitated and aerated to enable oxygen transfer from one phase to another [12,15]. However, the major problem encountered was the sky-high costs involved in processing. This was owing to raw materials or substrates which are expensive and difficult to obtain and maintain [10,12,16].

23.2.4.3 Major applications in medicine

The promising results in research obtained with BSs helped in aiding its use in various industries. This included the petroleum-oil refineries, bio-remediation and wastewater treatment services, food

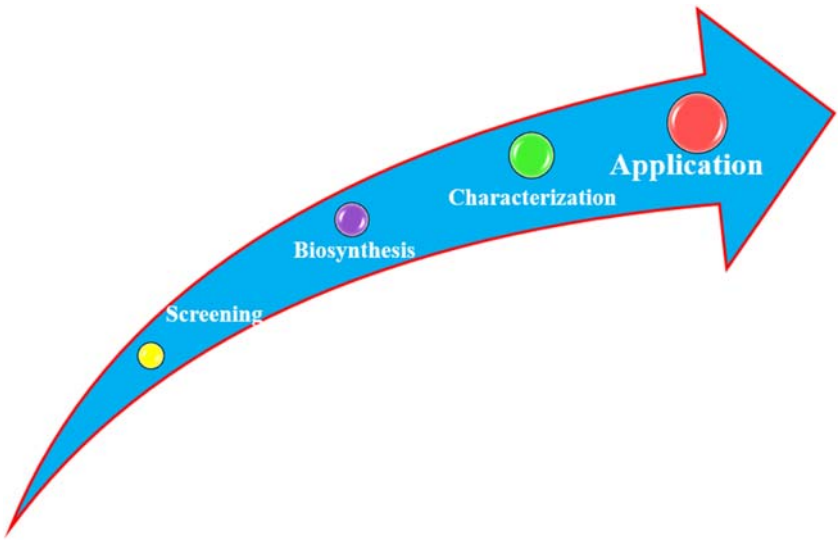

FIGURE 23.2

The overview of the steps involved in the production of biosurfactants.

and agriculture, textiles, pharmaceuticals and cosmetics, detergents and soaps manufacturing units. The relevance of their unique properties will provide answers especially as the world is running out of options for treatment of infections be it bacterial especially the multidrug-resistant bugs, fungi, parasites, and viruses [11,17,18]. Researchers started intensifying the search for its possible application and exploitation in the field of medicine too [6,19]. The major applications of BSs in the field of medicine are depicted in Fig. 23.3.

23.3 Viruses and biosurfactants

Viruses belonging to the gray zone between the "living" and the "nonliving." They take over the host cell apparatus to replicate. The highly infectious virus particle outside the host cell is called the "virion" [20,21].

23.3.1 Different classes of viruses

Viruses may be classified as RNA/DNA virus (based on the type of nucleic acid they possess), icosahedral or helical (their symmetry), whether enveloped or nonenveloped (presence or absence of the lipoprotein envelope), and six types (based on the replication mechanisms). Details were depicted in Table 23.2.

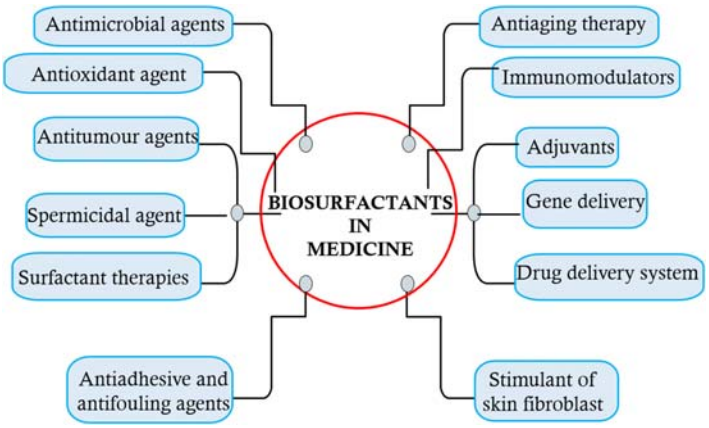

FIGURE 23.3

The major applications of biosurfactants in the field of medicine.

Table 23.2 Baltimore classification of viruses based on replication mechanisms [22].

Class	Nucleic acid	Type of mRNA	Examples	Remarks
I	(±) ds DNA	+ mRNA	Majority of the DNA viruses (*Adenoviridae*, *Herpesviridae* and Papova*viridae*)	DNA replication → *Nucleus* mRNA transcription and protein translation in the cytoplasm
II	(+) ss DNA		*Parvoviridae*	DNA enters the host cell nucleus and forms a duplex by using host enzymes
III	(±) ds RNA		*Reoviridiae*	RNA replication → *Cytoplasm* ds RNA is transcribed to mRNA by viral polymerases
IV	(+) ss RNA		*Picornaviridae, Coronaviridae, Togaviridae, Flaviviridae, Caliciviridae, Asteriviridae*	Since same polarity as mRNA they can directly translate to form early proteins
V	(−) ss RNA		Orthomyxoviridae, Paramyxoviridae, Rhabdoviridiae	Since opposite polarity as mRNA they cannot directly translate into proteins
VI	two copies of (+) ss RNA		*Retroviridae*	Initially converted to RNA: DNA hybrid by reverse transcriptase enzyme following which ds DNA is synthesized. Then its integrated into host cell chromosome

±, Both positive and negative strands in a helix; +, positive strand of nucleic acid;−, negative strand of nucleic acid; ss, single stranded; ds, double stranded mRNA.

23.3.2 Respiratory viruses and Coronavirus (severe acute respiratory syndrome Coronavirus-2)

The majority of respiratory viruses are enveloped, single-stranded RNA viruses except for *Adenoviridae* which has DNA. The RNA genome may be segmented or unsegmented depending on the virus. Details were depicted in Fig. 23.4.

The various families causing respiratory illnesses include the following:

1. *Orthomyxoviridae*: Influenza virus.
2. *Paramyxoviridae*: Parainfluenza virus, Respiratory syncytial virus (RSV), Human Metapneumovirus.
3. *Picornaviridae*: Human Rhinovirus.
4. *Coronaviridae*: Human Coronaviruses 229E, NL63 OC 43, HKU1, SARS-CoV, MERS-CoV, SARS-CoV-2.
5. *Adenoviridae*: Type 1,2,3,4,7 and 21serotypes of adenovirus.

The spectrum of these viruses can range from common cold, coryza, sore throat, pneumonia to acute respiratory distress syndrome (ARDS). These infections can in turn lead to complications like secondary bacterial infections, coagulopathies, and septic shock.

The crucial route of transmission in most of these infections is supposed to be droplets (particles ≥ 5 μm, settles in a distance of 3−6 ft). However, airborne transmission (particles <5 μm, remain as a suspension in the air) has also been noted especially associated with procedures generating aerosols. Direct contact with persons or articles or fomites contaminated with virus droplets is another route [21,23].

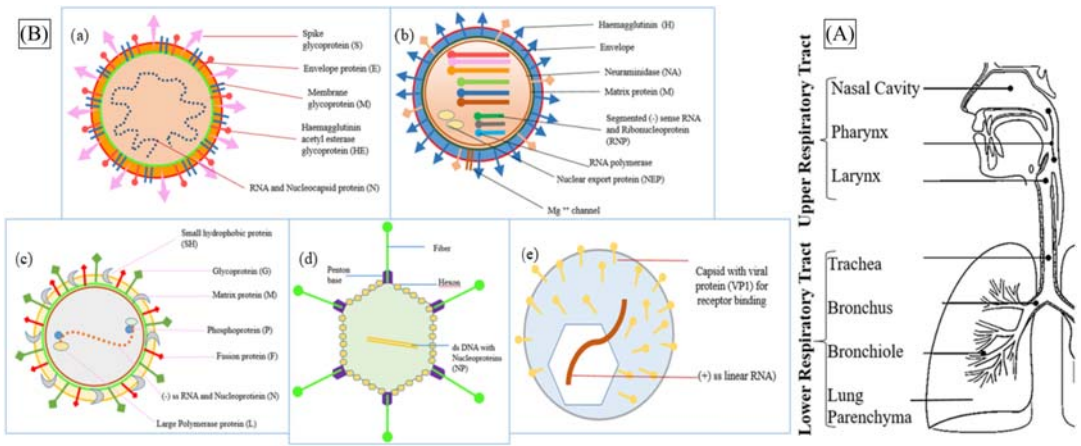

FIGURE 23.4

(A) The different parts of the human respiratory tract and (B) the common viruses causing illness: (a) Coronavirus, (b) Influenza, (c) RSV, (d) Adenovirus, (e) Rhinovirus.

23.3.3 Mode of action of biosurfactants on viruses

The elements which connive for allowing BSs to work in case of viruses range from the presence of envelope in most of these respiratory viruses to the weak links in the replication cycle. One must remember that these organisms are dependent on the host. It has to first enter the host cell, use different enzymes for the genome multiplication, and then produce the different structural and nonstructural components, wreak damage and then leave the cell in huge numbers by lysis or budding [21].

One of the most studied BS is surfactin which has been noted to have a broad spectrum antiviral property especially with human immunodeficiency virus (HIV-1) and Herpes simplex virus (HSV). It is a cyclic lipopeptide produced by *B. subtilis*. In comparison to nonenveloped viruses, at very low concentration can preferentially disrupt the membrane of enveloped viruses owing to the interplay of various physical and chemical factors. The outermost part of the envelope of the virus is the lipid bilayer (host-derived component) which interacts with the membrane active BS and acts as fusion inhibitors. Moreover, the permeability vicissitudes add to the viruses' woes and finally lead to a cleansing effect and disrupt the entire membrane system which had preserved the integrity of its structure. The peptidoglycan derivative of the surfactin has better antiviral action especially with Coronavirus, H1N1 and H3N2 Influenza viruses, Nipah, Ebola virus. The elevation of temperature hastens the action. Since the majority of the respiratory viruses are enveloped, this naturally available molecule could serve as the basis of the development of future models of antiviral molecules [24–26] (Fig. 23.5).

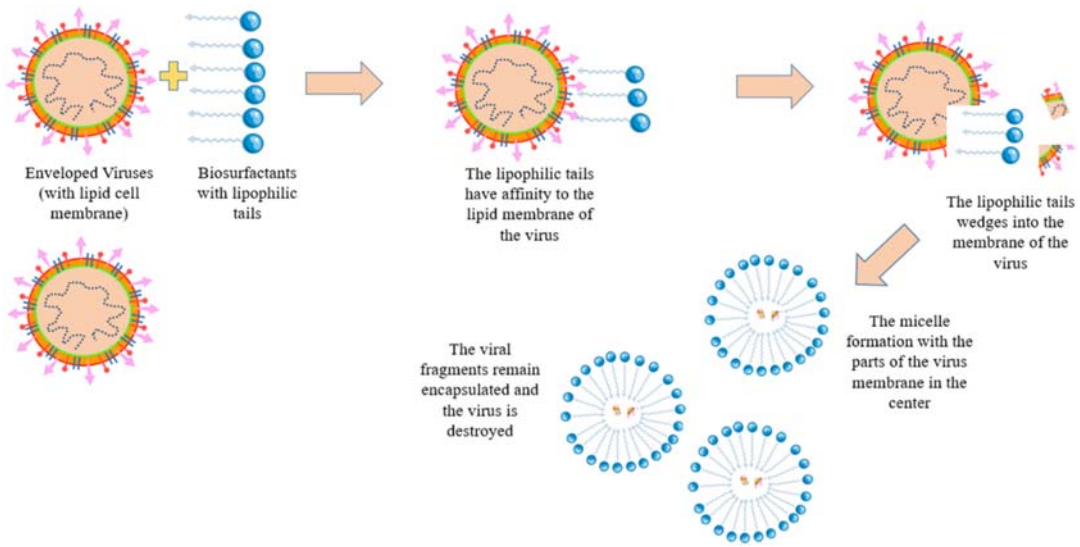

FIGURE 23.5

The mechanism of action of biosurfactant against enveloped viruses where the viral destruction occurs owing to disruption of its membrane integrity.

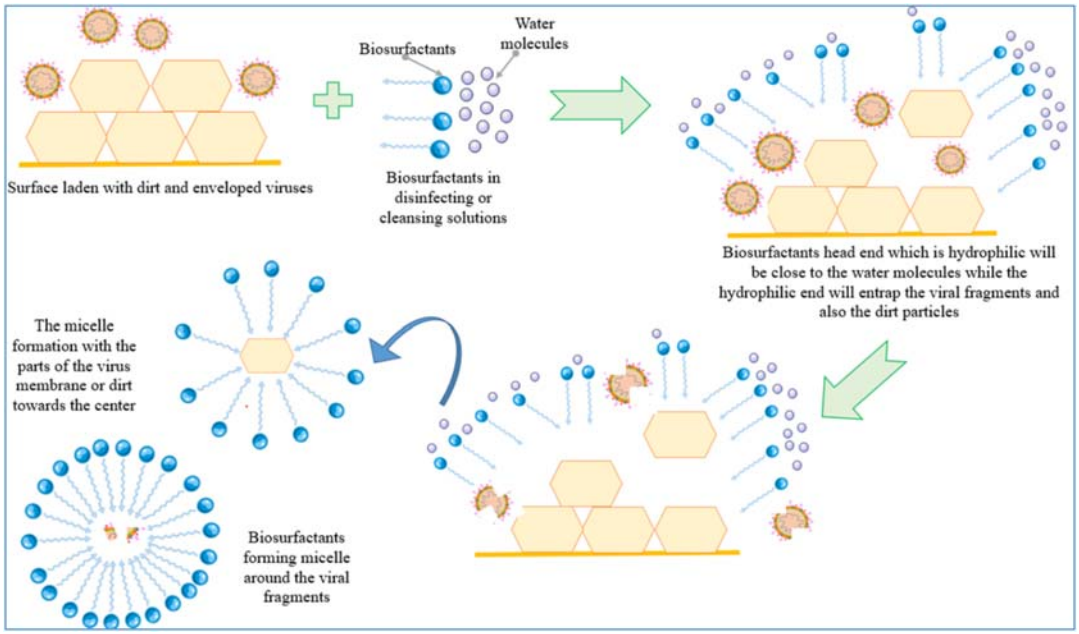

FIGURE 23.6

The mechanism of action of biosurfactant cleaning solutions both against contaminated surfaces with dirt and enveloped viruses where both get trapped in the micellar formation and is pulled away from the surface.

Other BSs with the aforesaid mechanism of virolytic activity are pumilacidin, rhamnolipids, and succinyl trehalose lipid mainly against HSV. However, the latter is reported to have additional action against the Influenza virus too. The former surfactin analoge is formed by *Bacillus pumilis*, followed by *Pseudomonas* sp., and *Rhodococcus erythropolis* respectively [19,27]. The sophorolipids of *Candida bombicola* is said to have activity against HIV-1 [5,6,9].

The cleansing property of the BSs also is based on the micellar formation and the ability to remove contaminants from various surfaces. This reduces the settling of viruses on the surface. The lipophilic part of the BS binds to the lipophilic part of the microbe. Concurrently the lipophobic part of the BS will bind to H_2O with high affinity. Thereby resulting in an emulsification response which will make sure the surface is free of that microbe. Hence this is of much relevance in the present situation where large-scale disinfection and cleaning are desirable to reduce transmission and limit propagation! This property may also be harnessed while using them as surface coatings too [8,14,28]. Details were depicted in Fig. 23.6.

23.3.4 Different roles of biosurfactants in respiratory viral infections including Coronavirus disease 2019

When proposing a role for BSs in viral infections many dynamics are to be considered -whether the BS could be used as an antiviral drug or its ability to reduce transmission by either acting as a

molecule delivery system or to eliminate the threat of the virus as surface modifications or coatings or as a cleansing or as a disinfecting agent.

23.3.4.1 Virucidal effect on enveloped viruses

The traditional classification of antivirals is those which are specific for a target in a particular organism while others nonspecifically acting antivirals can cross ranks. Major sites at which they act include the following as depicted in Fig. 23.7.

However, here the prospective antiviral BSs behave mainly as an inhibitor of membrane fusion where the initial attachment of the viral glycoprotein to the host cell receptor is blocked. This prevents the entire replication cycle from occurring and thereby reduces the infectivity and severity of the illness.

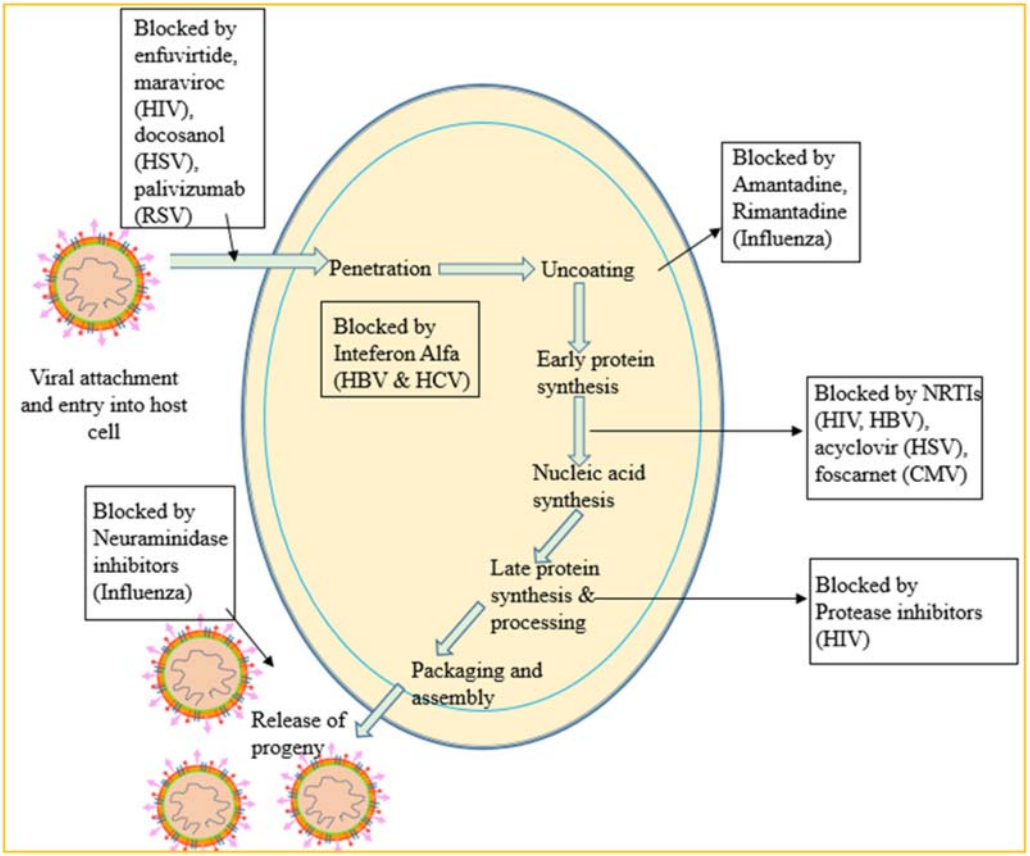

FIGURE 23.7

The major sites where antivirals act upon in the replication cycle of the virus.

A different biopeptide, Cyclosporine A from *Tolypocladium inflatum* is capable of inhibiting the protein synthesis, processing, and steps thereafter. This will limit the spread of infection by the progeny virus to the next host cell.

A different perspective in therapy especially as immunomodulators in COVID-19 is to use them to reduce inflammation and injury by reducing cytokine storm which results in multiorgan failure and shock.

23.3.4.2 Surfactant in acute respiratory distress syndrome

The mortality is high once ARDS sets in COVID-19 owing to the absence of appropriate therapy for this condition. In neonatal ARDS, evidence points in favor of surfactants to use for a reduction in the surface tension and thereby prevent lung collapse. Hence are recommended as therapyin neonates [29]. This rescue therapy can be tried in COVID-19 too [14,30,31].

23.3.4.3 Disinfection and cleaning applications

As discussed earlier the BSs can be safely used as detergents, household cleaning solutions, disinfecting agents. The torrential need for this during COVID-19 is ever-growing. New and combination products are being marketed and researched which can supplement or on theirown be a disinfecting agent [14,18].

23.3.4.4 Drug delivery systems, adjuvants, and vaccine development

For emerging viral infections especially in the current scenario of COVID use of antivirals, disinfecting agents and surfactant therapy in ARDS can help us stall the virus for some time. However, the inevitable doom is looming on us unless an effective vaccine or an adjuvant to boost up our immune cells is discovered.

To improve the action of the existing antivirals they could be delivered through these BSs. It could increase the half-life, efficacy and improve the concentration availability at the site. Hence a drug delivery molecule could be developed.

There are synthetic lipopeptides known to be able to induce virus-specific CD8 response to influenza virus, HIV-1, etc. This could be emulated with respect to BSs too. The sophorolipids BSs after improving their hydrophilicity by acetylation of its head have revealed countering activity to HSV and HIV.

Assured roles as an adjuvant that improves antigen presentation through good responses are witnessed in-vitro by increasing the differentiation of dendritic cells (DCs) and their maturation. This selective action which increases its exposure to aid the DCs needs to be exploited for vaccine therapy [14,28].

23.4 Conclusion

Judging based on the number of COVID cases increasing manifold, we need to take a closer look at the prospects of these BSs. Though there are many in vivo and in vitro data on BSs, a clarion calls for its consolidation and conformation via clinical trials in human beings. We should keep in mind their merits, especially owing to their long-term association with other sectors and domains.

True research is foreseeing the need of the future and trying to find answers at the earliest. The initial fire has been lit and one needs to take the torch to the destination!

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CHAPTER

Biosurfactant as an intervention for medical device associated infections

24

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24.1 Introduction

Recent advancement in medical science; in-dwelling medical devices (IMDs), has improved the clinical outcomes for patients and have been widely used in the healthcare industry. However, most of these medical devices are prone to biofilm formation hence play a vital role in healthcare-associated infections [1]. Common device-associated infections (DAIs) involving biofilms, has been associated with intravenous catheters [2], implantable cardiac valves [3], urinary devices like Foley catheters [4], orthopedic devices [5], cardiac pacemakers [3], intrauterine devices [6], biliary tract stents [7], breast implants [8], contact lenses [9], and voice prosthesis [8].

The medical surface provides a favorable ecosystem for the adhesion, proliferation, and growth of mixed species of organisms, mainly Gram-negative, positive and fungal in nature [10]. IMD-associated infections have a tremendous economic impact due to the higher healthcare costs, prolonged hospital stay, and loss of employment days [10]. Additionally, these infections are resistant to eradication strategies, since the majority of these infections are linked with biofilms and these biofilms contain pathogens with multidrug resistance [11].

Currently, there are no strategies that can effectively prevent DAIs in patients. Strategies for the prevention of DAIs are measures to delay the onset of microbial adhesion and biofilm formation. Regarding delaying the adhesion and biofilm formation, some plans are still in development, and others are already used in patient care. In general, novel preventative strategies should be strategized and developed to prevent and/or inhibit biofilm-linked DAIs in health care settings [12]. One of those strategies is the use of biosurfactants (BSs).

BSs are biological compounds with significant surface and emulsifying properties. They constitute broad physic-chemical features, such as glycolipids, lipopeptides, polysaccharide-protein

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complexes, phospholipids, fatty acids, and neutral lipids [13–17]. Depending on their physical and chemical properties their functions are also varied. In comparison with the other chemical surfactants, BS poses advantages like less or no toxicity, higher biocompatibility, and tolerance to extreme conditions [17–20]. BSs of microbial origin guarantee significant potential as antiadhesive, biofilm inhibition, and eradication of preformed biofilms in clinically relevant pathogens [17]. However, the amount of data required to suggest their usage in the medical field is lacking.

One of the major hurdles associated with the use of BS is the high economic burden associated with their identification, characterization, purification, and mass production. Though an array of low-cost production of biologically active BS is reported earlier [17], efforts are warranted in the direction for the process standardization and scaling up for engineering and biological evaluation [17].

This chapter discusses the role of BSs in biofilm development, as well as their usage in biofilm suppression and antiadhesion compounds. In addition, a diverse class of BSs produced from various sources, with potent antiadhesive and biofilm inhibition properties, that are effective against biofilm-lined device-associated infections are also detailed [17].

24.2 Nosocomial device-associated infections

Modern health care facilities have advanced a long way from the ancient form of simple organizations meant for conducting diagnostic and providing therapeutic services to the patient population. On the contrary, modern hospitals are complex institutions offering specific yet sophisticated care for the needs with the help of well-qualified health care staff and high throughput instruments [21]. However, even with this sophistication, environments at the health care facilities still serve as the reservoir for diverse pathogens capable enough to cause nosocomial infections (NIs) in patients during their hospital stay and/or visit [22]. An infection acquired by or occurring in a patient at a healthcare facility, in whom there was no infection or incubating at the time of admission or appearing after discharge is defined as NIs [23]. In addition to the patient population, health care workers are also prone to NIs.

Irrespective of the organizational complexity of the health care facilities, the rates of NIs are mounting day by day and always associated with the elevated socio-economic burden. Prolonged hospital stay is always associated with the NIs which ultimately results in overcrowding, lack of drugs, staff, equipment, and other related facilities [22]. Patients under life-supporting medical devices, implants, postsurgical care, and intensive care units (ICUs) are at the highest risk for developing NIs [22]. As a matter of fact, the fore mentioned population has a three times higher rate for NIs than other patients [22,24]. The vulnerability to the NIs was an indirect association with the compromised immunological status, microbial adhesion, and biofilm formation on medical devices, and implementing hygiene practices. In addition, practices like the use of broad-spectrum antibiotics at higher doses aid in the development of resistance and ultimately result in untreatable NIs [25].

Even after the ever-occurring advances in health care settings in high-income countries, NIs have exhibited an elevated prevalence rate of 3%–12%. According to the report of the European Centre for Disease Prevention and Control (ECDC) the mean incidence of NIs in Europe was 7.1%, with more than 4 million affected patients annually [22,24]. Similarly, the incidence rate of

NIs in the USA was estimated to be 4.5% with more than 1.5 million affected patients every year [26]. Though the data available from the low and middle-income countries are scarce, around 25%−30% of the hospital visiting patients develop NIs. As a matter of fact, the incidence and socio-economic burden of NIs is significantly high in these countries in comparison with the high-income countries [27,28]. Surveillance of NIs by the International Nosocomial Infection Control Consortium (INICC) in 8 low and middle-income countries (including India) revealed an incidence of 14.7% [22,24].

NIs can be classed as nonmedical device-associated and medical device-associated infections, depending on the cause. Pathogens are disseminated via contact in the event of nondevice related diseases, such as contacting an infected health care worker or touching contaminated materials [29]. Another major possibility of pathogens spread is by contact with an infected person's blood or other fluids [29].

Transmission of microbes from the medical devices result in medical DAIs and approximately more than half of all the NIs is a medical device associated in nature [29,30]. Major NIs caused by medical devices include (1) Bloodstream infections associated with central lines (CLABSI), (2) Urinary tract infections due to catheters (CAUTIs), (3) Pneumonia associated with ventilators (VAP), and (4) Infections on the surgical site (SSIs).

Around 65% of the medical device-associated infections are biofilm linked [29] and it is been estimated for 2% of breast implants; 2% of joint implants; 4% of the heart valve devices; 4% of the pacemakers; 10% of the ventricular shunts, 25% of the urinary catheters and 40% of the ventricular devices [29]. Microbes secrete extracellular polymers to develop a conditioning film on the abiotic surfaces of medical devices. Conditioning film facilitates microbial adhesion and provides polymeric matrixes which glued onto the device surface due to which they are resistant to break down and/or removal. Biofilms prevent antimicrobial penetration by acting as a barrier due to which biofilm-linked pathogens are found to be 1000 times more resistant than their planktonic counterparts. In addition, the biofilm matrixes aid the linked pathogens to evade the host immune system. Biofilms can act as a reservoir of associated pathogens leading to recurrent infections. In addition to device-associated infection, microbial attachment, and biofilm mode of growth can alter the functional status of the devices. In fact, biofilm-affected bone implants and prosthetics have been shown to loosen in comparison to uncontaminated implants [29,31].

24.3 Role of biofilms on device-associated infections

In DAIs, biofilm development has been recognized as a crucial step [32], in which a colony of bacteria adheres to a surface and secretes extracellular polysaccharides to create an architecturally complex film. Interestingly, biofilm formation involves a sequence of steps. Once the medical device or implant is inserted into the host milieu, components like proteins, other organic molecules, and ions get adhered to the abiotic surface and form a conditioning film [10]. Such a phenomenon results in the alteration of surface characteristics of medical devices and promotes the initial adhesion of microbes. Reversible attachment of microbes takes place at this stage and their adhesion is dependent on the surface properties of the microbes and the medical device. Characters like motility, cell surface hydrophobicity, and the ability to access the substratum are some of the

essential requirements for successful substrate attachment [33]. Microbes get adhered to the surfaces through physical forces (like Van der Waals forces) or with the aid of specific molecules like adhesions [32]. Over time, these reversibly attached microbes secrete diverse virulence factors like extracellular polysaccharides, BSs like rhamnolipids, and a variety of bio-macromolecules to facilitate a mechanically stable adhesion, resulting in irreversible attachment. With constant microbial proliferation, replication, and virulence factor production, a matured biofilm with three-dimensional complex structural features like nutrient/water channels carrying substances to the microbial microcolonies was formed within [30]. Later, the biofilm-linked microbes can detach from the matured biofilms and return to the planktonic mode of life [29]. With the help of motility, these biofilm-associated microbes move to other sites to initiate the biofilm mode of life.

24.4 Role of biosurfactants in biofilm mode of growth

BSs are metabolic biomolecules produced by the microbes with amphiphilic properties (containing hydrophilic and hydrophobic regions) which aid in reducing surface tension and interfacial tension [33]. Based on the hydrophilic regions present, BS is named as rhamnolipids (BS containing rhamnose), sophorose containing sophorolipids, and glycolipids, with a carbohydrate moiety [34].

Biosurfactants play major roles in cellular metabolism, motility, and defense. They are found to be an integral part of biofilms, molecules for the cell to cell communication mechanism, secondary metabolites, and virulence factors [34]. BSs act as a major component for the adherence to interfaces, facilitating the gliding movement of microbes through the wetting surfaces. In addition, a surface-active molecule like BS enhances the interaction between natural and synthetic organic polymeric surfaces and microbes [33]. Opportunistic pathogens like *Pseudomonas aeruginosa*, BS are involved with colonization promotion and migration dependent structural development [33].

Some of the major roles played by BS in bacteria are indicated in Fig. 24.1 [33]. As detailed earlier, BS production regulates the motility of organisms by affecting the development of flagella and influencing the attachment capability of bacteria. Attachment to varied surfaces is considered as a strategy to protect them from ecological stress and aid in the survival of the attached microbes. Bacterial adhesion to surfaces helps in energy-saving and biofilm matrix and the secreted components will shield the biofilm-linked microbes from external unfavorable conditions and maintain a microenvironmental niche [33]. One of the major factors which play a role in bacterial adhesion is the cell surface hydrophobicity which is influenced by the type and concentration of BS produced by the respective microbes [33].

24.5 Application of biosurfactant specific to device-associated infections

BSs are of various types and levels produced by Gram-negative organisms will invariability is unsuccessful against the Gram-positive organism, and vice versa [33]. In addition, bacterial cell surface hydrophobicity affected by the secreted BS accordingly varies the other organism's adhesion on the solid surfaces. Such properties have drawn the attention of many researchers in the

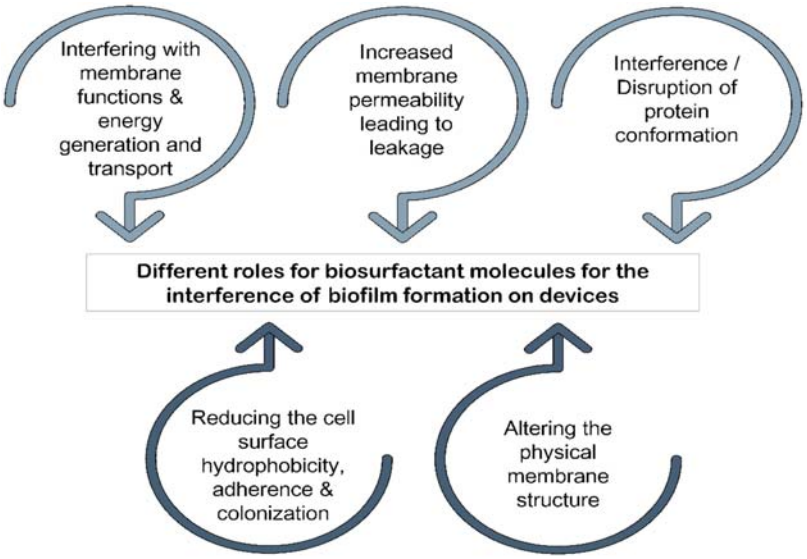

FIGURE 24.1

Roles played by biosurfactants in biofilm formation on polymeric surfaces.

field of antimicrobial and antibiofilm field and considered BS molecules as a representative candidate [33].

24.5.1 Biosurfactants with antiadhesion property

BSs play a major role in biofilm-forming phenotypic features like swarming motility, cell surface hydrophobicity, and cell adhesion and increase the possibility of biofilm-associated DAIs [35]. Among the other characters, reversible and irreversible adhesion is critical to DAIs. However, some BS was found to exhibit antiadhesion properties against pathogenic organisms to both biotic and abiotic sites. Thus the use of BS in the infections sites and/or solid surfaces might be an effective strategy to combat microbial colonization and biofilm formation [17]. As a matter of fact, there is an array of studies that confirmed the role of BS produced by microbes in postadherence struggle with other strains and/or species [17,36,37].

It is been found that surfactin, a secreted BS, found to decrease the biofilms on polyvinyl chloride platforms produced by pathogenic organisms like *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhimurium*, and *S. enterica* [17,36,37]. In another study, surfactin reduced biofilms on vinyl urethral catheters [19,35–37]. Precoating of solid medical surfaces with BS was considered a new and effective method to counter microbial adhesion and colonization. Understanding the role of opportunistic pathogens in DAIs these results have potential practical applications [19,35]. Interestingly, there was a significant reduction in biofilm formation when surfactin is incorporated into the growth media and as a precoating for catheters [19,35].

Members of the natural human microbiome also produce BSs with significant antiadhesion properties. *Lactobacilli*, being an important member of the healthy urogenital flora, are found to secrete diverse BS and compete with other microbes for epithelial cell adhesion [17]. In a healthy woman, the urogenital microflora comprises around 50 different species of organisms. Catheterization being an important and regular procedure in the current health care system, catheter-associated urinary tract infections have a significant part in the incidence of DAIs. Catheter surfaces are prone to microbial adhesion and through biofilm formation on catheter surfaces, microbes gain direct entry into the urogenital system bypassing the host immunity. Biofilm-associated uropathogens infect all the parts of the urogenital system and include *E. coli*, *P. aeruginosa*, *Staphylococcus*, *Saprophyticus*, and *Enterobacteriaceae* [38]. Members of the microflora may colonize the vagina and reduce the risk of infections by hampering the growth and adhesion of pathogens to the urogenital system [39]. Members of human microbiota impart the adherence and biofilm formation of uropathogens by various mechanisms. Either the presence of these microbes (members of the human microbiome) and or their cell wall fragments prevent uropathogens from uroepithelial cells. Host microbiome members produce inhibitors, thus forming aggregates with pathogens leading to the competitive exclusion from surfaces. In addition, the majority of these organisms synthesize numerous metabolites like lactic acid, H_2O_2, BS, and other antimicrobial peptides with the capability to eliminate disease-causing agents. Definite *Lactobacillus* strains are capable to produce certain types of protein-rich "surlactin" BS that can interfere with the adhesion of uropathogens [40].

Studies confirmed that the BS produced by *Lactobacillus acidophilus* protected silicone rubber surfaces from the biofilms of uropathogens and yeast [41]. Similarly, *L. fermentum* RC-14 produces BS that prevented the initial attachment of uropathogenic *Enterococcus faecalis* [42]. Velraeds et al. reported a dose-dependent response of BS produced by a *Lactobacillus* strain on the inhibition of initial adhesion by *E. coli* and other adherent microbes on surfaces with different physicochemical properties [41]. Reid and coworkers demonstrated the BS produced by *L. acidophilus* NCFMTM to be used directly to the vagina as an antiadhesion agent [43]. Velraeds et al. [41] detailed the antiadherence property of BS from *L. acidophilus* RC14, *L. fermentum* B54, *L. casei* subsp. *rhamnosus* 36 and ATCC 7469 against uropathogenic *E. faecalis* 1131.

Voice prosthesis is a medical device used for speech convalescence in patients with laryngeal tumors and receives a laryngectomy. The preferred material for the voice prosthesis is silicone due to its mechanical and elastic properties. Silicone rubber processed voice prosthesis is prone to thick biofilms of yeast and bacterial strains ultimately leading to replacement due to blockage, leakage leading to devise dysfunction and replacement [44]. Studies have confirmed that the uses of BS can reduce microbial adhesion and associated biofilm formation thereby improving the life span of voice prosthesis [20,45]. Rodrigues et al. have studied the design and improvement of approaches to avert microbial adhesion to silicone voice prosthetics [17,46,47]. They have tested the BS from *L. lactis* 53 and *S. thermophiles*. A coated silicone rubber voice prosthetics against multiple bacterial and yeast strains from explanted voice prosthetics. Within 4 hours of incubation, the precoated prosthetic could reduce the rates of initial adhesion by all the tested organisms [17]. Recently the authors have identified the use of BS containing solutions for prostheses cleaning aiming toward improving the prostheses life and directly benefit the implanted patients. In another study, BS was synthesized by *L. casei* subsp. *rhamnosus* CCM 1825 impregnated solution treated surface exhibited a reduction in the adhesion of *Klebsiella pneumoniae* cells by 50%, within 3 hours of incubation [46,47].

Two different studies have confirmed the role of BS produced by *S. thermophilus* dairy isolate against bacterial and yeast strains from infected voice prosthetics [17,48]. These findings confirm the potential of probiotic microbes, isolated from diverse human sites as formulations for the prevention of biofilm-associated NIs [43,49].

24.5.2 Biosurfactants with antibiofilm property

Dental caries is one of the major biofilm-associated infections initiated by the adhesion and proliferation of pathogens like *Streptococcus mutans*. The use of antimicrobial agents could result in the extermination of beneficial oral microflora in addition to the possibility of resistance development. The use of BS secreted from various beneficial microbes like *Lactobacillus* is found to have a potent effect against these biofilm-associated infections and is considered an effective strategy against biofilm-linked DAIs. *L. acidophilus* BSs were shown to downregulate the genetic factors for *S. mutans* adhesion to tooth surfaces [50]. Dalili et al. [51] identified a novel BS from *Corynebacterium xerosis* strain NS5 (named as "coryxin") which exhibited inhibition and eradication of biofilm by *S. aureus*, *S. mutans*, *E. coli*, and *P. aeruginosa*. Similarly, biofilm production by the fungal pathogen *Candida albicans* strains was hampered by BS from *Lactobacillus* sp. CV8LAC [52].

Based on the roles played by BS on biofilms they can be considered as a "necessary evil for biofilms." BSs are necessary for the production of biofilms. Some BS, on the other hand, operate as strong chemicals that hinder biofilm formation [53]. The present focus of this section is on the diverse array of BS with antibiofilm properties.

24.5.2.1 Lipopeptide biosurfactants as antibiofilm agents

One of the largest classes of BS with the antibiofilm property is lipopeptides (LPs). Chemically they are composed of 3 or more variations of homologous or congener particles consisting of members like surfactins, polymixins, fengycins, and fusaricidins [54,55]. Structurally, LPs have a hydrophilic peptide moiety attached to the hydrophobic lipid and fatty acid group which is aliphatic branched, or cyclic. The majority of the LPs with the potent antibiofilm property are isolated from *Bacillus* or *Paenibacillus* [56–58].

24.5.2.2 Polymyxins

Polymyxins are the secondary metabolites of *Bacillus* or similar species, formed as a group of nonribosomally synthesized cyclic LPs with the typical cyclic structure attached to a fatty acid tail or sometimes amino acids of bacterial origin [57]. Polymixins have been reported for complete inhibition of biofilms by *P. aeruginosa* over 24-hours duration at a dose of 20 μg/mL [59]. Another variant of polymyxin (Polymyxin D1) has been found operative against the polymicrobial biofilm mode of growth [58]. In addition, this BS was also effective against biofilms produced by Gram-positive: *S. aureus*, *S. bovis*, *Bacillus subtilis*, and *Micrococcus luteus*, and Gram-negative (*P. aeruginosa*) pathogens. The mechanism of action for the antibiofilm potential of polymyxins remains undefined; it is speculated to do with their high affinity for lipopolysaccharides [60]. Elevated surface charges due to the lipopolysaccharide (LPS) aggregation may lead to internalization and induce leakage of cellular components in microbes [60].

24.5.2.3 Fengycin-like lipopeptides

BSs derived from *B. subtilis* and *B. licheniformis* mainly constitute Fengycin-like LPs. Structurally, they are cyclic LPs containing 8 to 10 microbial amino acids attached to a fatty acid. These BS are reported to exhibit biofilm inhibition and eradication of *S. aureus* and *E. coli* biofilms, respectively [61].

24.5.2.4 Putisolvin

They are putisolvin I and II, which are cyclic lipodepsopeptides obtained from *Pseudomonas putida*. Both the forms are four membered cyclic peptides where putisolvin I contain valine residue instead of the leucine or isoleucine in putisolvin II [62]. They were shown to be efficient in biofilm suppression and dispersal by other *Pseudomonas* sp. strains, in addition to a substantial role in biofilm development by *P. putida* [63].

24.5.2.5 Pseudofactin

Pseudofactins are cyclic lipodepsipeptides derived from *P. fluorescens*, where palmitic acid is attached to the end amino acid peptide chain. Another form of Pseudofactin; Pseudofactin II, is reported to be potent against the biofilms of five different microbial species formed on different surfaces like glass, polystyrene, and silicone. Among these strains are *E. faecalis*, *E. coli*, *S. epidermidis*, *E. hirae*, and *P. mirabilis*. At dosages of 0.5 mg/mL, *C. albicans* and yeast biofilms had a comparable impact [64]. Pseudofactin is also documented with potent eradication property of preformed biofilms of *E. hirae*, *E. coli*, *E. faecalis*, and *C. albicans* to urethral catheters made of silicone [64].

24.5.2.6 Surfactins

It is one among the important BS that separated from *B. subtilis* consisting of a cyclic heptamer peptide attached to a beta-hydroxy fatty acid chain of 13−15 carbons. Indiscriminately, surfactins found to cause cytotoxicity and hemolysis due to the interaction with the cellular membranes [65]. In addition to the reported antiadhesion property, biofilm generation by a variety of therapeutically important pathogens on urinary catheters was shown to be inhibited by surfactants [66]. This antibiofilm effect might be due to the mechanism of hampering with cellular permeability probably through the formation of channels and pores [67].

24.5.2.7 Complexes of lipopeptides

Lipopeptides are usually a group of similar compounds that have characterized as pure compounds only for experimental purposes. Such a kind of complex lipopeptides was extracted from heavy metal tolerant *B. cereus* and reported by Sriram and coworkers in 2011 [68]. In addition to the significant antibiofilm property exhibited by this complex polypeptide, it was tolerant of extreme conditions like pH, temperature, and high osmolality due to heavy metals. Another complex BS includes a mixture that consists of polymyxin D1, fusaricidin B, and trace amounts of surfactin [58]. Laboratory testing of such preparation at a dose of 2 mg/mL was found to inhibit the biofilm formation in both Gram-positive and Gram-negative pathogens [58]. More interestingly, this combination of lipopeptides exhibited approximately 99% of biofilm inhibition and around 74% of preformed biofilm eradication [34]. Lipopeptides were combined with other conventional treatment

strategies like antibiotics and tested for the possible synergy and effects of inhibition [34]. Lipopeptides separated from *B. licheniformis* strain V9T14 were put to the test against uropathogens in synergy with diverse antibiotics and some of the combinations resulted in 100% biofilm removal, according to the researchers in comparison to the inhibitory effect exhibited by the antibiotics by its own [69].

24.5.2.8 Rhamnolipids

Rhamnolipids are BS with mono or di rhamnose fatty acid chains with sugars connected. Rhamnolipid was originally separated from *P. aeruginosa*, its analogs were isolated from *Burkholderia* as well [70], *Renibacterium salmoninarum*, *Cellulomonas cellulans*, Nocardioides, and *Tetragenococcus koreensis* [71]. Rhamnolipid is reported to have significant antimicrobial effects, however; their biofilm inhibiting property was not well documented. Rhamnolipid is found to have notable antibiofilm properties against *Bordetella bronchiseptica* [72]. They were found to disrupt preformed biofilms by *B. pumilus* on polystyrene surfaces [73]. Rhamnolipids were tested to eradicate preformed biofilms by *P. aeruginosa* PAO1 with reduced adherent microbes [46]. Approximately, 66% of the reduction in *S. salivarius* and *C. tropicalis* adhesion was observed in silicone rubber surfaces that were conditioned with rhamnolipids. In addition, rhamnolipids could reduce the initial adhesion (4 hours) by *S. epidermidis*, *S. salivarius*, *S. aureus*, and *C. tropicalis* by 48%. Rhamnolipids seem to be effective eradication agents (approximately 67%) for biofilms of *Yarrowia lipolyticaon* glass surfaces and found to be more effective than other surfactants like cetyl-trimethyl ammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) [73].

24.5.2.9 Sophorolipids

Candida yeasts are responsible for the production of Sophorolipids, a glycolipid BS substance, containing sophorose sugar dimer and a lengthy chain fatty acid. Joshi-Navare and Prabhune [74], have reported the synergy between sophorolipids and antibiotics on the effect of disrupting the biofilms of *E. coli*. Two days old *B. subtilis* biofilms that grown on coverslips after sophorolipid treatment (5% v/v concentration) exhibited significantly reduced biofilms in comparison with the untreated biofilms.

24.5.2.10 Other glycolipids with antibiofilm properties

Glycolipids comprising glucose and palmitic acid generated by the marine *Serratia marcescens* have been reported to be efficient against biofilms formed by *C. albicans* and *Pseudomonas aeruginosa* PAO1. This effect was evident with preformed biofilms of *C. albicans* and *P. aeruginosa* PAO1 on polystyrene microtitre plate [34]. Similarly, other glycolipid complexes synthesized by *Brevibacterium casei* MSA19 have been effective against both individual and mixed polymicrobial biofilms [75]. Plant-derived complex glycolipids: hydroxyproline-rich glycopeptides *Datura stramonium*, have also found to possess significant antibiofilm activity against multidrug resistant *C. albicans* [76].

24.5.2.11 Complex surfactant mixtures

Natural BSs are rarely originated in pure forms. They are generally seen in association with components that share similar physical and/or chemical characters. Due to which their purification is both extensive and/or financially not viable [34]. However, these BS combinations may have a greater

advantage than the individual pure components. A BS combo like this was separated from *Robinia pseudoacacia* and *Nerium oleander*, which the attachment was hampered and biofilm formation of *C. albicans* on medical surfaces like silicon, voice, and prosthetic for dentures at different antibiofilm doses [34,77]. Gakhar and coworkers [77] extracted similar complexes of BS from probiotic bacteria *Lactococcus lactis* 53 and *S. thermophiles* which greatly hampered the adhered microbes from preconditioned voice prostheses inducing reduced airflow resistance due to the biofilm formation [34,77].

24.5.2.12 Biosurfactants from fungi with antibiofilm property

BSs like sophorolipids of fungal origin are also found to inhibit biofilm formation by different microbes of clinical importance. Sophorolipid BS produced by *C. bombicola* has significantly reduced biofilm formation by *V. cholerae* [18]. Other yeast strains like *C. sphaerica* have reported producing BS such as Iunasan, which inhibit the biofilm formation of *P. aeruginosa*, *S. agalactiae* and *S. sanguis* at significant amounts [78]. Similarly, BS; rufisan, extracted from *C. lypolytica* inhibited biofilm formation by *S. aureus*, *S. agalactae*, and *S. mutans* NS at doses approximately equivalent to 0.75 μg/mL [79].

24.5.3 Biosurfactant assisted surface modification to prevent device-associated infections

Significant efforts are needed to create polymeric coverings that are functional for devices which possess antiadhesive and/or limited biofilm production by inhibitory characteristics. and microbial eradication [32].

Fig. 24.2 represents currently available surface processing commercial technologies used for achieving medical surfaces with antifouling and biofilm inhibition properties. These methods try to incorporate active antifouling and/or biofilm inhibitory compounds that interact physically and/or chemically to inhibit biofilm development with the basic polymer. For modified medical devices to

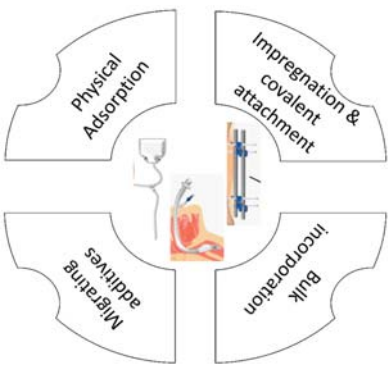

FIGURE 24.2

Current commercial processing methods for attaining antifouling and biofilm inhibition biosurfactant coated/impregnated polymeric surfaces.

inhibit biofilm formation and adhesion primarily should release active molecules that are impregnated into the polymeric substances. Physical absorption can be attained by submerging the medical services into the active agent-containing solution and allowing it to bind to the base polymer through chemical interactions. Thermo stable antibiofilm compounds shall be impregnated into the polymeric surfaces through melt processing, after swelling the base polymer. This method helps in the uniform distribution of the active antibiofilm compound to the surface. These methods shall be utilized for devices like urinary catheters. Bulk incorporation is another promising method used recently instead of melt processing which involves end functionalization or functionalization of the side chains or polymerization of monomers into the base of the polymer. Other industrial methods used for incorporating antifouling and antibiofilm compounds onto the surfaces include covalent attachment and migrating additives [32].

24.6 Conclusion

Biofilm formation on medical devices poses a significant threat for device-associated infections that are difficult to eradicate and often result in recurring infections and ultimately loss of functionality. The importance of the use of BSs in the health care field has been accumulating, due to the consequence of an arsenal of studies that are reported worldwide. However, the extraordinary cost associated with the identification, characterization, and standardization of the extraction process of BS is considered a huge constrain for their large-scale usage. Even though BS guarantee significant potential as antiadhesive, biofilm inhibition, and eradication of preformed biofilms in clinically relevant pathogens, the amount of data that is required suggesting for their usage in the medical field is lacking. Therefore, BS research aiming toward clinically relevant applications needs to be conducted. In addition, efforts should be taken to encourage studies that aim toward understanding the antibiofilm mechanism of BS to comprehend their fate in the human body. Such understanding will help to apprehend the interactions of BS with the host microflora, as well. Such results will confirm the usage of BS in biomedical and healthcare-related sectors to avoid biofilm-associated NIs. Significant efforts are needed to design functional polymeric surfaces or coatings for devices that possess antiadhesive and/or biofilm inhibition properties to inhibit the growth of biofilms and microbial eradication. All the same, there appears a great potential for BS in the healthcare sectors which needed to be exploited fully.

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CHAPTER

Biosurfactants for industrial applications

25

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25.1 Introduction

Surfactants are widely known as surface-active reagents that can be found in the food that we eat to hand sanitizers that we utilize to kill germs and in pills that we consume for ailment or general health. Their main role is to decrease the surface and interfacial tension between solids, liquids, and gases. Surfactants are amphiphilic which means that they have both hydrophobic and hydrophilic moieties in their structure [1–3]. Surfactants' different structures and properties enable their use in various areas such as detergent, paint, adhesives, and many more [3,4]. The market for surfactants is growing faster and is estimated to reach $52.4 billion by 2025 [5]. Surfactants can be classified based on having either synthetic/chemical origin or biological (biosurfactants) [1].

Chemical surfactants can be broadly classified into four groups based on their ionic properties: nonionic, cationic, anionic, and Zwitterionic as shown in Fig. 25.1 [1,3,4]. Nonionic surfactants, as the name suggests, do not dissociate or ionize in aqueous solutions. This is because of their nondissociative hydrophilic head, which could be of different hydrocarbon compounds like alcohol, ester, phenol, ether, and amide. Cationic surfactants dissociate into positively charged hydrophilic head (attached to a hydrophobic tail) and an anion (commonly halogen) in aqueous solutions, while anionic surfactants provide a negatively charged surface-active amphiphilic compound and a cation. The cation is usually an ammonium ion or an alkaline metal (such as K^+, Na^+). Zwitterionic or amphoteric surfactants are among a rare type surfactant as they possess both negative and positive charges on the same molecule [1,4]. Nonionic surfactants are commonly used in production areas like fiber, glass, dye, medicine, and food. Cationic surfactants are mostly used for corrosion inhibition, sterilization, rust, and many more. They are very effective for corrosion protection as the positively charged head readily gets adsorbed on surfaces that are negatively charged making a strong coating. Anionic surfactants can be found in detergents, dispersants, foaming agents, emulsifiers, etc. Zwitterionic surfactants are milder and less irritative than the other types of surfactants, therefore largely being used in personal care like shampoo, shower gels, and cosmetic products [3].

Synthetic surfactants are generally classified based on their ionic properties. Unlike synthetic surfactants, biosurfactants can have a rather complicated order of classification. They can be roughly grouped based on their molecular weight or microbial origin or chemical nature or other features [7]. Based on their molecular weight, biosurfactants are classified as Class I and Class II,

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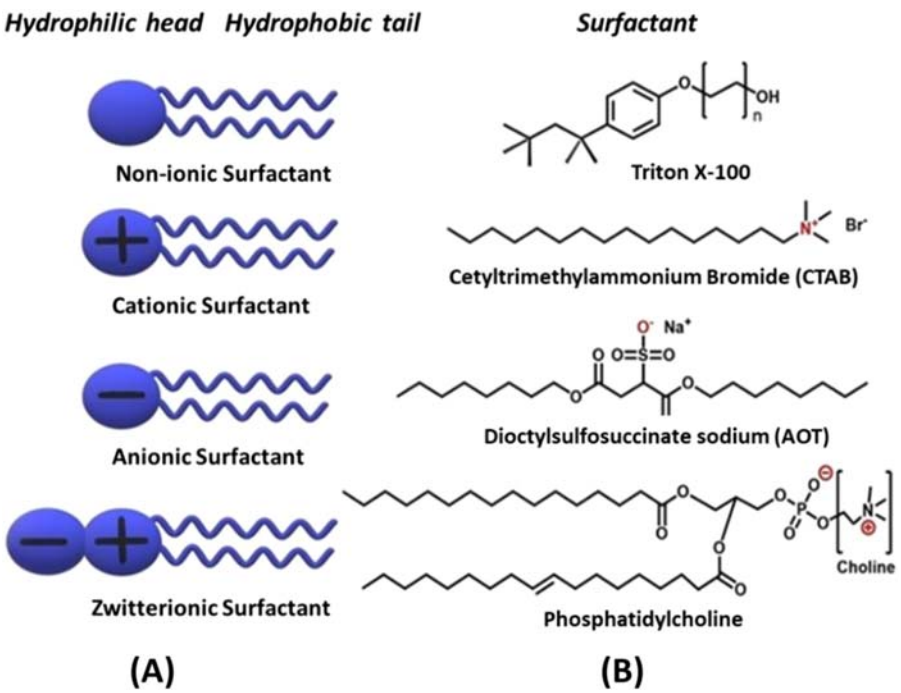

FIGURE 25.1

(A) Different types of surfactants and (B) corresponding examples of chemical structures [6].

where biosurfactants with low molecular weight fall in Class I and high molecular weight microbial surfactants are in Class II. The low molecular weight microbial surfactant group includes biosurfactant types like glycolipids, phospholipids, and lipopeptides. The Class II biosurfactant group contains microbial surfactants with high molecular weight like particulate and polymeric surfactants. These different biosurfactants are efficient in different areas of application; for instance, Class I biosurfactants are competent at minimizing interfacial and surface tension while class II biosurfactants are efficient at stabilizing emulsion [1]. Table 25.1 shows common examples of Class I and Class II biosurfactants. Rhamnolipids, sophorolipids, trehalolipids, and mannosylerithritol lipids are the best-known glycolipid biosurfactants. Carbohydrate linked to long-chain hydroxy aliphatic acids is a common characteristic of such biosurfactants [2]. It should be noted that rhamnolipids single-handedly are a big group of molecules with more than 60 congeners with different structures produced by different microorganisms [8]. One can expect various functionality in these microbial surfactants, different levels of efficiency, and suitability for multiple applications.

Conventional synthetic surfactants play an essential role in the modern industry and ranked the third highest in terms of mass production in the synthetic chemicals category. As a result, to keep up with the surfactant's supply and demand, the primary source of synthetic surfactants is petrochemical origin [9]. Biosurfactants are amphiphilic like any other conventional surfactant, but what sets them apart is their high surface activity, low toxicity, biodegradability, effectiveness at low

concentrations, and environmentally-friendly aspects which makes them very suitable for industrial applications including in biomedical fields [1,10,11]. Theoretically, biosurfactants or microbial surfactants can be produced in the presence of any hydrocarbon sources under favorable physicochemical conditions. This concept has brought scientists to utilize industrial wastes for biosurfactant production. However, there could be some complications when microorganisms are used for large-scale production. Microbial surfactant production faces difficulties in simulating favorable conditions for microbes to produce surfactants as part of their secondary metabolite, and challenges in delivering a finished biosurfactant product because of high-cost downstream processing. These are among many other difficulties [12]. Despite such complications, there is a continuous research effort directed toward biosurfactant production because of their prominence in different industries and their desirable qualities.

Biosurfactant global market witnessed a $1.7 billion market value in 2011 which increased to $3.99 billion in 2016 [13]. The statistics were projected to reach $5.52 billion by 2022. The Asia Pacific is witnessing the fastest-growing biosurfactant market. The leading companies in the biosurfactant industry are Jeneil Biotech (US), Ecover (Belgium), Evonik (Germany), among many others. Table 25.2 shows different global manufacturers who are focusing on various biosurfactant production for different applications. Despite all the interest and development in microbial

Table 25.1 Classification of biosurfactants and their common examples.

Biosurfactant group	Biosurfactant class
Glycolipids	Rhamnolipids, Trehalolipids, Sophorolipids
Fatty acids, sopholrolipids, and neutral lipids	Corynomyccolic acid, Spiculisporic acid, Phosphatidyethanolamine
Lipopeptides	Surfactin, Lichenysin
Polymeric biosurfactants	Emulsan, Alasan

Table 25.2 Global manufacturers of biosurfactants and their potential applications.

Manufacturers	Biosurfactant manufactured	Location	Niche
TeeGene Biotech	Lipopeptides & Rhamnolipids	UK	Biotechnology, medical (anticancer, antimicrobial, ingredients), pharmaceutical
Ecover, Belgium	Sophorolipids	Belgium	Household cleaning products, laundry, dishwashing, personal care
Jeneil Biotech, Inc.	Rhamnolipids	USA	Agriculture, antimicrobial, bioremediation, household, personal use
Saraya Co., Ltd.	Sophorolipids	Japan	Health and hygiene, food, environmental sanitation
Fraunhofer IGB	Glycolipids, Cellobiose lipids, MELs	Germany	Health, sustainable chemistry, environment
AGAE Technologies LLC	Rhamnolipids	USA	Pharmaceuticals, cosmetics, enhanced oil recovery, Personal careproducts, the reference standard for lab

surfactant technologies; it is no hiding that the cost of biosurfactant production is three to ten times more than chemical or synthetic surfactant production [13]. Although biosurfactants are expensive, their biocompatibility and their efficiency at reducing surface and interfacial tension make them very suitable for biomedical applications. Biosurfactants are being used in the petroleum industry; cosmetics; antimicrobial and medicine; and bioremediation [13,14]. Microbial surfactants are particularly known for biomedical and biotechnological applications due to their nontoxic and biodegradability. Some biosurfactants have shown potential as an alternative to viral vectors for gene transfection. Some microbial surfactants have been demonstrated in reducing biofilm formation by infectious microbes [13].

25.2 Materials and methods for biosurfactants

Glycolipid biosurfactants such as rhamnolipids and sophorolipids have gained much attention in recent years for their potentials in large-scale production due to their high fermentation yields [15,16]. On the other hand, lipopeptide biosurfactants are generally known for their low volume production but are considered a high-value product for their outstanding biological properties [17]. Daptomycin for example is a clinically approved lipopeptide antibiotic for its strong antimicrobial activities [16,17]. To better illustrate the differences among these biosurfactants, here are some examples. Lang et al. had demonstrated a rhamnolipid production yield of 112 g/L from a starting raw material of soybean oil (160 g/L) [18]. The production cost of such rhamnolipids was estimated as $5 kg with its potentials in environmental cleanup. Rhamnolipids with 95% purity, on the other hand, produced by AGAE technologies, LLC has an estimated market price of $1500 g [16], recommended for use in cosmetics or pharmaceutics [19]. Additionally, in the case of lipopeptide, their manufacturing costs range from $10,000 to $15,000 g [16].

These examples are used to compare three general concepts: first, high-volume low value, as in the case of Lang et al.'s rhamnolipid; second, high purity and high value in the case of AGAE technologies, LLC's rhamnolipids; and third, low yield high value in lipopeptide's production. These indicate that the different biosurfactants are suitable for application areas based on their purity and potentials [17]. Despite all the differences, the universal commonality among different biosurfactant production is that when it comes to its commercialization efforts, continuous research is geared toward obtaining biosurfactants with low toxicity, improved quality, and economically feasible production [10,16,20–22]. Here are some of the common strategies discussed for the enhancement of quality and yield of rhamnolipids [10]. The five general subtopics will be used as a framework for discussing general approaches toward improving biosurfactant production.

25.2.1 Exploring cheap sources/substrate

There is an interdependent relationship between the biosurfactant producer, the microorganism; the substrate on which the microorganisms grow, like the hydrocarbon sources; and the conditions established while they are put through the fermentative process, like a bioreactor [22]. Biosurfactant production being a multistepped process, reduction of the cost of biosurfactant production at every step is desirable for its feasibility. One of the many ways in cutting the cost of raw

materials is by utilizing suitable industrial wastes that are rich in lipids or carbohydrates. Some of the commonly used industrial waste products in biosurfactant production are (1) animal fat; (2) soap stocks; (3) frying oil; (4) olive oil mill effluent; (5) corn steep liquor; (6) whey; and (7) starchy substrates [12]. To put things into perspective, here are the percent breakdown of the different sectors contributing renewable substrates in biosurfactant production: the oil industry constitutes 35% of renewable substrates used in biosurfactant production followed by agro-industrial wastes at 20%, dairy products at 18%, food industry contributes 15%, and industrial waste contributes 12%. [23]. Fig. 25.2 illustrates an example of a cheap source, cooking oil employed as a substrate for the production of lipopeptide surfactin (high-performance liquid chromatography confirmed surfactin) biosurfactant and its application in heavy metals removal [24].

25.2.2 Manipulating/fine-tuning the manufacturing conditions

25.2.2.1 Carbon source

Different species of microorganisms thrive on various carbon sources or a mixture of different carbon sources. For example, *Torulopsis bombicola* was able to obtain only low yields of biosurfactant in the presence of only either glucose or vegetable oil; however, when both glucose and

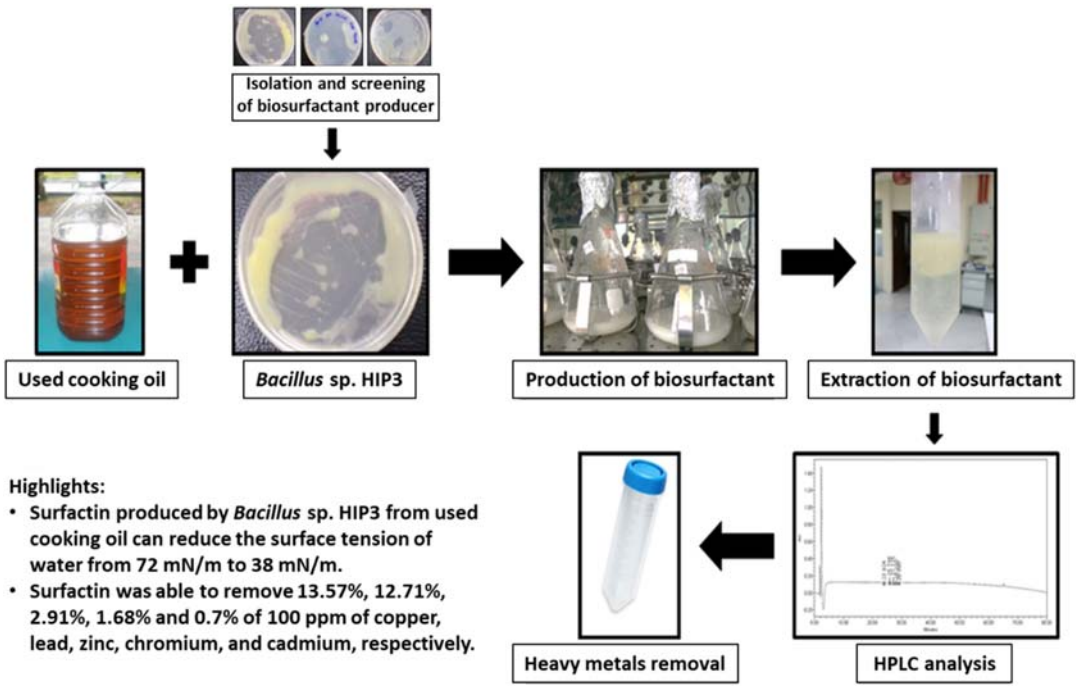

FIGURE 25.2

From raw material, used cooking oil through production and extraction to applications in heavy metals removal [24].

vegetable oil carbon sources were present, there was a higher yield of biosurfactant production [12]. This indicates that the nature of the carbon source affects the quality and quantity of biosurfactant production [17].

25.2.2.2 Nitrogen source

During a typical fermentation process for biosurfactant production, the carbon to nitrogen ratio affects biosurfactant productivity. High carbon to nitrogen ratios and carbon to inorganic phosphorous for instance enable high volumetric productivity for rhamnolipid biosurfactant production [22].

25.2.2.3 Solid-state and submerged fermentation

Two general fermentation types are employed in different biosurfactant production: Solid-state fermentation and submerged fermentation [25]. In solid-state fermentation, biomolecules are produced by microorganisms that are grown on a solid support. This usually takes place in the presence of limited water or the absence of water. This fermentative technique not only generates biosurfactants but also other biomolecules that are used in fields like biofuel, pharmaceuticals, and food industries. The submerged fermentation process takes place in excess of water [26]. These two processes have their advantages or disadvantages over each other [25,27,28].

25.2.3 Exploring nonpathogenic microbial strain that produces natural products

In microbial surfactant production, the pathogenic nature of some strains of biosurfactant-producing microbes can be anticipated. Even though there are numerous well-documented nonpathogenic biosurfactants producing microbial strains, continuous attention needs to be given toward screening for microorganisms that can not only produce biosurfactants with better surface-active characteristics but also produce nontoxic biosurfactants for industrial production [29].

25.2.4 Surveying improved low-cost separation and purification methods (multistep downstream processing)

The biosurfactant recovery/purification process is complicated; the purification cost of biosurfactant production depends highly upon whether the application of that biosurfactant requires a high purity grade or not [22]. In purification or the recovery processes, numerous purification methods have been used, such as precipitation methods like ammonium sulfate precipitation and acid precipitation; chromatography methods like ion-exchange chromatography, and froth chromatography; and other methods like solvent extraction [10,12,22]. Research in the biosurfactant field is continuously channeled toward achieving low-cost separation and purification methods that do not employ hazardous organic solvents [22].

25.2.5 Metabolic and cellular engineering for microbial strain improvement

In biotechnology, it is not simple to translate benchtop scale experimental success to a large-scale production, which is the same for biosurfactant production. The recent development in high-throughput technologies in computational biology and bioinformatics have enabled access to information for a

better understanding of complicated biological processes. Bridging the gap between this knowledge obtained from cutting edge technologies to designing new strategies for high yield and low-cost microbial surfactant production is highly sought after [22]. In the next section, industrial applications of biosurfactants in biomedical and biotechnology will be discussed.

25.3 Industrial applications of biosurfactant in biomedical area

Biosurfactants are natural surface-active reagents derived from microorganisms that help reduce the surface and interfacial tension. Efficient biosurfactants can obtain low critical micelle concentration (CMC). Above the CMC, micelle formation occurs and micelles affect the solubility of the nonpolar portion of solute in an aqueous solution. To understand this concept, a generic illustration of the micelle formation process is used in Fig. 25.3. If biosurfactant concentration (g/L) is thought of as the independent variable and the surface tension of the liquid (mN/m) as a dependent variable; there is a negative correlation between surface tension and biosurfactant concentration up to the CMC point. At the beginning of this process, the surface tension of the liquid is high with no monolayer of the biosurfactant molecules formed. Gradually, monolayers of biosurfactant molecules are formed until it has reached the CMC. At this CMC point, the biosurfactant molecules have helped achieve the lowest stable surface tension in liquid [12].

This functionality of biosurfactant where it can solubilize/encapsulate both polar and nonpolar molecules has a lot of positive implications on where they can be used. Biosurfactants find their applications in industries like petrochemicals, pharmaceuticals, mining, beverages, cosmetics, food, nanotechnology, textiles, and cleaning, etc. as shown in Fig. 25.4 [11,12]. For example, in the food industry, biosurfactants are being used as a food additive and preservative to modify the texture of food and preserve them [30]. Biosurfactants or microbial surfactants are also being utilized in heavy metal and hydrocarbon bioremediation. In the case of toxic hydrocarbon cleanup, the biosurfactants enhance the area of the hydrophobic part of the substrate which can then attract microorganisms or other living forms that utilize contaminants as nutrients [1]. In the introduction,

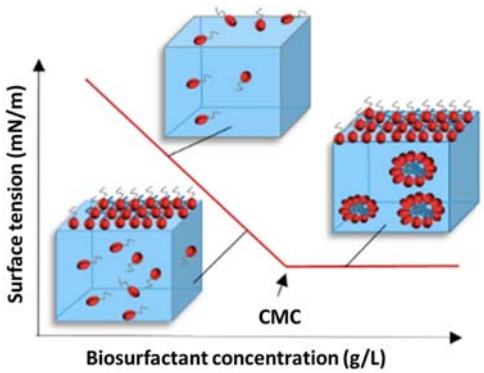

FIGURE 25.3

Typical micelle concentration concept [12].

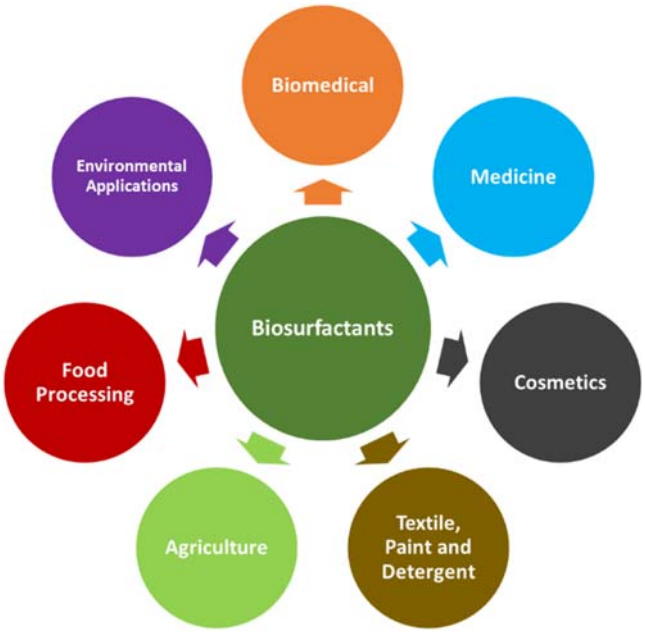

FIGURE 25.4

Primary industrial applications of biosurfactant [23].

the classification of microbial surfactants and their properties were discussed. In these following sections, industrial or commercial biomedical applications/potentials of different biosurfactants will be explored. It is noteworthy to state again that, diversity of biosurfactant groups makes it difficult to generalize their applications because of their diverse features and hence, their diverse surface activities [31].

25.3.1 Biosurfactants for antimicrobial activities

There are two general areas of concern in the scientific community when it comes to antimicrobial drug discovery: firstly, searching for effective drugs for novel pathogenic microbe and for existent pathogens that are showcasing antibiotic resistance. These concerns have brought much attention to the use of biosurfactants for antimicrobial applications because of their strong biological activities and clever structures. There is a consensus on how biosurfactants have the potentials in being used as a safe and effective alternative to the present synthetic commercialized medicine available in the market [32]. Glycolipids and lipopeptides, which are part of biosurfactants with a low molecular weight group, are important types of biosurfactants not solely for their various commercial applications, but also for their commercial therapeutic potentials [22,31,33]. There are numerous patents filed in biosurfactants' potential commercial usage for their antimicrobial activities. However, the fact remains that their actual applications in the pharmaceutical and biomedical industries are relatively restricted. One of the examples of commercial success of a biosurfactant in the antibiotic

drug category is a cyclic lipopeptide Daptomycin. Daptomycin was first produced by Cubist Pharmaceuticals (Cubicin) which was approved for skin infection treatment in 2003 [12].

What is the biosurfactant's general mode of action when it comes to antimicrobial activities? The simplified answer is that these microbial surfactants possess membrane permeabilization properties that enable them to disrupt the original microbial cellular membrane which then eventually leads to cell lysis and cell death of the microbes [32,34]. To put it in other words, the relevance of microbial surfactants in antimicrobial applications is generally because biosurfactants can damage the cell wall or the plasma membrane of the microbial cell. Another general factor for biosurfactant's antimicrobial activities is that sometimes the fatty acid part of biosurfactant gets introduced into the cell membrane causing several changes in the original cell structure [35,36]. What investigators know so far about the antimicrobial activity of rhamnolipid, for example, is that the microbial cell membrane is the proposed site of rhamnolipid's antimicrobial activities [37]. In general, to understand the effectiveness of these biosurfactants in antimicrobial activities, minimum inhibitory concentrations (MICs) is determined against the target bacterial culture, [37] as shown in Fig. 25.5. A lower MIC score indicates the effective antimicrobial functionality of the antimicrobial agent. Fig. 25.5 showcases decent bacterial inhibition facilitated by rhamnolipids and sophorolipids. A point to be noted here is that sodium dodecyl sulfate (SDS) is a well-known detergent used for denaturing proteins with moderate toxicity and is not recommended for ingestion [38]. On the other hand, the biosurfactants have potentials in industrial biomedical applications for their antimicrobial activities because they're relatively safe owing to their biological origin [32]. Additionally, the fact that some biosurfactants like surfactin exhibited lower MIC than a polypeptide like a polylysine by five times has positive implications in terms of economic feasibility as less amount of such potential antimicrobial reagent will be needed for commercialization [39].

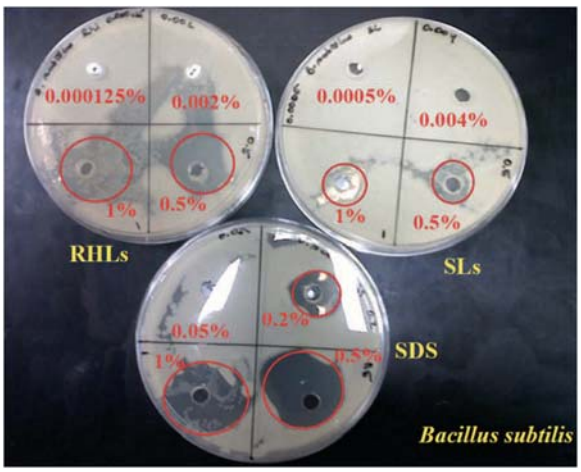

FIGURE 25.5

Illustrates the antimicrobial activities of rhamnolipids (RHLs), sophorolipids (SLs), and synthetic anionic surfactant (SDS) at different concentrations employed against *Bacillus subtilis*. The concentrations are below and above the CMC and the red circles show that the *B. subtilis* bacterial culture in those circled zones are cleared due to these surfactants [32].

Microbial surfactants have not been discussed much when it comes to their antifungal properties against pathogenic fungi that affect human [32]. However, glycolipid biosurfactant groups like sorpholipids, mannosyl-erythriol-lipids, and cellobiose lipids are involved in broad-spectrum antifungal activities against plant-based pathogens, making them useful in agriculture for plant protection [34]. Lipopeptide biosurfactant was investigated for their antifungal and antibacterial activities [40]. Fig. 25.6 shows the effect of lipopeptide biosurfactant with different concentrations on test organism *Botrytis cinereal*, a pathogenic fungus. With the increase in the concentration of lipopeptide biosurfactant, an increase in the inhibition zone diameter can be observed. Table 25.3 sheds light on antibacterial and antifungal activities of lipopeptide biosurfactant against microbes like *Micrococcus luteus, Salmonella typhimurium, Staphylococcus aureaus, B. cereus, Escherichia coli, B. cinerea, Fusarium moniliforme, Colletotrichum truncatum*, and *Penicillium candidum*. With the disk diffusion method's help, investigators were able to look at the effect of different concentrations of lipopeptide biosurfactants on different microbial organisms. *B.cinerea* exhibits the largest diameter of inhibition zone due to lipopeptide biosurfactant, among all the other test organisms. When lipopeptide biosurfactant was of 0.8 g/L concentration, the zone of inhibition diameter was 38.2 mm in *Botrytis cinereal*, which then increased to 48.8 mm when lipopeptide biosurfactant concentration was increased to 3.2 g/L [40].

Antiviral activities are reported for surfactin, sorpholipids, and rhamnolipids [32]. For instance, Vollenbroich et al. suggested that the antiviral activity of their biosurfactant surfactin could be due to physicochemical based interactions between the microbial surface-active surfactant and the lipid membrane of the virus [41]. With the help of an electron microscope, they were able to observe disruption of the lipid membrane of the virus facilitated possibly by surfactin (lipopeptide). Shah et al. proposed a hypothesis where they suggested that there is an inverse relationship between virucidal activity and the carbon chain length of sorpholipids [42,43]. Biosurfactants like rhamnolipids

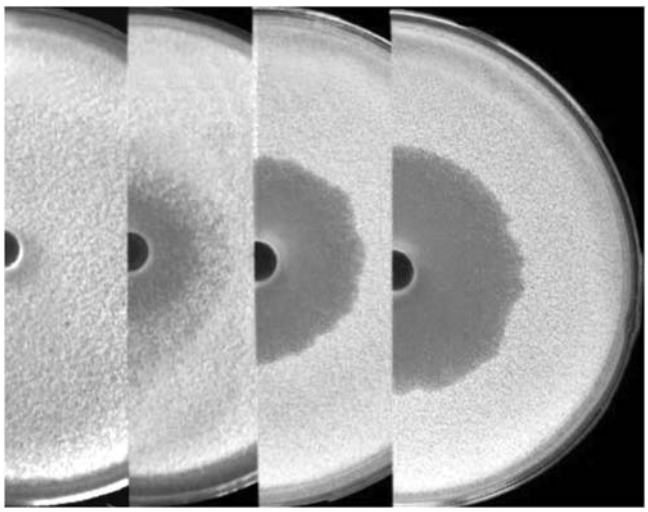

FIGURE 25.6

Lipopeptide exhibits antifungal activities against *Botrytis cinerea* [40].

Table 25.3 Increase in the concentration of lipopeptide biosurfactant shows an increase in the diameter of the microbial inhibition zone [40].

	Zone of inhibition diameter (mm)		
Microorganisms	**0.8 g/L**	**1.6 g/L**	**3.2 g/L**
Bacteria			
Micrococcus luteus	8.1	13.4	18.0
Salmonella typhimurium	7.4	10.3	12.0
Staphylococcus aureaus	7.7	9.0	11.4
Bacillus cereus	–	–	–
Escherichia coli	–	–	–
Fungi			
Botrytis cinerea	38.2	41.3	48.8
Fusarium moniliforme	16.5	20.1	28.4
Colletotrichum truncatum	6.7	7.3	11.4
Penicillium candidum	6.1	8.4	10.8
Gibberella zeae	–	–	–

"—" = no inhibition.

have shown antiviral activities toward herpes simplex type 1 and 2 viruses. Some sophorolipids have been claimed to have antiviral activities against the human immunodeficiency virus [32].

25.3.2 Biosurfactants for antibiofilm

It has not been too long since strategies for microbial inhibition were designed solely based on the physiology of free-living bacteria (planktonic bacteria). The problem here is that bacteria are not only in planktonic form but are also in biofilm. The physiology of planktonic bacterium is fundamentally different from microbial biofilms, as the biofilms have multicellular organism-like characteristics. These biofilms are formed by microorganisms on solid surfaces to protect themselves from external toxic components [44]. In the US, healthcare-associated infection loss amounted to approximately $28 − 45 billion per year. Out of such losses in the biomedical industry, 60% of it is happening due to medical equipment-related infections.

The medical equipment-related infections are connected to microbes inhabiting them as slimy layers of biofilm coatings are often seen on the surfaces of the medical devices, as shown in Fig. 25.7 [45]. These microbial biofilms are being formed on medical equipment such as catheters, heart valves, intrauterine devices, hip prostheses, and many more [32,36]. Biofilms are highly resistant to existing antimicrobial agents due to their resilient nature [36]. Therefore, the interest in biosurfactants in the biomedical industry, particularly in the area of biofilm prevention, dispersion, and/or inhibition is primarily due to multiple potentials biosurfactants exhibit in that area. Potentials such as biosurfactants' antimicrobial activities against pathogens and potentials in reducing the adhesion of biofilms on surfaces [36,44]. Here are some of the examples of biosurfactants used in fighting biofilms. Surfactins for instance have

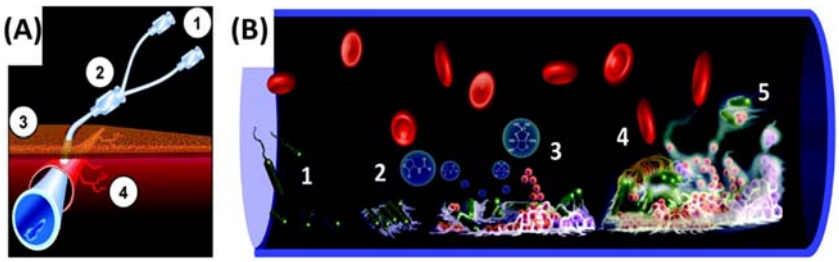

FIGURE 25.7

(A) Potential infection sources of a percutaneous intravascular device. Medical devices introduce a vulnerable biointerface into normally well-protected organs and vasculature. Contamination can come from (1) infusate (2) from nonsterile catheter materials, (3) the skin, or (4) from distant hematogenous infections. (B) Dynamic biofilm life cycle on a medical device: (1) transport and initial attachment of microbes, (2) irreversible adhesion or attachment, (3) microcolony formation, (4) maturation of the biofilm, and (5) detachment and dispersion of the cells.

Adapted with permission from A. Vertes, V. Hitchins, K.S. Phillips, Analytical challenges of microbial biofilms on medical devices, Anal. Chem. 84 (2012) 3858–3866. [45]. Copyright (2012) American Chemical Society.

reported stopping the bacterial biofilm growth present on polyvinyl chloride-based urethral catheters and microtiter wells [44]. Another interesting group of live microorganisms called probiotics and their derivatives have received a lot of research interest in combating biofilm. Such probiotics can be found in dairy products and fermented food. They have been reported to not only prevent the formation of pathogenic microbial biofilm but also target them by forming nonpathogenic good biofilms. Some probiotics are known to release biosurfactants in combination with other metabolites like hydrogen peroxide, carbon peroxide, and many others with potentials in antibiofilm [32,46].

25.3.3 Biosurfactants as antitumor/anticancer agents

Cancer is one of the main causes of death worldwide. According to the International Agency for Research on Cancer (IARC), 17 million new cancer cases, and 9.5 million cancer deaths happened worldwide in 2018. To give an overall perspective on the prevalence of different cancer types, here are the statistics provided by Miller et al. affiliated with the American Cancer Society [47]. According to the report, as of 2019 in the US, the three most common cancer types among the male population are melanoma of the skin; colon and rectum; and prostate. The three most common cancer types among the female population are colon and rectum; breast; and uterine corpus [47]. Increasing cost related to cancer treatment has multiple factors associated with it and one such factor is the increase in cancer prescription drugs [48]. Many different chemotherapy drugs are available for different cancer types and chemotherapy is the conventional method of cancer treatment. However, numerous researches have discussed the cytotoxic nature of chemotherapeutic drugs with a nonspecific manner of targeting not only the cancer cells but also other tissues in the body. In recent years, microbial surfactants have gained much attention for their potentials in the antitumor or anticancer biomedical field/industry because of their unique structures and desirable properties like biocompatibility, low toxicity, and biodegradability [34,49,50]. The proposed mechanism of action of surfactin biosurfactant is shown in Fig. 25.8. Surfactin can manipulate the lipid composition of the cell

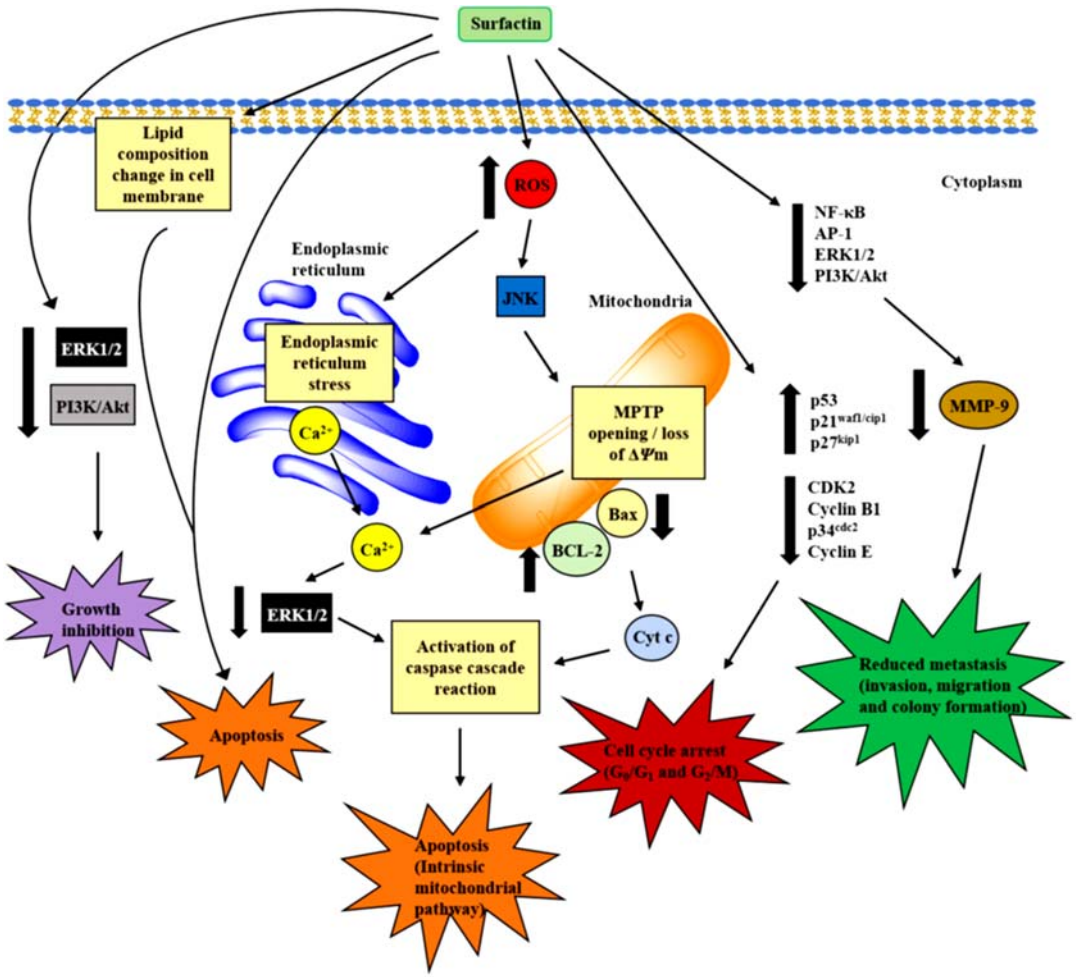

FIGURE 25.8

Proposed mechanisms involved in vitro anticancer activity of surfactin [49].

membrane of cancerous cells and disrupt them or get involved in programmed cell death of cancer cells, among many other proposed mechanisms of anticancer activities [49].

One of the early evidence that backed the biosurfactant's antitumor/anticancer activities was when Zhao et al. showed the activity of mannosylerythritol lipids, a glycolipid biosurfactant type in mouse melanoma which is chemotherapy-resistant cancer [51]. Other important biosurfactant types known for their potentials in antitumor activities are lipopeptide biosurfactants specifically, iturin, surfactin, and fengycin [50]. Out of those lipopeptide biosurfactants, surfactin, for example, has been shown to have anticancer activities on different cancer types like colon, breast, hepatoma, and leukemia [49]. It is suggested that the anticancer activity of surfactin-like lipopeptide is credited

toward the interactions between the nonpolar fatty acid part of the surfactin and the nonpolar acyl chain of the membranal phospholipids, while the polar peptide moiety of surfactin interacts with the polar portion of the cancer cells' lipid membrane [52,53]. Another lipopeptide biosurfactant type, Iturin A of marine bacterial origin for instance had been tested in vitro and in vivo for their anticancer effects on breast cancer. This is a promising result for the actual clinical development of such lipopeptide-based therapy for breast cancer in the near future for applications in the biomedical field/industry [54]. Fig. 25.9 shows the antitumor potentials of Daptomycin. Daptomycin as

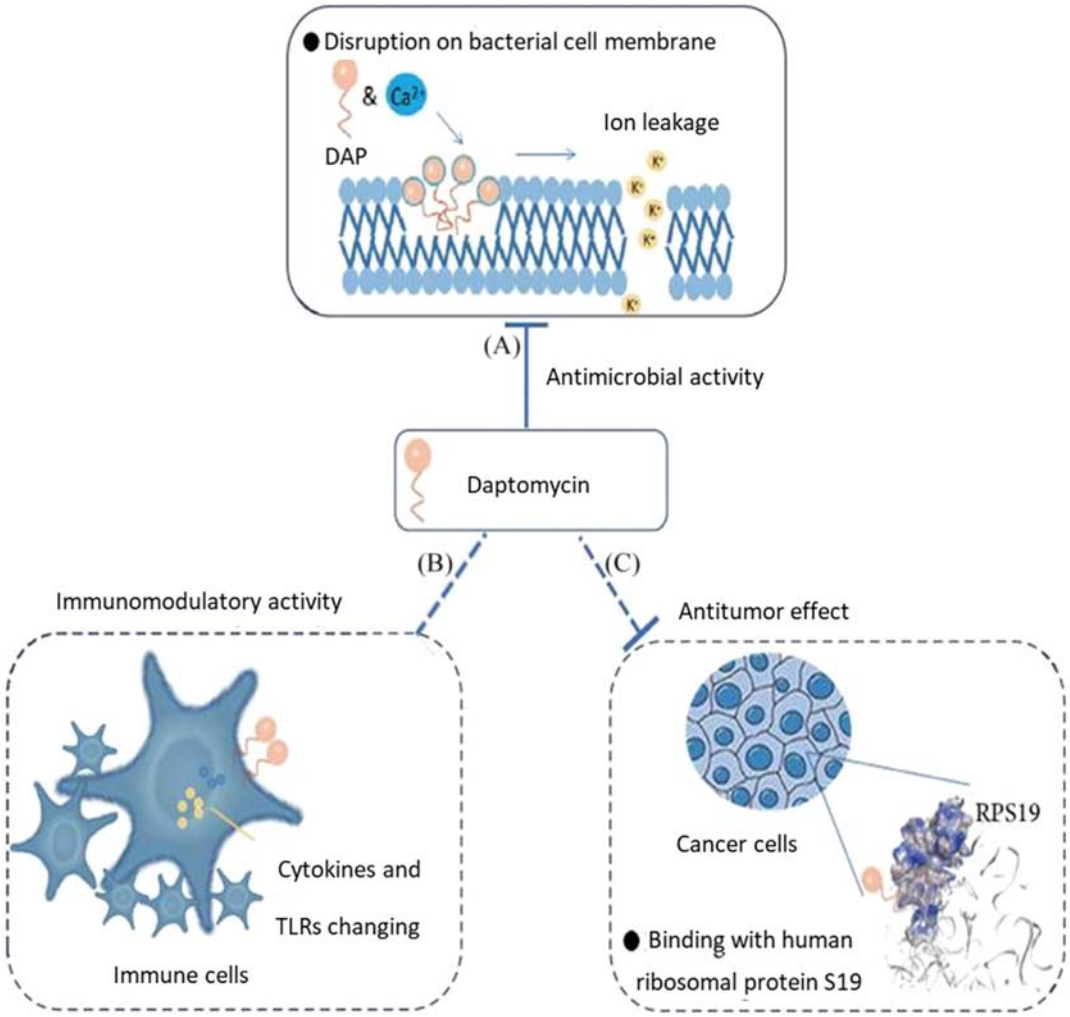

FIGURE 25.9

Multifunctional properties of commercial lipopeptide microbial surfactant daptomycin [55].

previously discussed is the first commercial cyclic lipopeptide antibiotic that works against infections caused by gram-positive bacteria. They not only show antimicrobial activity but are also associated with potentials in antitumor and immunomodulatory activities [55].

A biosurfactant synthesized from *Acinetobacter indicus* M6 showed a promising behavior for anticancer [56]. The effect of biosurfactants on the antitumor activity was studied using cells from lung cancer. The lung cancer cell viability was studied using different amounts of biosurfactant. Fig. 25.10 shows the effect of the amount of biosurfactant on cell viability for A541 (lung cancer cell line) and MCT-3 T3-E1 (nontumor cell line). The effectiveness of biosurfactants for antitumor activity is quite evident. Table 25.4 shows the numerous reports published in the past about lipopeptide biosurfactant surfactin and demonstrates its potentials in being an anticancer agent [50]. The surfactin biosurfactants in Table 25.4 are from the different microbial origins with a different mode of action against battling different cell lines of distinct cancer types. Different strains of *Bacillus*; a genus of gram-positive bacteria; synthesize surfactin that is effective against different cancer cell lines [50].

25.3.4 Potential applications of biosurfactants in immunomodulatory activities

Biosurfactants or microbial surfactants have potentials in broad-spectrum immunomodulatory activities where they can either help in activating or suppressing the body's immune system. Biosurfactants are widely known essential reagents with potentials in immunological adjuvant applications, among other immune modulation biomedical applications [69]. Our body inherently has a sophisticated immune system that helps in fighting infections caused by pathogens. The concept of vaccines is discussed to understand the role of immunological adjuvants. Vaccines lower the risks of infections by helping the

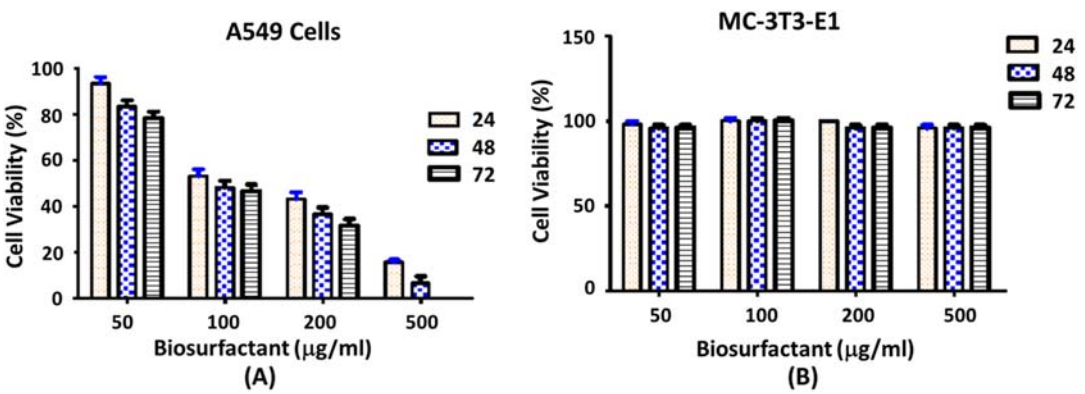

FIGURE 25.10

Cell viability study for (A) A549 cell lines (B) MC-3T3-E1exposed to different concentrations of biosurfactant at different time intervals.

Adapted with permission from Y. Kameda, S. Ouhira, K. Matsui, S. Kanatomo, T. Hase, T. Atsusaka, Antitumor activity of Bacillus natto. V. isolation and characterization of surfactin in the culture medium of Bacillus natto KMD 2311, Chem. Pharm. Bull. (Tokyo) 22 (1974) 938–944 [56]. Copyright (2020) Elsevier.

Table 25.4 Surfactin biosurfactant showcasing anticancer activities against different cell lines and their mechanism of action.

Cancer type	Cell line	Origin	Mode of action	References
Ehrlich ascites	Ehrlich ascites		Cytolytic activity	[57]
Colon	HCT-15 HT-29	*B. circulans* DMS-2	Growth inhibition	[58]
Colon	LoVo	*B. subtilis*	Growth inhibition Cell cycle arrest Apoptosis Induction	[59]
Breast	MCF-7	*B. subtilis* CSY 191	Growth inhibition	[60]
Breast	MCT-7	*B. subtilis natto* TK-1	Growth inhibition Cell cycle arrest Apoptosis Induction	[61–63]
Breast	MCF-7	*B. subtilis*	No growth inhibition or cytotoxicity Inhibition of invasion, migration, and colony formation	[64]
Breast	T47D MDA-MB-231	*B. subtilis* 573	Growth inhibition Cell cycle arrest	[65]
Breast	MDA- MB-231	*B. subtilis*	No growth inhibition or cytotoxicity Inhibition of invasion, migration, and colony formation	[64]
Cervical	HeLa	*B. subtilis* HSO121	Growth inhibition	[53]
Cervical	HeLa	*B. subtilis*	Growth inhibition	[66]
Leukemia	K562	*B. subtilis natto* T-2	Growth inhibition	[67]
Leukemia	K562	*B. subtilis natto* T-2	Cell cycle arrest Apoptosis Induction	[68]
Hepatocellular HCC	BEL7402	*B. subtilis* HSO121	Growth inhibition	[53]
Pancreatic	SW-1990	*B. subtilis* HSO121	Growth inhibition	[53]

Adapted with permission from M. Rofeal, F.A. El-Malek, Valorization of lipopeptides biosurfactants as anticancer agents, Int. J. Pept. Res. Ther. (2020) 1–9 [50]. Copyright (2020) Springer.

body's natural immune system grow immunity toward different infectious diseases. Billions of vaccines are administered yearly as preventive measures. Different vaccine types have been licensed in the past with new ones under development for various disease preventions. Here are examples of two primary vaccines: (1) subunit vaccines that contain only a part of disease-causing microbe, and (2) live attenuated vaccines that generally contain whole weakened microbes. Subunit vaccines are newer vaccine types and are relatively safer than traditional vaccines like the live attenuated vaccines. However, subunit vaccines come with reduced effectiveness and require immunological adjuvants. Immunological adjuvants in general are compounds that aid in boosting the immune response of the body to a vaccine. Immunological adjuvants could potentially reduce the cost of production of vaccines and could reduce vaccine-related side effects [70–72].

The potential role of biosurfactants in the immune system can be better understood by learning how the immune system of an organism is activated. The host organism cells, in general, possess membrane receptors working as sensors that recognize the pathogens or their components. These membranal receptors are responsible for activating the immune system in a multistepped process.

The immune cells (dendritic cells, neutrophils, macrophages) have pathogen recognition receptors (PRRs), with prominent Toll-like receptors (TLRs) that participate in recognizing pathogens like bacteria, viruses, fungi, and parasites. Biosurfactants are generally sensed by the TLR-2 receptors that play an essential part in the immune system [73]. Lipopeptide biosurfactant, for example, Daptomycin, participates in activating the immune response by interacting with the TLRs, as shown in Fig. 25.11 [55,73]. Here are some examples of the immunomodulatory activities of biosurfactants. In a study, the investigators concluded that biosurfactants could potentially help stimulate the immune system [69]. It was suggested that a high concentration of biosurfactants administered in laboratory animals aided in an animal's ability to resist bacterial infections [69]. When lipopeptide (herbicolin A and Iturin AL) microbial surfactants were mixed with conventional antigens, multiple beneficial properties were observed. Microbial surfactants were shown to not only aid in obtaining an improvement in antibody-mediated-immunity responses but also bring desirable properties like low toxicity and lesser side effects in their applications [74]. Surfactin's potential role in

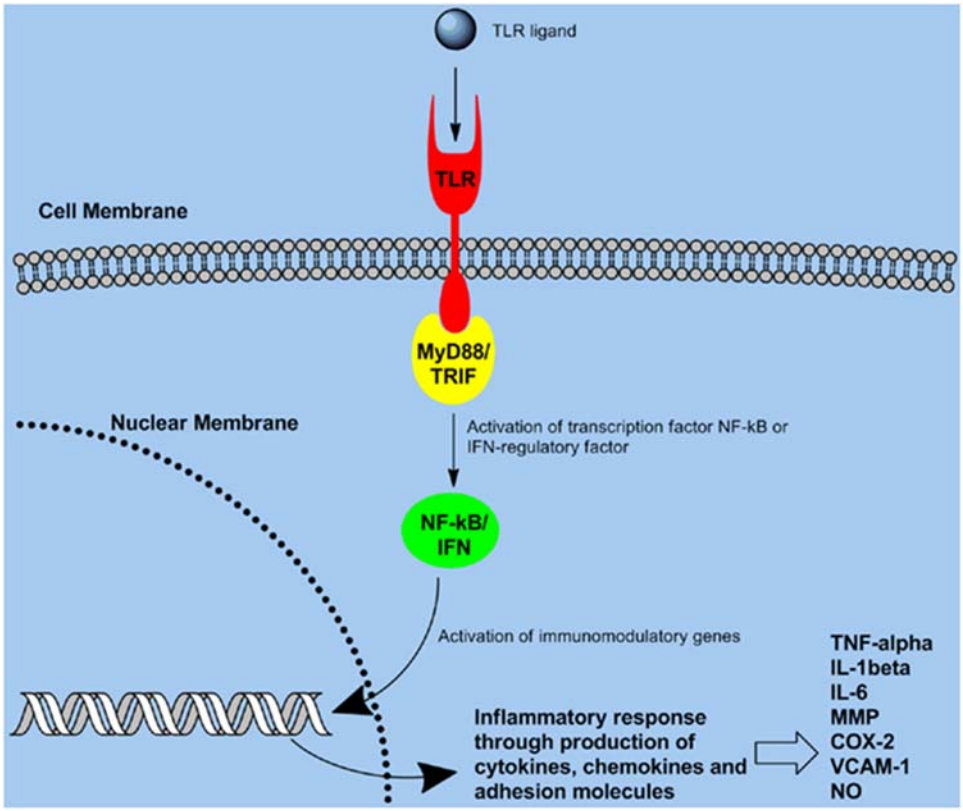

FIGURE 25.11

Summary of the Toll-like receptor (TLR) signaling pathway. Lipopeptides get involved in the TLR pathway that enables their proposed adjuvant activities [70].

autoimmune diseases and transplantation was discussed [75]. This is an example of a biosurfactant's role in suppressing the body's immune system. The surfactin biosurfactant derived from *B. Subtilis* (gram-positive bacteria) has immunosuppressive capabilities that have positive implications like potentials in therapeutic applications for autoimmune diseases like allergy, arthritis, and diabetes, and transplantation [75].

25.3.5 Potential applications of biosurfactant in gene transfection and drug delivery

Gene transfection is a method of introducing foreign genetic material into the target mammalian cells. This technique or technology is being employed not only in basic science like cellular and molecular biology but also in clinical applications like gene therapy [32]. In the area of gene therapy, there are numerous debates regarding the advantages and disadvantages of using viral or nonviral vector methods [76,77]. However, since the viral and nonviral method of gene transfection is not the scope of this chapter, the focus will be on the biosurfactant's role in aiding gene transfection. One of the early findings that suggested the potential use of biosurfactant in helping improve the efficiency of gene transfection was when mannosylerythritol lipid A (MEL-A) biosurfactant was shown to have a positive effect on gene transfection efficiency. The MEL-A, a glycolipid type biosurfactant improved the cationic liposome-mediated gene transfection efficiency in the mammalian cells by five to seven times compared to the control cationic liposome that did not contain MEL-A. Such results indicate biosurfactants' potential use in gene therapy applications and specifically nonviral gene therapy [78]. As shown in Fig. 25.12, it is suggested that the nano vector liposome (DOPE) with MEL-A biosurfactant can transfect the genetic material into the nucleus of the target cell via membrane fusion pathway [79]. MEL-A's advantageous surface-active properties aid in improving gene transfection efficiency in vivo and in vitro studies. On top of improving gene transfection efficiency, biosurfactants like MEL-A are preferable for such biomedical applications due to reduced unwanted immunological responses and reduced toxicity associated with their usage [80]. In Fig. 25.13, confocal laser scanning fluorescence microscopy (CLSM) images show the difference between NIH3T3 cells treated with cationic liposomes containing MEL-A biosurfactant and NIH3T3 cells treated with cationic liposome that did not contain MEL-A [81]. The CLSM images showed that NIH3T3 cells treated with cationic liposomes containing MEL-A biosurfactant had genetic material inside the nucleus of the target cell and liposome-DNA complex containing MEL-A present on the plasma membrane of the target cell. This indicates a successful in vitro gene transfection facilitated by a glycolipid-type biosurfactant.

In 1990, a patent was obtained for liposomes synthesized by two different types of rhamnolipids [19]. The rhamnolipid liposomes showcased desirable properties like biodegradability, high bioactivities, and low toxicities. These microbial surfactants exhibited their usefulness as microcapsules for proteins, nucleic acids, and drugs among many other applications [32]. Another unique biosurfactant type with desirable functionalities like sophorolipids is speculated to be useful in facilitating the entry of drug molecules. Sorphorolipid biosurfactants that are usually synthesized by yeasts are known to form micelles that can encapsulate drugs. This action is possible due to the interactions between the water-soluble drug and sophorolipid's hydrophilic head. As shown in Fig. 25.14, if the sophorolipid biosurfactant is administered with drug molecules, sophorolipids can help extend across the cellular phospholipid bilayer membrane and deliver the drug compounds inside the cell.

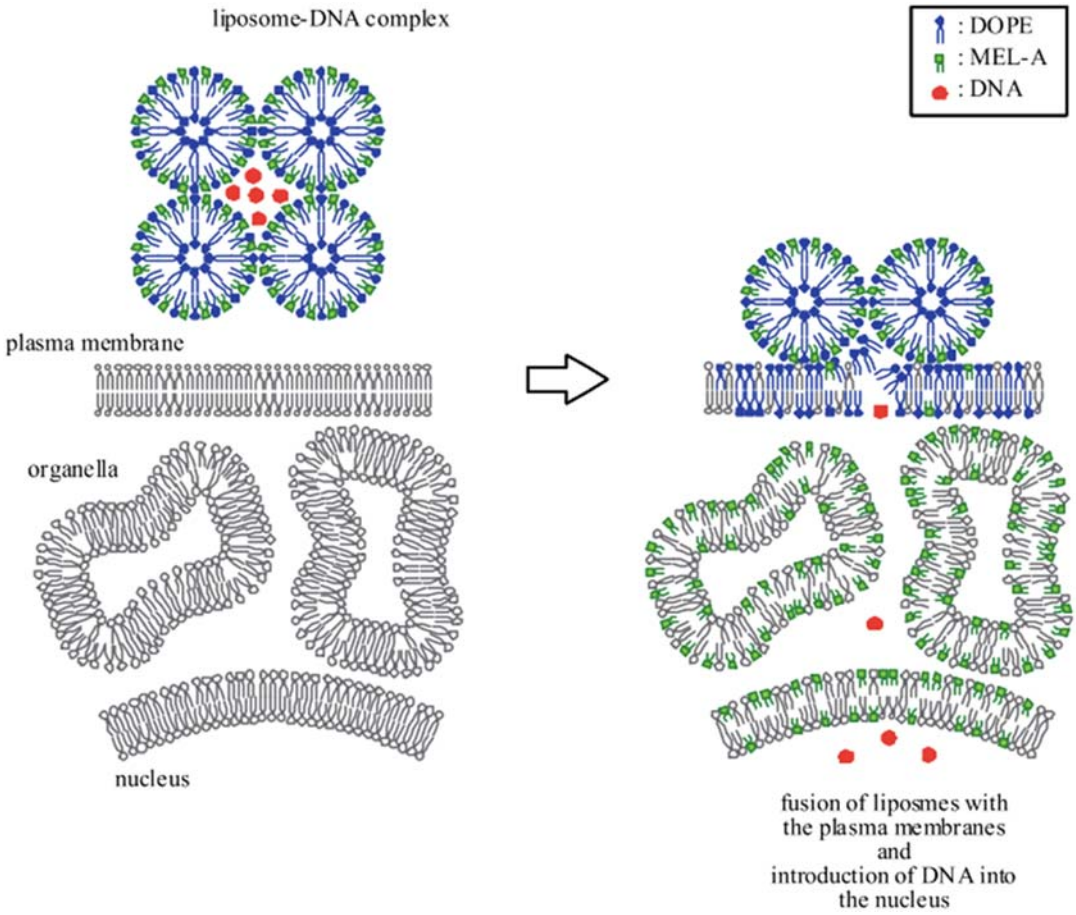

FIGURE 25.12

Schematic diagram of gene transfection by cationic liposome nano vector (DOPE) with MEL-A biosurfactant.

Adapted with permission from M. Nakanishi, Y. Inoh, D. Kitamoto, T. Furuno, Nano vectors with a biosurfactant for gene transfection and drug delivery. J. Drug Deliv. Sci. Technol. 19 (2009) 165–169 [79]. Copyright (2009) Elsevier.

This indicates that biosurfactants like sophorolipids can enhance the solubility of the drug molecules and drug delivery system in general [82].

25.3.6 Wound healing and dermatological applications

Our skin is the largest tissue of our body and it combats multiple external stressors like mechanical and chemical injuries and harmful ultraviolet rays. Skin wound healing is a complex multistepped process in which new tissues replace damaged tissues in our bodies. The wound healing process constitutes mainly four phases that include hemostasis, inflammatory, proliferation, and maturation phase. These

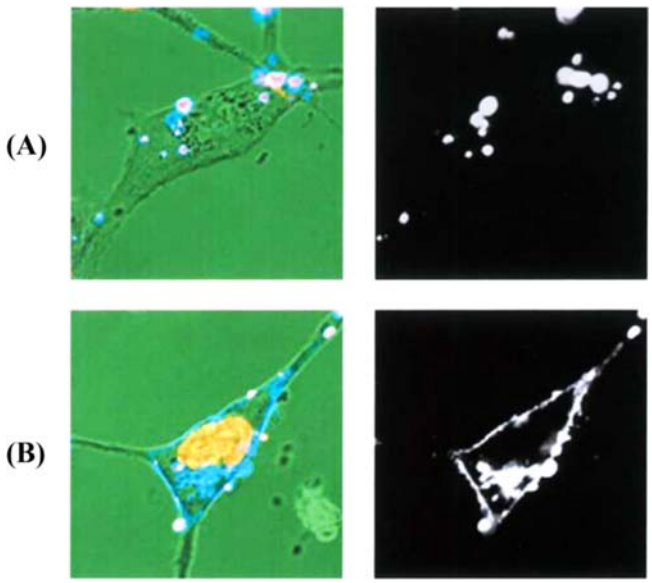

FIGURE 25.13

Confocal laser scanning fluorescence microscopy images of NIH3T3 (fibroblasts) cells (A) without biosurfactant MEL-A (glycolipid) after one-hour incubation time and (B) with MEL-A after one-hour incubation time (the yellow indicates DNA, pink shows liposome-DNA-complex, and blue indicates liposome) [81].

wound healing events are highly coordinated and precise biological activities. Multiple factors can disrupt the wound healing process, leading to improper wound healing [83,84]. Improper wound healing affects nearly 25 million people in the US constituting more than 7% of the US population. In 2014, the US medicare fund spent $35.3 billion on wound care-related expenditures [85].

Biosurfactants are potential candidates for many different biomedical applications, including dermatology and wound healing areas owing to biosurfactants' biological origin and desirable biological properties [33]. When an ointment containing glycolipid type biosurfactant was used on in vivo wound model Wistar albino rat, fast wound healing was observed with rapid collagen deposition in the wounded area, as shown in Fig. 25.15. Fig. 25.15 briefly describes the process of synthesizing and understanding the effect of a novel wound healing agent, a biosurfactant containing ointment. The biosurfactant producer *B. licheniformis* SV1 was isolated from oil-contaminated soil samples, and after extraction, the biosurfactant produced was confirmed to be glycolipid. Other than glycolipid biosurfactant type, lipopeptides are known to be useful in wound healing and dermatological applications [84].

Lipopeptide-based gel exhibited promising wound healing capability owing to its antioxidant, antifungal, and antimicrobial properties. In a study, rat models were used to understand the wound healing properties of lipopeptide gel [86]. The models compare the differences between untreated rat skin, rat skin treated with a conventional reference drug CICAFLORA, rat skin treated with glycerol, and rat skin with different lipopeptide-based gel quantities. Visual diagnosis of wound healing as shown in Fig. 25.16 showcases that out of all the experimental rat groups, groups treated

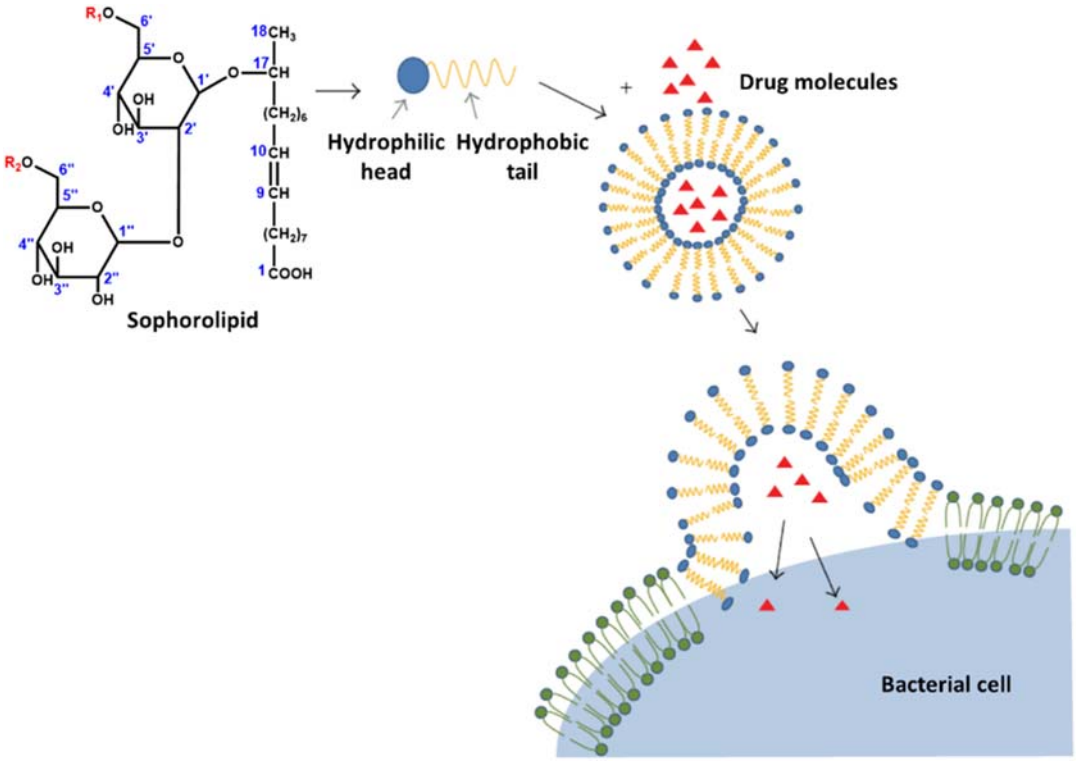

FIGURE 25.14

Diagram of the hypothesized mechanism of sophorolipid biosurfactant facilitated drug delivery [82].

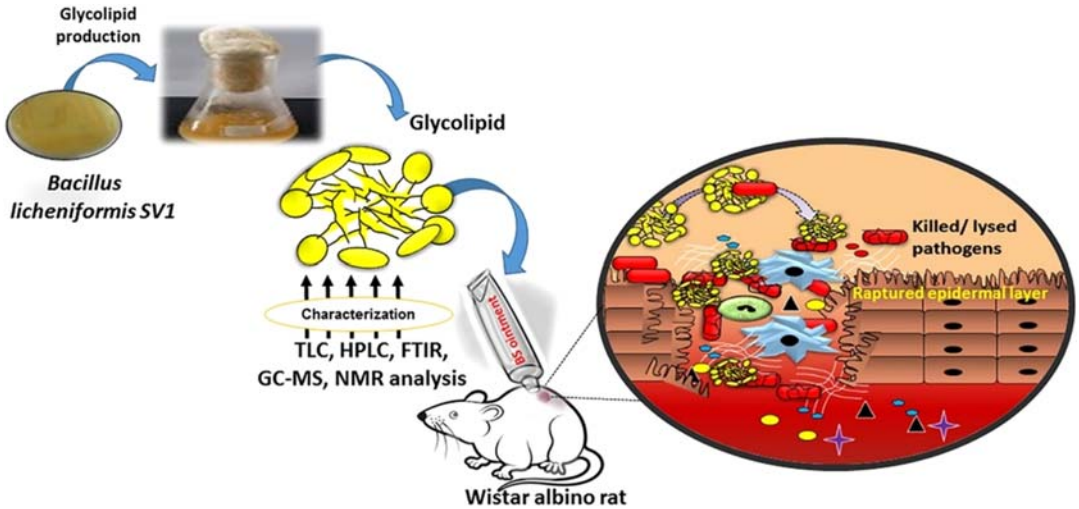

FIGURE 25.15

Synthesis of novel glycolipid and its wound healing properties.

Adapted with permission from S. Gupta, N. Raghuwanshi, R. Varshney, I.M. Banat, A.K. Srivastava, P.A. Pruthi, et al., Accelerated in vivo wound healing evaluation of microbial glycolipid containing ointment as a transdermal substitute, Biomed. Pharmacother. 94 (2017) 1186–1196 [84]. Copyright (2017) Elsevier.

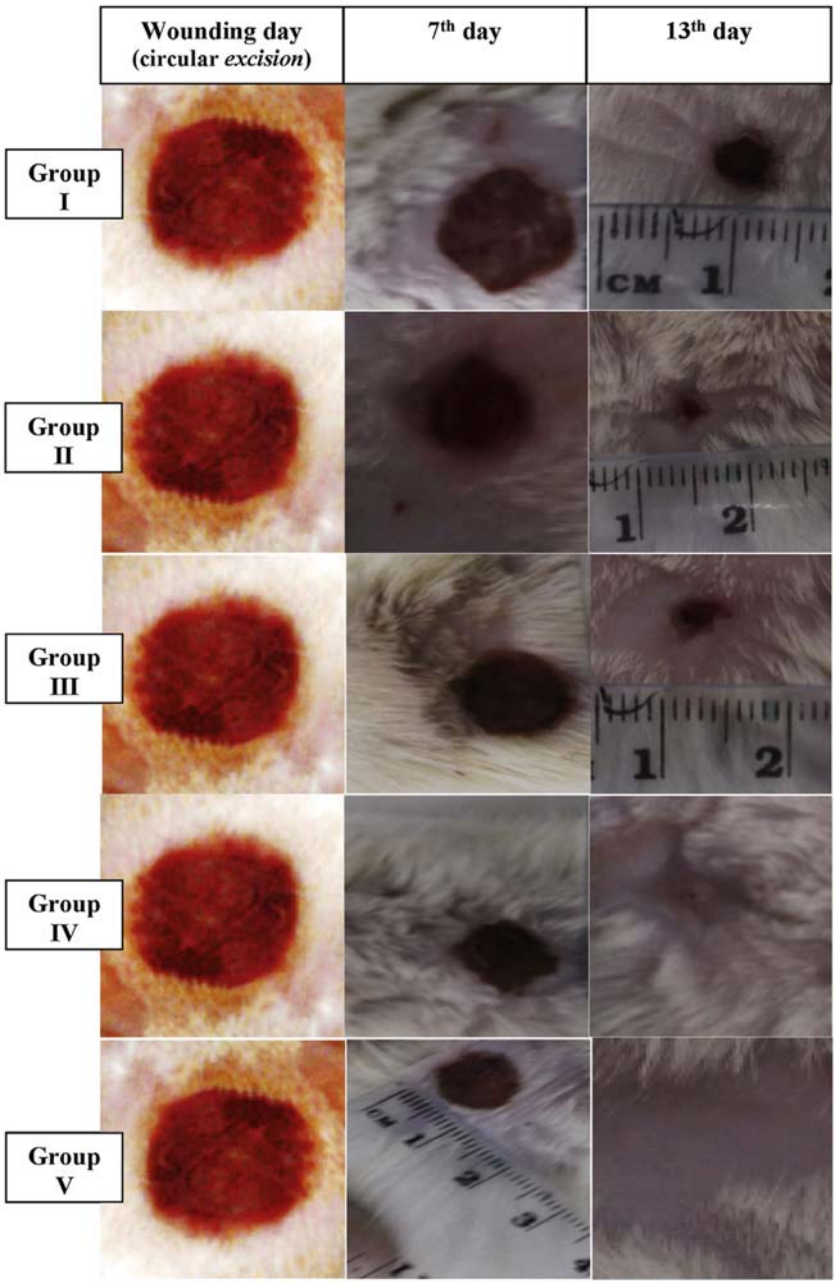

FIGURE 25.16

Visual appearances of rat model with induced excision wound (1.5 cm × 1 cm) at day 0, 7 and 13. Group I control untreated rat model skin; Group II treated with conventional drug CICAFLORA; Group III treated with glycerol (100%); Group IV treated with 5 mg/mL lipopeptide biosurfactant based gel; Group V treated with 15 mg/mL lipopeptide based gel [86].

Adapted with permission from R. Zouari, D. Moalla-Rekik, Z. Sahnoun, T. Rebai, S. Ellouze-Chaabouni, D. Ghribi-Aydi, Evaluation of dermal wound healing and in vitro antioxidant efficiency of Bacillus subtilis SPB1 biosurfactant, Biomed. Pharmacother. 84 (2016) 878–891 [86]. Copyright (2016) Elsevier.

with CICAFLORA and lipopeptide-based gel showed a clean wound area without inflammation [86]. Biosurfactant MEL-A was shown to leave positive effects on damaged, dry skin induced by SDS treatment. MEL-A shows potentials in being an effective skin moisturizer that can compete with natural ceramides. Natural ceramides are of low-yield and high production cost, while MEL-A could be produced cheaply with yeast and renewable substrates like vegetable oil [87]. These studies indicate excellent potentials for the usage of biosurfactants in general skincare and different skin-related issues.

25.4 Conclusion and future perspectives

Multiple biosurfactants are seen in the market for different applications; however, biosurfactants' full potential usage has not been fully realized. Many global companies are producing biosurfactants like rhamnolipids and sophorolipids for skincare, detergency, and food additives. There are multiple reports discussing biosurfactants' undeniable potentials in biomedicine and biotechnology. One of the highly discussed concerns regarding their unrealized potentials is that structurally different biosurfactants are produced by the same microbe, making it difficult to analyze their specific properties and mechanism of action in biomedicine. Another concern is that more optimization and better biosurfactant production strategies are required to reduce the cost of production and improve the yield and volumetric productivity of biosurfactants. Although multiple limiting factors hinder large-scale biosurfactant production, further exploitations of these microbial surface-active reagents could be highly profitable and beneficial in different industries.

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CHAPTER

Antitumor and anticancer activity of biosurfactant

26

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26.1 Introduction

Surfactants are organic molecules that bind to each other in a spontaneous manner to form sealed bubbles [1]. These compounds decrease the surface tension, that is, tension force between two interfaces that may exist between surfaces of two different liquids, between surfaces of a liquid and a gas, or between the surfaces of a liquid and a solid [2]. Surfactants have found many practical applications as emulsifiers, foaming agents, antifoaming agents, deinking of papers wetting agents, washing, and enzymatic process and as dispersants [3]. Surfactants besides being used as cleaning agents have also found their use as stabilizers for colloidal suspensions. They also found their uses in different products like adhesives, detergents, fabrics, emulsions, soaps, paints, inks, antifogs, etc. [4]. Further, surfactants show a lot of applications in agricultural formulations such as herbicides, pesticides, biocides, etc. in addition to personal care products like shampoos, cosmetics, toothpastes, etc. [5]. One of the interesting features of surfactants is reducing in thickness of crude oil. The surfactant when present in low concentration on the surface of a system adsorbs over the surfaces or interfaces of the system [6]. This adsorption of surfactant on the surface of a system alters the different properties of surfaces and their interfacial free energies [7]. The structural composition of surfactants showed that they are amphiphilic, that is, consists of hydrophobic as well as hydrophilic groups, and because of this nature they become very useful in daily life [8]. The hydrophilic part of the surfactant interacts with a solution or hydrophilic phase and hydrophobic part to the oil phase [9]. The surfactants are cationic, anionic as well as neutral (Fig. 26.1). Surfactants are when dissolved in water, organize themselves in a manner to produce micelles and lyotropic liquid crystals at their high concentration [10]. These properties of surfactants are very important according to research purposes. The structure of some surfactants is given in Fig. 26.2.

These surfactants besides being synthesized chemically are also produced by certain microorganisms. The surfactants produced by microorganisms are biooriented, hence defined as biosurfactants [11]. The biosurfactants are less toxic and more biodegradable as compared to chemically synthesized surfactants [12]. These biosurfactants are mostly produced extracellularly and on little scale intracellularly as well. Microbial biosurfactants can also be produced by taking immiscible liquids in surfactants growing medium [13]. Some microbes produce biosurfactants on large scale and are of commercial interest. Some of the biosurfactants are bile salts present in the small intestine, lecithin found in egg yolk, soybean,

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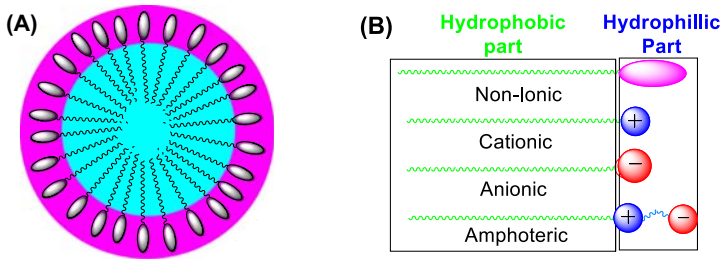

FIGURE 26.1

(A) Micelle formation by surfactants, (B) General structure of nonionic, cationic, anionic, and amphoteric surfactants.

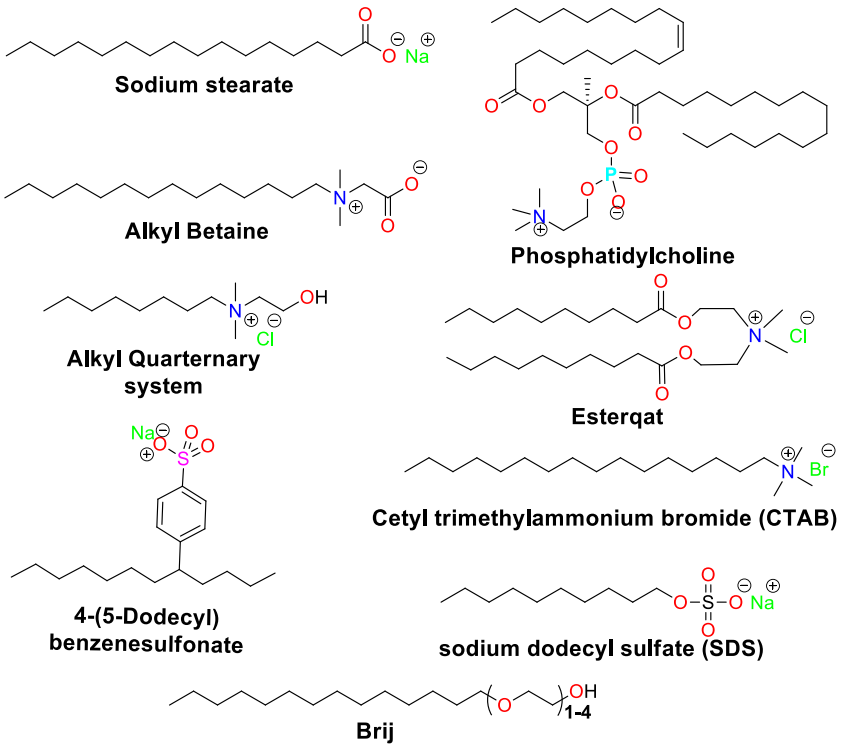

FIGURE 26.2

Chemical structure of few surfactants.

Emulsion, Sophorolipids, Rhamnolipids, etc. [14,15]. The biosurfactants have found their applications in herbicide and pesticide formulations, healthcare and cosmetics, food industries, Uranium ore processing, and mechanical dewatering of peat. One of the potential applications of biosurfactants is their use in environmental protection, that is, oil spill remediation and enhanced oil recovery [16]. The emulsification of

hydrocarbons (oil) is enhanced by biosurfactants that lead to an increase in the solubility of hydrocarbons contaminants and make them more available for microbial degradation [17]. Chemical structures of some important biosurfactants are shown Fig. 26.3.

Because of significant biological responses, biosurfactants have found a lot of industrial applications and therefore are of great commercial interest, and because of this, there is an increase in the study of their biological and physicochemical properties. The basic working of biosurfactant is based on its property of reducing surface tension, which is simply measured by drop collapsing and oil displacement tests. The standard concentration of biosurfactants considered to reduce surface tension between air and water (pure) is 72 to 32 N/m. The surface tension of water can also be reduced by surfactin, rhamnolipids, sophorolipids, and mannosylerythritol lipids (Table 26.1).

Concerning the emergence of novel viral and bacterial diseases during the past few decades, there has been less production of antibiotics to fight these pandemics. In cases, the medicines are present or discovered diseases are becoming resistant to these and making situations more

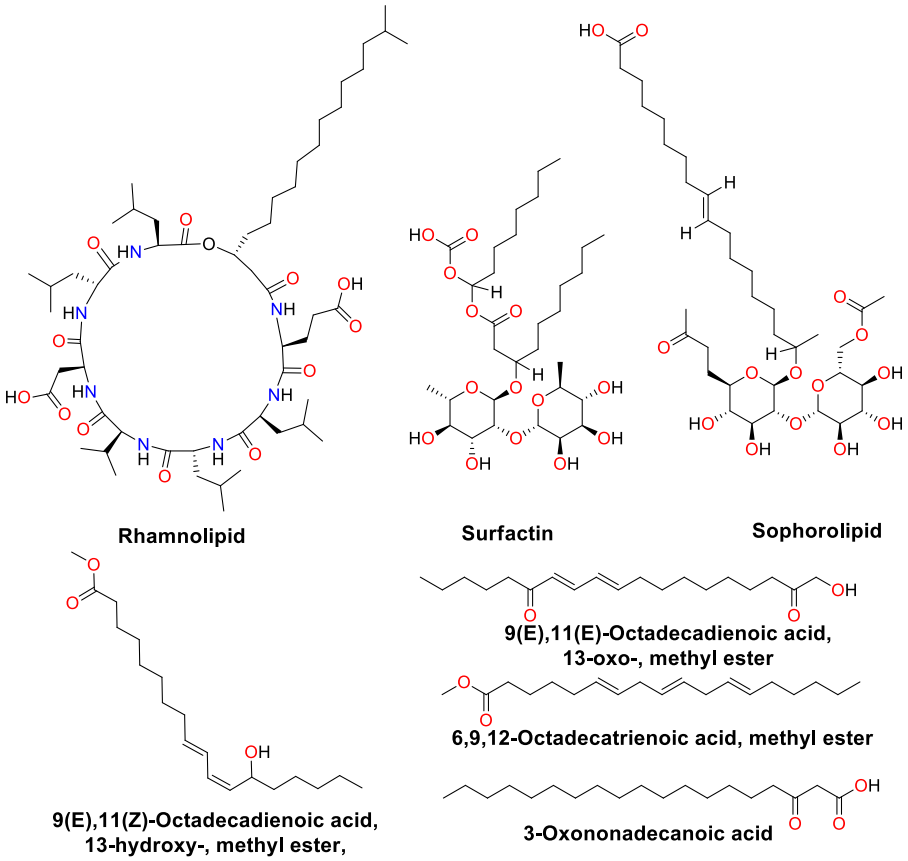

FIGURE 26.3

Chemical structure of some of the biosurfactants.

Table 26.1 Some biosurfactants and their concentration range required to reduce surface tension between air and pure water.

S. No.	Biosurfactant	Concentration range	References
1	Surfactin and rhamnolipids	72−30 mN/m	[18]
2	Sophorolipids	72−27 mN/m	[19]
3	Mannosylerythritol lipids	72−33 mN/m	[20]

Table 26.2 Biosurfactants with their sources and functions that are perform in the body.

S. No.	Biosurfactants	Source	Functions	References
1.	Bile salts	Small intestine	Absorption through small Intestine	[21]
2.	Lecithin	Soybean/egg yolk	Important food ingredient	[22]
3.	Pulmonary surfactant	Type II alveolar cells	Alveolar size regulation and many more	[23]
4.	Sophorolipids	Nonpathogenic yeasts	Biomaterial applications	[24]

critical. The emerging field of biosurfactants and their use as medicines can be a good alternative in controlling these pandemics. The biosurfactants have also been found useful in biological functions occurring naturally in the body (Table 26.2). In this chapter, we will discuss the potential applications of biosurfactants in the medical field and in developing an alternative effective therapy for treating patients, with the main focus on their antitumor and anticancer activity. Biosurfactants have the potential to be used as an antimicrobial agent, effective therapeutic agent, binding to heavy metal, cell adhesion, cell aggregation, the pathogenesis of bacteria, quorum sensing, producing antibiofilm, and antimicrobial compounds. Because of antifungal, antibacterial, antiviral, and antiadhesive properties biosurfactants are a good alternative to antibiotics used against disease caused by food-borne pathogens. The diverse use of biosurfactants in the biomedical field is shown in Fig. 26.4.

The various types of cancer are rising across the world with time. One of the big problems in the treatment of these cancers is the use of synthetic drugs that show the toxic effect on normal cells also. Another problem is the use of these synthetic drugs is their efficacy. A lot of studies have shown a less toxic effect of biosurfactants as anticancer and antitumor agents. As we know that the process of apoptosis results in the malignant cancer, however biosurfactants perform the apoptosis specifically in cancer cells or affect them by inhibiting their growth. Studies reveal that biosurfactants mannosylerythritol lipid obtained from glycolipids showed apoptosis and inhibited the growth of mouse melanoma cells [25,26]. A biosurfactant namely, monoolein showed antiproliferative activity against cervical and leukemia cancer cells [27]. Christovata and his team have shown that a biosurfactant trehalose lipid showed antiproliferative activity against the human cancer cell which further lead to the death of these cancer cells [28]. In this study, they isolated biosurfactants from Nocardia farcinica strain BN26 and the cancer cells studies were BV-173, HL-60,

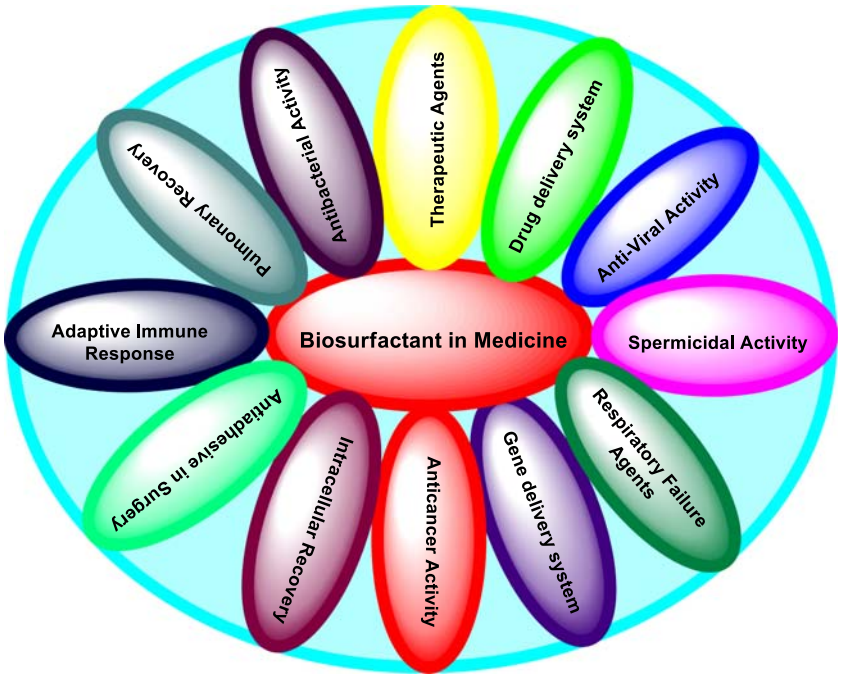

FIGURE 26.4

Biological activity of biosurfactant multimedicinal fields.

KE-37, HL-60/DOX, and JMSU-1. A lot of studies reported for biosurfactants have shown their ability to control various functions of mammalian cells and hence act as antitumor agents. Further, these biosurfactants have found their applications in intercellular molecular recognition like cell differentiation, signal transduction, and cellular immune response, etc. [25–29].

It has been observed from various studies that biosurfactants show apoptosis and can be used in the differentiation of various cancer cells. One such example was studied by Matsuyama and his coworkers in 2011 in which serratamolide AT514 was found to be used as a powerful catalyst in apoptosis of various human tumor cells and lymphocytic leukemia cells [30]. Waghmode et al. have been recently synthesized biosurfactant from *Planococcus maritimus* (SAMP MCC 3013) which showed cytotoxicity on cancerous cell lines of HeLa, MCF-7, and HCT with IC_{50} values of 41.41 ± 4.21, 42.79 ± 6.07 and 31.233 ± 5.08 μg/mL, respectively [31]. Similarly, Zhao et al. have been found that lipopeptide obtained from *B. subtilis* also shows antitumor effects by inhibiting human breast cancer cells by disruption of protein Kinase B [32]. The mechanism of protein disruption was discussed by Dey and his coworkers in 2015 [33]. Zhao et al. have been investigated to provide a good interpretation of studied lipopeptide which can completely kill K562 cells by increasing reactive oxygen species (ROS) in K562 cells (Fig. 26.5) [34]. It has been found that the surfactant (iturin) has inhibited the cancer cells like alveolar adenocarcinoma (A549), breast cancer cells (MDA-MB-231) [33] renal carcinoma (A498), and colon adenocarcinoma (HCT-15) [35].

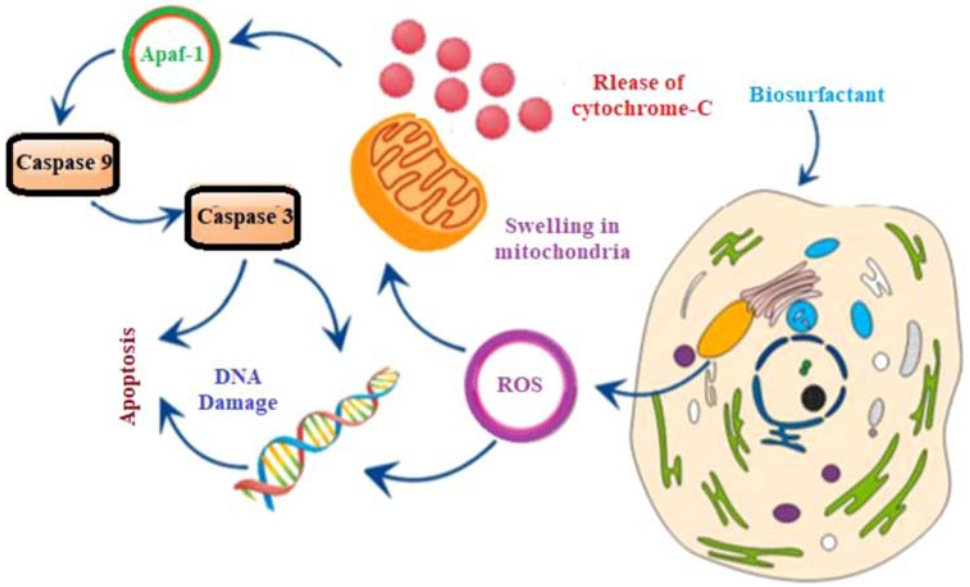

FIGURE 26.5

ROS/JNK-mediated mitochondrial/caspase pathway. Reactive oxygen species, Cyto-C, Apaf-1 [34].

Similarly, another biosurfactant (surfactin) has found critical in suppressing TPA-induced breast cancer cell invasion and human colon carcinoma cells [36] by inhibiting metallopeptidase-9 (MMP-9) matrix expression [37]. It was also observed that it uses ROS/c-Jun N-terminal kinase (ROS/JNK)-mediated mitochondrial/caspase pathway to kill MCF-7 (a breast cancer cell line) [25]. Another important surfactant named fengycin has shown repression of lung malignancy cell (95D) and restrained the development of 95D cells (xeno-united) in bare mice [38].

We have been arranged the current chapter based on the action of surfactants on various types of cancer cells.

26.2 Anticancer and antitumor activity of biosurfactants

26.2.1 Breast cancer

Surfactin, a biosurfactant has found its use in repressing many types of cancer cells. For example, Lee et al. have been reported that surfactin, when extracted from *B. subtilis* (CSY 191) can repress MCF-7 cells (breast cancer cells) with N IC50 value of 9.65 μM. High surfactin production was seen on cofermentation of CSY 191 stain with fermented soybean paste. The compound obtained after cofermentation showed 4−5 times more cytotoxic effect as compared to the normal one [39]. Similarly, the surfactin obtained from *B. subtilis* 573 was used significantly for inhibition of MDAMB-231 and T47D at 72 and 48 hours, respectively [40]. Abedali and his coworkers extracted surfactin from *B. safensis* F4, have shown antitumor activity against breast cancer cells (T47D) with IC50 value of 0.66 mg/mL [41].

Liu et al. have been studied the cytotoxicity of surfactin extracted from *B. subtilis* Hs0121 on Bcap-37 (the human breast cancer cells). It was found that it showed cytotoxicity at IC50 value of about 29 ± 2.4 μM nearly after 24 hours [42]. Meena and his team have been extracted surfactin from *B. subtilis* KLP2015 that showed cytotoxicity against mammalian breast cancer cells MCF-7 with a death percent of 78.91% ± 2.09% [43]. Cao et al. have been found that surfactin obtained from strains of *B. subtilis* TK-1 showed antiproliferative activity against MCF-7 cancer cells with IC50 value of 14.8 μM after 72 hours [44]. The surfactin extracted from *Micromonospora* marina (marine actinomycete) has been found to act as a potential anticancer agent against breast cancer cells. The cytotoxic activity was exhibited by surfactin on human breast cancer cells by process of apoptosis, and increasing ROS therefore, cleaving potential of mitochondrial membrane [45].

Iturin A, a biosurfactant which has been extracted from *B. megaterium* (marine bacteria) by Dey and coworkers [33]. Iturin A is a cyclic peptide whose chemical structure can be predicted as Asn-Tyr-Asn-Gln-Pro-Asn-Ser which are connected by C16 lipid chain. This biosurfactant is found to act as antitumor agent against breast cancer cells. It inhibits a central oncogenic protein that promotes the development of cancer (AKT signaling pathway) [46,47].

26.2.2 Melanoma cells

In the elaboration of the study performed by Abedali and his coworkers, the abstraction of surfactin from *B. safensis* F4, besides antitumor activity against breast cancer cells (T47D) also showed the same activity against melanoma cells (B16F10) of a mouse with IC_{50} of 1.17 mg/mL [41]. Zhao et al. have been utilized a biosurfactant named Mannosylerythritol lipid (MEL), which was extracted from the yeast, which inhibited growth in tumor cells by the process of apoptosis [26].

26.2.3 Colon cancer

Meena and his team extracted surfactin from *B. subtilis* KLP2015 that showed cytotoxicity against mammalian cancer cells HCT-15 with cell death percent of 80.1% ± 1.92% [43]. The surfactin was acted as an antiproliferative agent against LoVo colon cancer cells at IC50 value of 26 μM after 48 hours. During the action of surfactin, the apoptotic effect was seen that was observed by studying modification in apoptosis degree, change in morphology, fracture of DNA, and cell cycle in administrative protein. The treatment of surfactin to infected cells produced 40% apoptotic cells and a cell cycle arrest in 10% G0/G1 phase. Further, it was observed that surfactin can suppress phosphorylation levels of PI3K/Akt signaling. It was seen subsequently that cell cycle arrest occurred via apoptosis induction and inhibition of cell survival signaling [36,48].

Cheng and his team have been extracted fmbJ-fengycin from *B. subtilis* which showed high potential as an anticancer agent against HT29 (colon cancer cell line). Several experiments were used to investigate its anticancer effect which includes ROS method, apoptosis, and apoptosis-related protein levels in this cell line. The published work has been shown that Fengycin represses the progress and development of this cancer by interrupting the process of apoptosis through Bcl-2/Bax pathway [49]. This activity occurs by simultaneous inhibition in cell proliferation, cell apoptosis followed by ROS generation, and arresting cell cycle at G1 phase. In one of the studies a type of iturin, that is, Bacillomycin D has induced apoptosis in mammalian cancer cells HCT-15 (Fig. 26.6) [35].

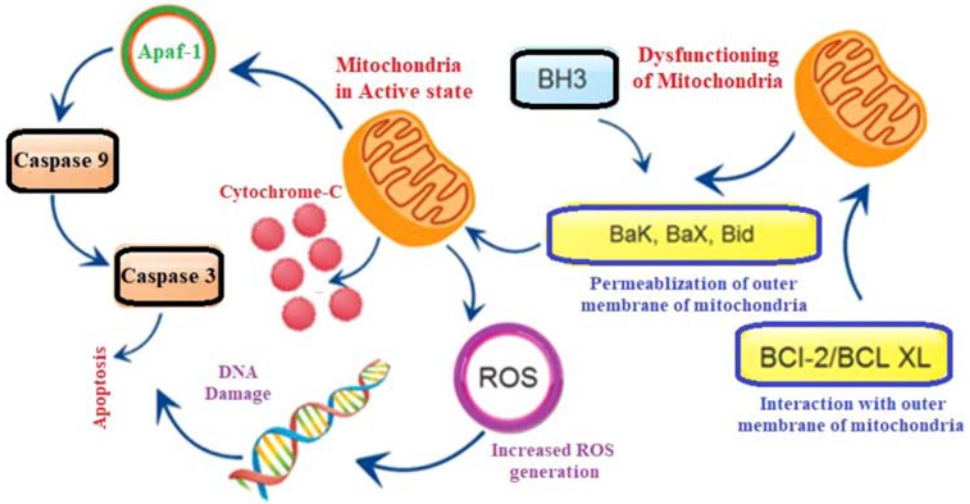

FIGURE 26.6

Apoptosis process by targeting Bcl-2/Bax pathway [35].

26.2.4 Hepatoma cancer

Meena and his team have been extracted surfactin from *B. subtilis* KLP2015 that showed cytotoxicity against mammalian cancer cells Hep2-C with cell death percent of 76.09% ± 1.32% [43]. Liu and his team have been shown surfactin-like lipopeptide which was isolated from *B. subtilis* HSO121 when used in purified form inhibits the growth of human Bel-7402 at an IC50 value of about 35 μM. They have further tested the same surfactant on normal human keratinocyte cells (HaCaT) and found an IC50 value of 97 μM after 24 hours. This study also indicated the specificity of anticancer activity of surfactin toward Bel-7402 cells [42]. In a similar study, Wang and his team have been proposed that process (mechanism) of apoptosis induced by surfactin in hepatoma cells is similar as seen in breast cancer cells. After treating with surfactin accumulation of $[Ca^{2+}]i$ and sustained generation of ROS occurs that results into apoptosis. This accumulation of $[Ca^{2+}]i$ is because of the release of Ca^{2+} ions from inositol 1,4,5-trisphosphate and ryanodine receptors channels. This accumulation further resulted in the stimulation of endoplasmic reticulum stress [50].

26.2.5 Cervical cancer

The extracted surfactin from *B. subtilis* KLP2015 showed cytotoxicity against mammalian cancer cells (L-132 and NIH/3T3) with cell death percent of 88.56% ± 2.41% and 77.84 % ± 1.96%, respectively [43]. Liu and his coworkers have been chosen HeLa cell line to study antiproliferation effect of surfactin on cervical cancer. It was observed that HeLa viability was reduced by about 50% at a concentration of about 4040 μM after 24 hours. Further, it was also reported that their nano form shows more reduction in cell viability [42] But Bacillomycin D does not show the same effect on mammalian cancer cells L-132 [35]. Karimpur and his team have been synthesized

Gemini surfactants loaded with Curcumin, which was reported to show anticancer activity against breast cancer [51]. €-poly-L-lysine, a biosurfactant was used as an anticancer agent against Cervix adenocarcinoma, that is, HeLaS3 cells by inhibiting the growth of these cells [52]. Monoolein is also a biosurfactant that has been used as an anticancer agent against HeLa (cervical cancer cells) by showing growth inhibition activity [27].

26.2.6 Human epidermal keratinocyte line

Dey and his team have been studied in one of the research that this does not show any significant toxic effect on HaCaT cancer cells [33]. It also didn't show any significant effect on normal HMEC cells [53].

26.2.7 Carcinoma cancer cells

Vo et al. have been reported the extraction of surfactin from *B. subtilis*, and used it as an anticancer agent against SCC4 and SCC25 cells (Human oral squamous cell carcinoma). It was found to work through ROS-regulated mitochondrial pathway [54]. Surfactin is also observed in hepatocellular carcinoma cancer cells (BEL7402) by inhibiting their cell growth and inducing apoptosis [45].

26.2.8 Leukemia cells

Surfactin extracted from *B. subtilis* natto T-2 showed the cytotoxic effect on human leukemia cancer cells (K562) at different concentrations between 2 and 62 μM with an IC50 value of about 20 μM. Surfactin also showed antiproliferative activity by inducing apoptosis and cell cycle arrest. During the process of apoptosis, the proteins inducing apoptosis such as PARP, caspase-3, p27kip1, and p21waf1/cip1 get activated. On activation of these proteins a lot of morphological changes occur in these proteins that regulate the progress of the G1 phase. It was also observed that the expression of cyclin D1 was declined [55]. The same group of scientists in 2009 showed that this process occurs through $[Ca^{2+}]$i/ERK-mediated mitochondrial/caspase pathway in which $[Ca^{2+}]$i increases on exposure (15 μM) for about 9 hours (Fig. 26.7) [56]. Surfactin was found to show anticancer activity against K562 (human myelogenous leukemia cell line). The results obtained suggested that surfactin downregulates the activity by growth inhibition, cell cycle arrest, and apoptosis induction [45]. Monoolein is a type of biosurfactant that has been used as anticancer agent against U937 (Leukemia cancer) by showing growth inhibition activity [27].

Isoda and his team have been investigated biosurfactant Mannosylerythritol lipid, which was known to induce granulocytic differentiation in HL60 (human leukemia cell line). The same surfactant was found to induce differentiation in K562 (human myelogenous leukemia cell line). The results obtained suggested that Mannosylerythritol lipid downregulates the activity of tyrosine in K562 cells to induce cell differentiation and inhibit proliferation in these cells [57]. In another study, the biosurfactants reveal that the succinoyl trehalose lipids (STLs), were used in growth inhibition and differentiation of Promyelocytic leukemia cancer cells (HL60) [58], and Basophilic leukemia cancer cells (KU812) [56]. Sophorolipids, one of the known biosurfactants have been found their use as anticancer agents against Promyelocytic leukemia cells (HL60) by interacting with the plasma membrane [59]. A biosurfactant named Serratamolide was used as an anticancer agent

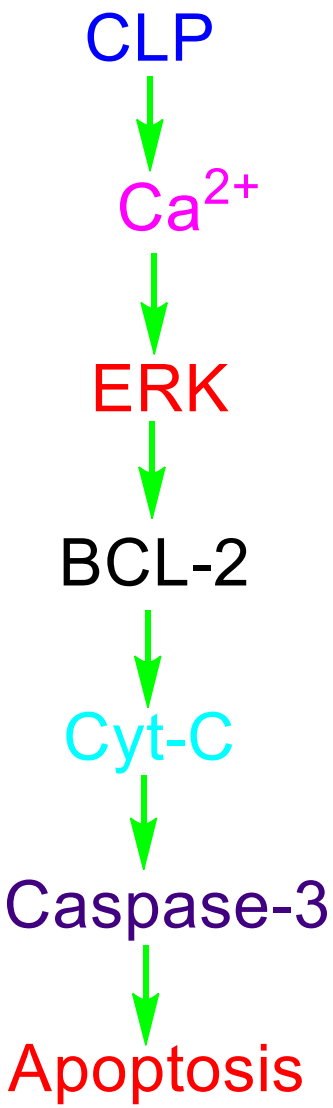

FIGURE 26.7

A tentative scheme indicating the pathways involved in apoptotic effects of cyclic lipopeptide (CLP) in K562 cells.

against BCLL a type of B-chronic lymphocytic leukemia cancerous cells. This biosurfactant was found to show apoptosis activity on these cancerous cells [60].

In one study, a mixture of iturin homologes extracted from *B. subtilis* was used to inhibit K562 cells (chronic myelogenous leukemia) at 42.75% concentration [34]. It was observed that the IC50 value required for inhabitation of K562 cells was 65.76 μM and complete inhabitation at a

concentration of 100 μM. It was found that K562 cancerous cells growth was inhibited by three different pathways.

1. Induction of paraptosis.
2. Inhibition of autophagy progress.
3. Induction of apoptosis.

26.2.8.1 Induction of paraptosis

When caspase inhibitor was introduced into the system, the process of paraptosis occurred in cytoplasmic vacuoles followed by mitochondrial swelling as well as Endoplasmic reticulum swelling, but without blebbing of the membrane.

26.2.8.2 Inhibition of autophagy progress

An upregulated expression of LCII and P62 was seen in a system that illustrated inhibition of autophagy progress

26.2.8.3 Induction of apoptosis

This step progresses by causing ROS burst. It can be illustrated by the upregulated expression of cytochrome *c* (Cyto-c), *bax*, and *bad*, together with downregulated expression of *Bcl-2* that system works by intrinsic pathway. The use of ROS inhibitors were showed that the process of apoptosis is ROS-dependent and the use of caspase inhibitor confirms that paraptosis is caspase-independent. It was also observed that the action of lipopeptide on K562 does not involve an extrinsic apoptosis pathway. In summary, the lipopeptide (containing mixtures of iturin) that were extracted from *B. subtilis* have the potential to act as an anticancer agent against myelogenous leukemia in vitro via the three steps paraptosis, apoptosis, and inhibition of autophagy occurring simultaneously. The mechanism of action is given in Fig. 26.8.

26.2.9 Lung cancer cells

The cytotoxicity effect of $Bi_2Zr_2O_7:Dy^{3+}$ (1−11 mol%) nanophosphors particles fabricated with a biosurfactant (Epigallocatechin Gallate) was studied by Rajashekharaiah and his coworkers. The cytotoxic effect of these biosurfactant fabricated nanoparticles was found to show high potential on cancer cell lines of lungs, that is, A549 [61]. Fengycin having nearly the same structure as surfactin also exhibits antitumor activity. Fengycin has also been extracted from *B. subtilis* and found to show an antiproliferative effect on human colon adenocarcinoma cell lines (LoVo cells). It was also observed that it shows high potential in inhibition of LoVo cell lines as a result of proapoptosis and cell cycle arrest [36]. Sophorolipids also have shown anticancer and antitumor activity against lung cancer cells A549 by inducing apoptosis in them [62]. In one of the studies a type of iturin, that is, Bacillomycin D has induced apoptosis in Adenocarcinomic human alveolar basal epithelial cells (A549) [35]. Studies reveal the antitumor activity of iturin was investigated on Caco-2 and was seen that inhibition occurred because of repression of bcl-2, releasing cytochrome-C to the cytoplasm from mitochondria and expressing bad and bax. As a result, the apoptosis mitochondrial pathway was induced in cells [63]. And parallel to this an increase in the concentration of Ca^{2+} was observed, ER dilatation was found as a result of activated caspase-12 followed by apoptosis in Caco-2 [64]. Kameda and his team in about 1974 investigated the anticancer activity of surfactin against Ehrlich ascites cancer cells. It

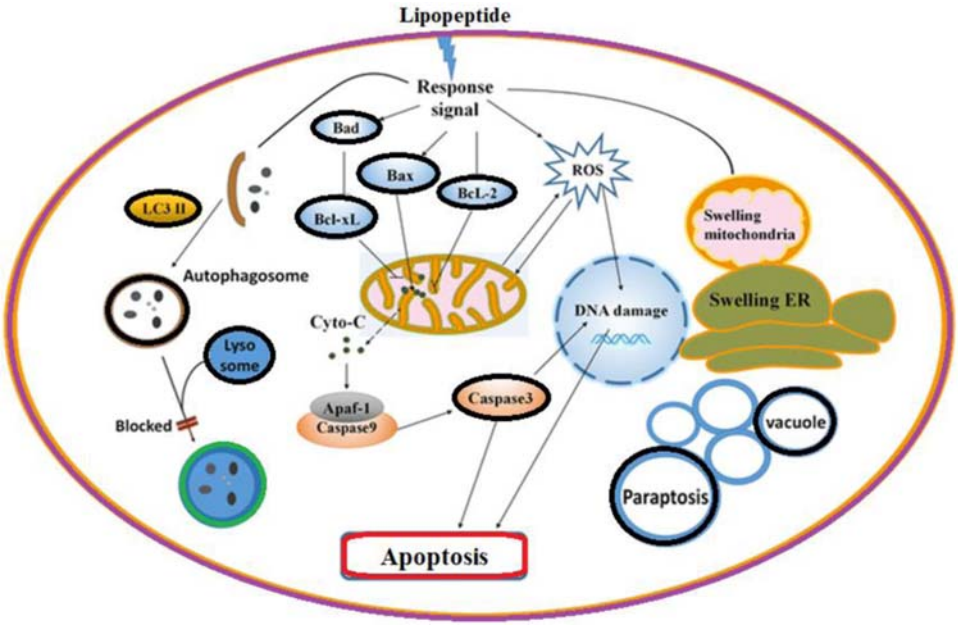

FIGURE 26.8

Overall mechanisms of the inhibitory effect of *Bacillus* lipopeptides on K562 [34].

was observed that surfactin showed cytotoxic activity against these cancer cells [65]. Sophorolipids have shown anticancer and antitumor activity against liver cancer cells H7402 by showing cell growth inhibition, cell cycle arresting and induction of apoptosis [66]. €-poly-L-lysine, a biosurfactant was used as an anticancer agent against Hepatocellular liver carcinoma, that is, HepG2 cells by inhibiting the growth of these cells [52]. Sophorolipids are one of the important biosurfactants that showed antitumor and anticancer activity against pancreatic cancer cells (HPAC) by showing Necrosis [67]. In the process of necrosis, the death of animal tissue occurs that is infected by a certain kind of disease or injury and in case the cells are infected by cancer. Shao et al. have been found that Sophorolipids act as an anticancer and antitumor agent against esophageal cancer cells denoted by KYSE109/KYSE450. It was observed that the Sophorolipids control this type of cancer by growth inhibition of these cells [68]. Viscosin a biosurfactant has been found its use as an inhibitor to the migration of PC3M cancer cells, that is, a type of prostate cancer [69].

26.3 Other biomedical applications

26.3.1 Biosurfactants as antibiofilm agent

It has been reported in various studies that biosurfactants can be used to destroy pathogenic biofilms in an environmentally friendly manner. A biosurfactant extracted from Lactobacillus acidophilus has been

used to down-regulate the gene expression that adheres to the *S. mutan* on the tooth surface [70]. In another study, Dalili and his team extracted a biosurfactant from Corynebacterium xerosis strain NS5 (Coryxin) that processed disruptive and inhibitory activity against biofilms of *S. mutans* (80%), *Staphylococcus aureus* (82.5%), *P. aeruginosa* (30%), and *E. coli* (66%) [71]. One more biosurfactant that was extracted from *Lactobacillus* sp. CV8LAC was used to inhibit the growth of a biofilm produced by *Candida albicans* strains upto 82% and 81% [72].

26.3.2 Biosurfactants as antimicrobial agent

There are fewer reports published on the antimicrobial activities of biosurfactants. In one of the study a biosurfactant derived from a *L. paracasei* sp. was used as antimicrobial agent against pathogenic *C. albicans*, *Staphylococcus aureus*, *S. agalactiae*, *S. epidermidis*, and *E. coli* [73]. Mandal and his coworkers have described that the biosurfactants like surfactins, fengycins, iturins, and kurstakins, have also shown antimicrobial activities [74]. It was also studied that a cell-bound biosurfactant that was extracted from *L. jensenii* and *L. rhamnosus* showed in vitro antimicrobial activity against stains of *S. aureus* (a multidrug-resistant microbe) [75]. Also, Mani et al. have been extracted glycolipid type biosurfactant from *L. lactis* which was found to show a lot of antibacterial activities [76]. Antifungal activity of surfactants Viscosinamide [77], Iturin [78], Rhamnolipids [79] has been published by a different group of researchers. Lichenysin a known biosurfactant is used as a chelating agent and has also shown antibacterial activity [68,80]. Another biosurfactant Pumilacidin was found to show Antiviral activity against HSV-1 [81]. It has been demonstrated by various studies that biosurfactants can be safely administered dermally or orally.

26.3.3 Biosurfactants in drug delivery

Because of the amphipathic property that is shown by biosurfactants they can be loaded over the nanoparticles, which can be further used to carry hydrophobic drugs. Huang and his team assembled surfactin to nanoparticles by solvent emulsion method to encapsulate doxorubicin an anticancer drug [73]. Now this assembled nanoparticle showed high toxicity against human breast cancer MCF-7/ADR cells (doxorubicin-resistant) as compared to doxorubicin. This enhancement in property of high toxicity can be attributed to high cellular uptake which can be used as a potential anticancer agent in cancer therapy [82]. In a similar way, other biosurfactants can also be assembled into nanoparticles and used for drug delivery purposes.

26.4 Conclusion

Biosurfactants have the potential to be used as an antimicrobial agent, effective therapeutic agent, binding to heavy metal, cell adhesion, cell aggregation, the pathogenesis of bacteria, quorum sensing, producing antibiofilm, and antimicrobial compounds. Because of antifungal, antibacterial, antiviral, and antiadhesive properties biosurfactants are a good alternative to antibiotics used against disease caused by food-borne pathogens. All the types of studied surfactin obtained from various microbes were showed relatively high antibacterial activity against multidrug resistant clinical pathogens. That is why nowadays, these have been very well used in pharmaceuticals or cosmetics

for dermal applications. Great effort by researchers focused on the clinical/cosmetic formulation of biosurfactants and their broad applications for commercial purposes. Microbial surfactants from *C. xerosis* have been shown to inhibitory and disruptive activities against biofilm formation that could be of use in bio-film-related menace. Conclusions have been drawn from various sources of studies that proved that biosurfactants strongly inhibited cell proliferation and induced apoptosis of colon cancer cells. Apoptotic analyses confirmed that the growth inhibition by biosurfactants was due to apoptosis induction and cell cycle progression arrest through the suppression of cell survival-regulating signals. Therefore, these studies will provide persuading evidence that biosurfactants may be considered as a new class of anticancer drugs.

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CHAPTER

Biosurfactant as antibiofilm agent 27

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27.1 Introduction

Biofilms are a practical relationship of surface-joined microorganisms that become encased in extracellular polymeric substances (EPSs). The biofilm is normally made out of different substances, including polysaccharides, proteins, nucleic acids (DNA), lipids, and humid substances [1]. Considerable proof exists supporting a significant part of polysaccharides in the cohesiveness of the EPS lattice. The biofilm can contain extensive measures of proteins that, generally, can far surpass the polysaccharide content, on a mass premise. Both cell surface-bound and emitted proteins add to the network arrangement and depend on their natural capacity [2]. Although at first observed as leftover material from lysed cells, it has become progressively clear that extracellular DNA not just is in certainty a necessary aspect of the grid yet, in addition, is liable for a few attributes characteristic for the biofilm method of life [3].

Extraordinary accentuation has as of late been given to the ecological effects brought about by compound surfactants because of their harmfulness and trouble in being debased in the earth. Expanding ecological concerns, the development in life science, and the rise of more severe laws have prompted biosurfactants to be an expected option in contrast to the synthetic surfactants accessible available [4]. Biosurfactant generally alludes to surfactants of microbial cause. The vast majority of the biosurfactants delivered by organisms are combined extracellularly and numerous microorganisms are known to create biosurfactants in huge relative amounts. Biosurfactants are required to be recognized as multifunctional materials of the 21st century, as they have applications in various modern cycles, just as possible new future uses are typically due to their different structures. Microorganisms generate surface dynamic mixtures to increase both the bioavailability of immiscible hydrophobic and normally blocked substrates allowing better endurance under low humidity conditions. The development of biosurfactants involves the existence of miscible hydrophilic and sleek/hydrocarbon carbon sources in the way of life medium. Financial aspects of the cycle and environmental certifications will make it attractive when using waste products as substrates [5].

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27.2 What is biofilm?

Biofilms are a collection of at least one form of microorganism that can grow on wet surfaces. Microorganisms that structure biofilms incorporate bacterial, algae and their spore, fungi, and spore, protists, etc. Biofilm is mostly present in aqueous conditions, such as subterranean aquatic objects and distant marine orifices. Biofilm arrangement starts when free-drifting microorganisms attach to a solid−liquid interface. "This initial step of connection happens when the microorganisms produce a sticky ingredient identified as an EPS. EPS associate with carbohydrates, peptides, spores, DNA, and RNA of the microbes, etc. It empowers the microorganisms in a biofilm to stay together. Further layers of microorganisms and EPS expand upon the primary layers. Eventually, they make a bulbous and complex 3D structure. Water channels befuddle biofilms and take into account the trading of supplements and waste items, as indicated by the article in Microbe. Different ecological conditions help to decide the degree to which a biofilm develops. These components additionally decide if it is made of just a couple of layers of cells or fundamentally more. For example, microorganisms that produce a lot of EPS can develop into genuinely thick biofilms regardless of whether they don't approach a ton of supplements [6]. Then again, for microorganisms that rely upon oxygen, the sum accessible can restrict the amount they can develop. Another natural factor is the idea of sheer pressure. On the off chances that have a high stream of water over a biofilm, as in a spring, the biofilm is generally genuinely slim. In the event that a biofilm in moderate streaming water, as in a lake, it can turn out to be exceptionally thick" [7].

27.2.1 Characteristics of a biofilm formation

Biofilms are astoundingly heterogeneous. Numerous estimations and perceptions have been made of different biofilms; they all highlight the variety of individual biofilm states. As we have referenced previously, in common, normally happening biofilms there is almost consistently an enormous number of various types of microorganisms living respectively. Also, extraordinary biofilms appear to show changed inner structures, distinctive synthetic properties, diverse electrical properties, and, undoubtedly, various properties of pretty much some other estimation or perception that can be made. Every one of these properties appears to add to the qualities of the biofilm in general that make it unique (e.g., difficult to slaughter) contrasted with managing every one of the microorganisms in confinement (not in a biofilm, but rather in a planktonic climate) [8].

Here is a portion of the more obvious qualities normal to all watched biofilms:

1. Biofilms are dynamic and receptive to their current circumstance; that is, they can adjust to changes in their current circumstance.
2. A wonder known as separation is by all accounts regular among all biofilms. Bacterial cells can separate from their biofilm province exclusively or in bunches.
3. At the point when singular microorganisms confine from a biofilm, these disengaged microorganisms are moderately simple to slaughter with synthetic substances intended for this reason.
4. At the point when microorganisms disconnect from their biofilm state in bunches, the clusters are bits of the biofilm that are right now not appended to a surface; for this situation, they keep up the defensive properties of the first biofilm and are accordingly considerably more hard to slaughter.

5. In the correct conditions, biofilms can relocate across surfaces throughout some period in an assortment of ways, as represented underneath.

27.2.2 Process of biofilm formation

The initial phase in the development of a biofilm is the connection of free-coasting microorganisms onto the surface, trailed by emission of EPS, fast multiplication, and collection of microscopic organisms in the sludge layer. Two significant variables to be considered are (1) reversible bacterial grip, which is a vague association between the microorganisms and material surface, and (2) irreversible explicit ligand−receptor connection between the microscopic organisms and the material Surface [9]. The initial step is the connection of the bacterial cells to the chosen abiotic or biotic surface. Microorganisms ordinarily stick to a molding film commonly made out of natural particles that can advance the adherence of microorganisms to the surface. Introductory connection is intervened through powerless reversible van der Waals collaborations between the cell surface and the base, which can prompt a more grounded adhesion-receptor interceded connection [10]. Bacterial cell surface structures, for example, flagella, fimbriae, and *exo*-polysaccharides partake in irreversible communications. These can be dipole, hydrogen, ionic, or hydrophobic. The subsequent advance relates to the improvement of microcolonies advanced by the development and division of the main appended cells [11].

The microcolonies dynamically amplify and combine to frame the principal layer of cells covering the surface. At the point when different layers of cells heap up on a superficial level, the third step of the development is gotten, demonstrated by the presence of a developed biofilm portrayed by the presence of macrocolonies encompassed by water channels that help appropriate supplements and flagging atoms. At last, to endure when supplements become restricted, or basically to spread and colonize to different specialties, some biofilm cells can isolate exclusively or in bunches. By and large, biofilm scattering happens in light of natural changes and is reliant on developing conditions [12] (Fig. 27.1).

FIGURE 27.1

A schematic diagram of biofilm formation.

27.2.3 Harmful effects of biofilm

Biofilms may happen on any surface, especially in seagoing and modern water frameworks. Incidentally, numerous advances in clinical innovation have given new and risky specialties to biofilm arrangement. Hence, embedded clinical gadgets, for example, catheters and counterfeit heart valves, give surfaces to biofilm development, hence supporting a hold of contaminating organisms coming about in biofilm-associated diseases, being assessed that around 65%−80% of all human microbial contaminations include biofilms, though biofilm arrangement isn't itself fundamentally a harmfulness factor, on the grounds that numerous nonpathogenic living beings additionally produce biofilms that don't cause infection [13]. In any case, since the improvement of protection from antimicrobial mixes related to the biofilm "method of life" is one of the most stunning clinical outcomes of biofilm development, biofilms are exceptionally pertinent for general wellbeing. The biofilm-shaping capacity of specific microbes shows up to encourage the endurance of these microorganisms in nature and the host, in light of the fact that to the collection and dispersal of an adequate number of microorganisms for an infective portion, which isn't normally found in a mass liquid. Furthermore, the heterogeneous microenvironments that happen inside biofilms may advance a separated populace of phenotypic and genotypic variations of microorganisms that guarantees endurance despite changing natural conditions and may likewise guarantee endurance and encourage infection. Nevertheless, biofilm frameworks likewise are particularly reasonable for the treatment of hard-headed mixes on account of their high microbial biomass and capacity to immobilize mixes [14].

27.2.3.1 Impact on human health

Biofilms can be built on embedded clinical gadgets, such as prosthetic heart valves, joint prosthetics, catheters, and pacemakers. The marvel was first seen in the 1980s when bacterial biofilms were detected on intravenous catheters and pacemakers. The explanation that the biofilm arrangement is an amazing reason for concern is that, inside a biofilm, bacteria are more impervious to antitoxins and other important disinfectants that you might use to monitor them [15]. It is true that, when compared with free-drift microorganisms, those that grow as a biofilm can be up to several times more impervious to antimicrobials and other organic and compound specialists. Parasitic biofilms can also cause contamination by the production of embedded gadgets. Yeast species, for example, individuals of the Candida variety grow bosom inserts, pacemakers, and prosthetic heart valves. Candida species also develop on human body tissues, causing diseases such as vaginitis and oropharyngeal candidiasis [16,17] (Fig. 27.2).

27.2.3.2 Food spoilage

Biofilms have as of late picked up consideration in food creation and readiness conditions. A few food waste and pathogenic microorganisms have been accounted for to append to and structure biofilms on food contact surfaces. The result of this can be food waste and additionally food harming. Indeed, even with cleaning techniques predictable with great assembling practice, these biofilm microorganisms can stay on gear surfaces. It includes the expulsion of soil also, related microorganisms by cleaning with cleanser plans intended for evacuation of specific soil types, for example, fat, protein, sugars, and starch [18]. The practicality of remaining microorganisms is then diminished by the expansion of a disinfectant. An expansion in temperature influences cleaning and

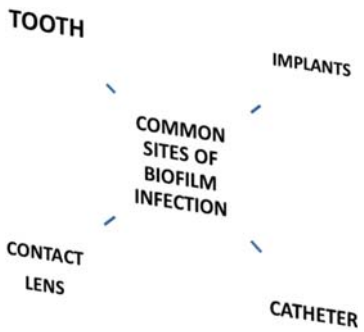

FIGURE 27.2

Common sites for biofilm infection in humans.

cleansing by expanding the substance impacts of cleansers and disinfectants and by encouraging the evacuation of fats and oils. High temperatures, be that as it may, increment the perseverance of protein soils due to their denaturation. On the off chance that the cleaning and purification program is incapable, at that point soil and microorganisms may recover influencing the quality and security of the food [19].

27.2.3.3 Ship biofouling

Bacterial attachment to pontoon bodies fills in as the establishment for biofouling of seagoing vessels. When a film of microorganisms structures, it is simpler for other marine living beings, for example, barnacles to connect.

Such fouling can diminish the greatest vessel speed by up to 20%, drawing out journeys and devouring fuel. Time in the dry harbor for refitting and repainting decreases the efficiency of transportation resources, and the helpful existence of boats is additionally diminished because of erosion and mechanical evacuation of marine living beings from boats' frames [20].

27.3 Biosurfactants

Biosurfactants can be described as surface-dynamic biomolecules supplied by microorganisms with a wide variety of applications. Lately, because of their novel properties, such as explicitness, low harmfulness, and relative simplicity of planning, these surface-dynamic biomolecules have become widely intrigued. Biosurfactants have been used in a few companies, including natural synthetics, gasoline, petrochemicals, mining, metallurgy, agrochemicals, manures, fruit, beverages, beauty products, medicines, and many others, due to their exceptional useful properties. They can be used as emulsifiers just as demulsifiers, wetting experts, scrubber operators, spreaders, utilitarian food fixing, and cleaners. Interfacial surface strain decreasing the ability of biosurfactants made them assume significant roles in the recovery of oil and bioremediation of heavy crude oil [21].

Including the major capabilities of biosurfactants, they were used to create the surface of the hydrophobic substrates. Biosurfactants are additionally used to expand the bioavailability of

hydrophobic substrates by solubilization/de-sorption. They also handle the relation and removal of microorganisms from the soil [22].

Biosurfactants have both hydrophilic and hydrophobic divisions, rendering them complete at interfaces between liquids with different polarities, for example, hydrocarbons, and water, resulting in a reduction of the interfacial surface pressure. In addition, they have discovered how to enhance additional transport through films and affect various host−organism partnerships [23].

In comparison to compounds or processed surfactants, biosurfactants have increased some favorable circumstances, including their biodegradability, biocompatibility, and edibility. Biosurfactants may be used in natural cleaning by biodegradation and detoxification of mechanical effluents and the bioremediation of sullied soils. Their particularity and accessibility of raw materials have made them the most preferred surfactants [24].

27.3.1 Types of biosurfactants

Biosurfactants are typically ordered by their structure and microbial inception. The main groups of biosurfactants are glycolipids, phospholipids, polymer biosurfactants, and lipopeptides.

27.3.1.1 Glycolipids

Glycolipids are segments of cell films that included a hydrophobic lipid tail and at least one hydrophilic sugar bunches connected by a glycosidic bond. For the most part, glycolipids are found on the external handout of cell layers where it plays not just an auxiliary function to keep up film security yet additionally encourages cell-cell correspondence going about as receptors, secures for proteins, and controllers of sign transduction (Fig. 27.3).

Glycolipids are found broadly appropriated all through all cells and essentially limited, however not solely, to the plasma layer. They are starches related to long-chain aliphatic acids or hydroxyaliphatic acids by the selection of esters. Biosurfactants are important glycolipids. Among the glycolipids, the most common are rhamnolipids, trehalolipids, and sophorolipids [25].

27.3.1.2 Phospholipids

Glycerol esters, fatty acids, phosphoric acids, and other alcohols are phospholipids. The most common phospholipids are phosphatidylcholine, phosphatidylthanolamine, and phosphatidylserine. Phospholipids are commonly used compounds that have major structural and metabolic functions in the cell because of their chemical properties. Phosphatidylcholine was the first phospholipid to be identified. Initially, it was known as lecithin after lekithos, namely egg yolk. Today, phospholipid's metabolic and physiological functions are an active area of study [26] (Fig. 27.4).

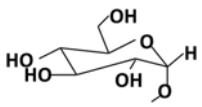

FIGURE 27.3

Chemical structure of glycolipid.

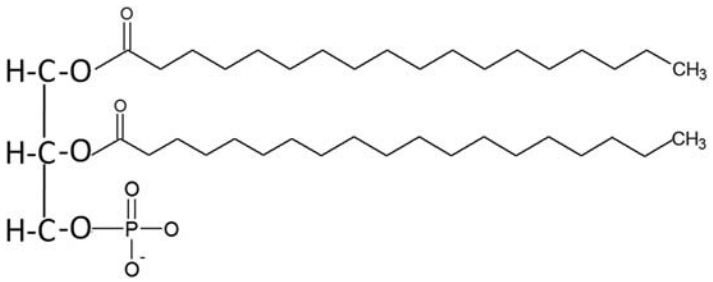

FIGURE 27.4

Chemical structure of phospolipid.

27.3.1.3 Polymeric biosurfactants

The most widely researched polysaccharide-protein complexes are emulsan, lipomanane, alasan, liposan, and other complexes. Emulsan was most common among bioemulsifiers because it has a large number of reactive groups, which keep the molecule close to oil droplets, creating barriers that avoid coalescence from degenerating. When a great amount of oil is needed to manufacture either the necessary quantity of pure emulsifiers or mechanical mixing with the high volume of the fuel oil [27]. Liposan consists of about 83% sugar and 17% protein. The heteropolymer that is a carbohydrate component composed of glucose, galactosamine, and galacturonic acid, as a consequence of acid and emulsifier's enzyme digestions. The emulsion of Liposan has been affected and cured with a wide range of commercial vegetable oil [28].

27.3.1.4 Lipopeptides

Lipopeptides were attempted to handle several plant pathogens as effective and flexible tools. For their opposite behaviors in a large variety of phytopathogens including bacteria, fungus, and oomycetes, all three families, surfactins, iturins, and fengycins, were investigated. There are antifungal activities in iturin and fengycin, while surfactin has a wide spectrum of strongly antibacterial activities that are often used as a larvicidal agent. Interestingly enough, lipopeptides are biologically suitable compounds [29].

27.4 Biosurfactants as antibiofilm agent

Biofilm disease in medical care is hard to handle and is equally critical for the agribusiness because it affects the resilience and viability of harvests. It is important to establish a methodology of biofilm management to combat pollution in the agribusiness associated with biofilms. Biosurfactants were recently exploited as future antibiotic film candidates to complain about the strength of biofilm producers by the precise destruction of biofilms [30]. Organisms are surface dynamic metabolites that have proven various roles in various areas, ranging from bioremediation to biomedical applications. Biosurfactants tweak the biofilm framing capabilities of microorganisms because of

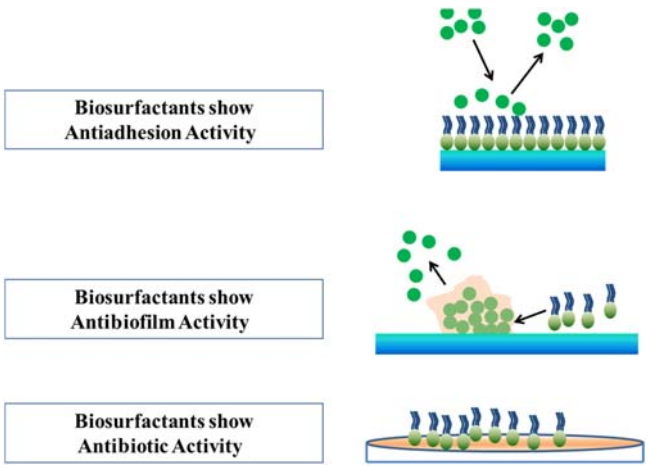

FIGURE 27.5

Schematic representation of biosurfactants as antiadhesive, antibiofilm and antibiotic characteristics.

their surface-changing properties, which directly precipitate microbial colonization and biofilm creation (Fig. 27.5).

During their travel to the antibiofilm operator various natural, processed and six-engineered mixtures were investigated, in which biofactants gain importance in extraordinary conditions as potential antibiofilm experts. Biofactants are complex surface amphipathic particles delivered by live cells that are designed to minimize fluid surface tension. They are mechanically, naturally, and biomedically important. Antibiofilm applications have been tested and found to be successful for the glue property of biofactants [31], Banat et al. studied the effect of various types of biosurfactants as antibiofilm agents which are described in the following sections.

27.4.1 Polymyxins biosurfactants as antibiofilm agent

Polymyxins are a family of cyclic lipopeptides that is nonribosomally incorporated. They are usually delivered to Bacillus or related organisms as optional metabolites. Its average composition is that of a cyclic, fat-related polypeptide. They can also contain bacterial interesting acids, including 2,4-diamine butyric (DAB), which are bacterial corrosive. Polymyxins in the treatment of gram-negative pathogens have a small therapeutic spectrum of obstructive activities. A few industry-accessible polymyxin concepts are available that can be given as a polymyxin B sulfate, including colistin, neocorporin, and polymyxin B. Polymyxin may also be consolidated into a combination neosporin antitoxin therapy with trimethoprim for eye disease and Neomycin and bacitracin [32]. Polymyxins constitute the new decision-making medicine in a few conditions, and are mostly prescribed with alertness because of concerns of harm; this determination, in any case, has been reconsidered in the light of more detailed tests. In the case of *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *P. aeruginosa* polymyxins are supported, either intestinally or topically as cream or powders. Polymyxin is responsible for decreasing *P. aeruginosa* biofilms by 99% in a 12 hours,

and completely longer than 24 hours at centralizations of 20 μg/mL. However, such findings are based on the viability and nondistribution of microorganisms, even if the modified morphology of the bacterial cells was noticed. The early intensity therapy of colistin is recommended to overcome chronic *P. aeruginosa* pollution with a biofilm or abnormal colonization in patients with cystic fibrosis, a combination of colistin internal breath with oral ciprofloxacin. Polymyxin D1 has proved to be potent against bacterial bodybuilding; however, it has occurred to us in previous work that the compound has been used in vague amounts in conjunction with fusaricidin and surfactin. In addition, this complex of biosurfactants has been discussed to inhibit biofilm formation of all Gram-positive microorganisms, for example, *S. aureus*, *Streptococcus bovis*, *Batillus subtilis*, *Micrococcus luteus*, and *P. aeruginosa*, for example, Gram-negative microorganisms. Strictly speaking, the biofactants were able to avoid the arrangement of biofilms for mixed species. In general, polymyxins are uncertain about their system of action on bacterial biofilms. However, a high lipopolysaccharide partiality is proposed for the framework of activities on planktonic microorganisms [33].

27.4.2 Surfactins as antibiofilm agent

Surfactin is one of the most excellent biosurfactants excluded effectively from Bacillus subtilis. Surfactin consists of a 13−15 carbon, beta-hydroxy chain with biofilm destructive ability. Tragically, owing to their cellular layer support, surfactins can also be purposelessly cytotoxic with hemolytic exercises. The production of Salmonella biofilms formed on PVC microtitre pools and urethra catheters has been minimized. They have been seen as an undulation effect on the lipid bilayer, possibly showing signs for the instrument that extends the susceptibility of biosurfactant activity or porous biofilm or integrity by organizing some form of channels inside the biofilm [34].

27.4.3 Putisolvin as antibiofilm agent

Putisolvin biosurfactant obtains from *P. putida* species which is generally found in two structures, putisolvin I and putisolvin II. This biological agent produces a 4-part cyclic peptide, which is exposed to leucine or isoleucine in putisolvin II as the valine build up in putisolvin I. While putisolvin is an integral part of *P. putida* biofilm, it controls the growth of other types of bacterial biofilm. These surfactants have also been found to be viable dispersal operators in the current expansion of biofilms from various Pseudomonas strains [35].

27.4.4 Pseudofactin as antibiofilm agent

Pseudofactin has been taken from *P. fluorescens* as a cyclic lipodepsipeptide. The composition of Pseudofactin relies on that of a palmitic corrosive added to the eight amino-peptide chain amino array. The carboxylic C-terminal set of the lactone with the hydroxyl of the third amino corrosive, threonine, from the last amino corrosive structure. The success of Pseudofactin II against five forms of bacterial biofilms on glass, polystyrene, and silicone substrates was 36%−90%. *Enterococcus faecalis*, are found in certain strains *E. coli*, *Staphylococcus epidermidal*, *Enterococcus hirae*, and *Proteus mirabilis* [36]. On the *Candida albicans* yeast biofilms, bond restraints were compared (92%−99%) at 0.5 mg/mL. Pseudofactin has been reported to provide 26%−70% viable dispersion

on previous untreated surface biofilms and appears to hinder the underlying attachment of *E. hirae* and *E. coli* on silica urethral catheters and absolute restriction of *S. epidermidis* biofilm formation [37].

27.4.5 Rhamnolipids as antibiofilm agent

Di- or mono-rhamnosis sugar bound to a variety of unfermented fats is present in the rhamnolipid. It is primarily enclosed from *P. aeruginosa*, *Burkholderia*, *Renibacterio salmoninarum*, *Cellulomonas cellulans*, *Nocardioides*, and *Tetragenoccus koreensis* additionally complement analogs. Rhamnolipids are a replacement of the syntactical used by particular oil applications and are used in the manufacture of petroleum polluted environments. Their capacity to monitor biofilms, however, has never been widely documented. They are eliminated as much as possible as barriers to bacterial growth. In *Pseudomonads* spp., rhamnolipids function to inhibit biofilm growth [38,39]. The development of biosurfactants to enhance water and oxygen supplement to biofilm, work like a barrier in their communication, and hence the rate of reduction in sustaining channels throughout the biofilm. The effectiveness of rhamnolipides against *Bordetella bronchiseptica* biofilms has also been studied. The biofilm boundary tool is thought to be cellular; other unconnected cells in either case now may be meaningful. Pre-determined biofilms, such as *B. pumilus* from marine climates have been documented to be disrupted which leads to concentrations of small MICs and to improve the ability for the removal of established biofilm. At the time the site, for instance, silicone elastics were infused with rhamnolipids using *S. salivarius* and *C. tropicalis* biofilm, a substantial decrease in bond (total 66%) was observed. Rhamnolipids were found to be compelling biological agents which disrupt *Yarrowia lipolytica's* biofilms from presuspension in glass portions with an ironic impact of 67% more than CTAB. Rheamnolipids have also been found to be associated with prolonged lung epithelial pore liability, rapid necrotic by neutrophils inhibition, and the inability in the respiratory systems of infected patients to transfer the normal tracheal ciliary movements [40].

27.4.6 Sophorolipids as antibiofilm agent

Sophorose lipidare common glycolipid biosurfactants consisting of a dimer of the indicated sophorose and a long-line unsaturated fat that is supplied with *Candida* class by yeasts. This way live MMOs are green with unblemished films, and dead microscopic specimens are red fluorescent with harmed layers. The detail of the effect on biofilm from *E. coli* of sophorolipds has been detected [41].

27.5 Conclusion

It was recognized that various refractory patient environments, the dispersal of airborne microbes, and the trapping of mechanical surfaces, are at the core of microbial biofilms. Moreover, the growth of healthy biofilms and the absence of optional annihilation structures are steadily exacerbating such problems. Biosurfactants talk of increasing therapy which has the characteristic to spread or disrupt certain biofilms effectively against bacterial, infectious, and viral properties. Their usage, alone or as adjuvants to other antimicrobial chemotherapies, may talk of the anticipated direction in biofilm processing later.

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CHAPTER

Rheological behavior of biosurfactants

28

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28.1 Introduction

In the past decades, the trends in material science are directed more and more towards green or biocompounds to diminish the damage resulting from planet pollution during industrial activities [1,2]. In this context, biosurfactants have gained increased attention owing to their advantageous properties which helped to advance several applicative areas, like the food industry [3], medicine [4], pharmacology [5], or environmental protection [6,7]. Proper processing of biosurfactants into products used in the aforementioned fields imposes deep knowledge on their solution features [8–10]. Given the structural peculiarities of these substances, the flow characteristics can be tailored through the action of external factors, such as the solvent type, system composition, ionic strength, temperature, pH, shear intensity, etc. [11].

Rheology is one of the most employed techniques for the investigation of flow kinetics of a large variety of materials prepared as dispersions or as simple/complex solutions [12–14]. Rheological experiments provide essential data that enables monitoring the changes in the sample composition as a function of time or shear deformation magnitude [15–17]. In this way, it is possible to perform a detailed characterization of the interactions leading to self-assembly and other phenomena observed in biosurfactant-containing systems [18]. Considering these aspects, the rheological behavior of biosurfactants in various environments is of paramount importance in many applications. For instance, when dealing with the petroleum industry the bioremediation of oil spills and related contaminants is affected by the interactions in the multiphasic system [18,19]. In medicine, the fungicidal and bactericidal actions of biosurfactants in solution could affect the therapeutic efficiency [20]. When biosurfactants are found in heterogeneous systems, they gradually begin to aggregate at the phase boundaries. As a consequence, an interfacial layer, which changes the wetting and surface energy features, appears. The resulted molecular layer, besides reducing the surface tension in liquids, is also responsible for the diminishment of the interfacial tension among the distinct liquid phases on the interfacial boundary formed in immiscible media; hence could influence the interfacial rheological properties and mass transfer [21]. On the other hand, biosurfactants can be employed as food additives to render some desired technological functions to edible products [22]. More specifically, these biocompounds can act as flavoring agents, preservatives, miscellaneous additives, coloring substances or texturizing agents. Emulsions are fundamental for food formulations solubilization, dispersion, and constancy, affecting the appearance and texture of the

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final products [23] Biosurfactants have deep involvement in the emulsion stability by controlling the agglomeration of phases. Biosurfactants are recognized for the diminishment of the interfacial tension among the system phases and can upgrade the consistency by tuning the rheological properties of the food product. The same impact can be studied when preparing cosmetics since biosurfactants are encountered in the composition of foams or emulsions categories of beauty products.

However, in spite of rheological importance in biosurfactants products formulation, the literature is not very abundant on such information. This work reviews the state-of-art regarding the rheological investigation of certain biosurfactants and related systems. The implication of the rheological functions on processing such materials is shortly presented.

28.2 Brief introduction on biosurfactants

The general sources of biosurfactants are represented by filamentous fungi, yeasts, and bacteria. The term "biosurfactants" denotes surface-active substances excreted or produced at the microbial cell surface. Structurally speaking, such biomolecules are mainly constructed of distinct hydrophilic and hydrophobic parts, which are making them aggregate in the presence of fluid environments having different polarities [24]. Biosurfactants are known to display some benefits regarding the lab-made ones, such as multifunctionality, diminished toxicity, combined with excellent biodegradability, while they remain active in various conditions of pH and salinity. Moreover, they have a remarkable capacity to form emulsions in certain immiscible fluids, like hydrocarbons and water. These features render materials capable of lowering surface and interfacial tension and producing microelusions that present good detergency, foaming, and dispensing attributes. The classification of the biosurfactants relies on accounting for many factors, such as structural common features, dimension, charge, hydrophobicity, degree of modifications, and specific physical and chemical characteristics [25,26].

Based on the *chemical structure* and *hydrophobicity*, these materials can be divided into:

- glycolipids,
- lipoproteins,
- fatty acids,
- macromolecular surfactants.

According to their *microbial origin* biosurfactants can be imparted as:

- hydrophilic sequence made of amino acids or polypeptides anions or saccharide-based cations,
- hydrophobic moiety made of fatty acids having unsaturated or saturated structure.

Based on the producing source, biosurfactants can be divided as:

- microbial biosurfactants,
- enzymatic produced surfactants.

The structure and composition of each biosurfactant determine its physicochemical and biological features. The synergism between bioactivity and interfacial activity determines a large spectrum of applications in several fields, for example in pharmaceutics, agriculture, food industry,

environmental preservation, and other practical situations that remain to be investigated for the overwhelming types of biosurfactant structures.

28.3 Rheological properties of some biosurfactants and their systems

The biosurfactant solutions display different behaviors as a function of concentration, pH, temperature, or other external factors. Given the amphipathic character of these compounds, they enable the reduction of the surface tension, in this way contributing to the emulsion of immiscible fluids. Biosurfactant effectiveness is affected by examining the hydrophilic/lipophilic balance, which reveals whether a biosurfactant has a great probability to generate water in oil or oil in water emulsion. Solution properties can be deeply characterized using rheology, which is widely known for its versatility in obtaining data regarding the microstructure, composition, and interactions in fluid samples. Moreover, elucidation of shear flow behavior is of paramount importance for designing efficient processing operations and desired attributes for the final product. The biosurfactant systems are studied mainly under the form of emulsions, but in some cases, they are foams, biofilms, solutions, or hydrogels. Depending on the morphology and fluidity of the studied system, rheological techniques can be adapted to extract information from bulk to interface of the surfactant derived material. Some quantitative parameters are extracted from rheological experiments, more specifically viscosity, elastic modulus, viscous modulus, yield stress, relaxation time, etc. Commonly, these properties can be obtained by performing two main sorts of measurements, namely steady-state rotational shear tests and small amplitude oscillatory shear tests [27,28]. The broadest outlook of liquid rheology is achieved through the oscillatory flow measurements at a fixed frequency since both elastic and viscous features are highlighted. Steady flow kinetics discloses information only viscous properties. The magnitude of shear stress, shear rate, and shear strain represents the foremost parameters for quantitative analysis of both the flow conditions and the deformation response of the liquid system. It is from these properties that the components of the viscoelastic modulus are acquired. These aspects constitute the basis for quantitative evaluation of the liquid's characteristics for quality control as demanded for the pursued applications.

28.3.1 Rheology of emulsions

Emulsions can be viewed as systems that contain several phases having immiscible character. Such systems often display in their composition a surface-active agent, which may be a biosurfactant, that displays two major functions: (1) to diminish the interfacial tension among the phases and in this way facilitate the faster appearance of the emulsion and (2) to enhance the steadiness of the dispersed phase regarding coalescence when is rendered [29,30]. Two broad categories of emulsions are known [30]:

- *Simple emulsions*: Small parts of a single fluid phase are spread in a different immiscible liquid medium;
- *Multiple emulsions*: The globule is made of several little droplets. In this situation, the dimension of a globule of this sort is considerably bigger than the magnitude of the constituent ones.

The rheology technique is useful to distinguish between the behavior of emulsions and suspensions of hard spheres. The contrasting flow characteristics of the first category of fluids are caused by the droplet deformation, surface mobility, and internal fluid movement. At very small volume fractions, the emulsion viscosity is affected by the fluid circulation inside the droplets. Given these considerations, rheological analyses can be employed to keep track of the variations in the structural organization and forces acting among the droplet in the system. For example, when the fluid system is subjected to conditions that are modified in a precise way, one can measure viscosity or elastic modulus as a function of the imposed shear deformation for elucidation of the emulsion properties and their evolution under the factors of interest [31,32]. This type of experiment provides essential data regarding droplet flocculation, gravitational separation, continuous phase gelation, and, in certain situations, it is possible to study the degradation of emulsions throughout the stages of preparation or storage [33,34]. Therefore the interactions taking place between distinct components within emulsions might be characterized via rheological tests.

On the other hand, it is widely known that fluids having suspended particles display a special structure, which is highly responsive to shear. As a result, steady-shear viscosimetry is not entirely adequate for probing the rheological behavior of a dispersion found in an unperturbed state. Stress can be exerted to material in many ways, such as simple shear, simple compression, and bulk compression, and thus the relation between stress and strain is utilizable for multiple situations, however, the magnitude of the stress, strain, and shear modulus are influenced by the nature of deformation [30]. An example is illustrated in Fig. 28.1A, which shows that at very low deformation, there is a linear interdependence among the applied stress and the achieved strain for an ideal

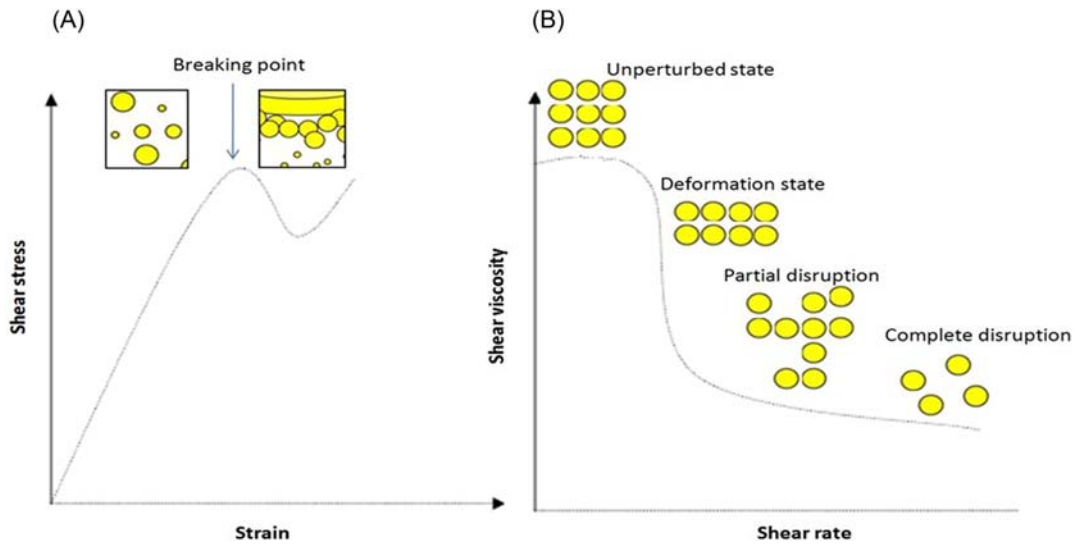

FIGURE 28.1

The illustration of stress–strain dependence strain for an ideal elastic material (A) and flow curve of an emulsion showing the deformation and disruption of flocs (B).

elastic material. As the deformation is higher, the stress begins to range in a nonlinear manner regarding strain and therefore the material is more susceptible and might break.

In Fig. 28.1B is depicted the case of an emulsion that has flocculated droplets and displays a shear-thinning behavior as a result of the deformation of the flocs and also their disruption in the presence of the shear field. When dealing with oscillatory rheometry, the examined emulsion is exposed to a very small oscillatory stress in such a way that it does not affect its structure, leaving it intact. A supplementary benefit of this test is that both the viscous and the elastic response characteristics of the specimen can be acquired. Most emulsions used in the formulation of a wide class of products present a combination of shear-thinning feature and pseudo-plastic flow, distinguished by yield stress [30,35]. Fluid systems displaying a higher degree of shear thinning at small shear rates tend to spread easily, whilst emulsions presenting higher values of yield stress have a bigger degree of resistance to an external deformation prior they begin to flow. These aspects mean that the higher the yield stress the higher the degree of emulsion structuring and the larger the degree of its stability. Overall, the majority of products present viscoelastic features often depicted by a combination of shear thinning, yield stress, and shear frequency dependence profiles of shear moduli. More specifically, in oscillatory shear mode, the prevailing storage modulus over the loss modulus at high-frequency range reveals good emulsion stability, while in the situation of prevalent viscous modulus in comparison to the elastic one at a low-frequency zone it points out that emulsions can be easily spread over a substrate. As specified by the viscoelastic model, the samples having Maxwell-like flow are often leading to a semicircular shape of the Cole−Cole plot and their storage and loss moduli scale up with f^2 and f^1, respectively. Ideal viscoelastic fluid is characterized by a phase angle (denoted δ) of 45 degrees at a large frequency domain. When the phase angle is 0 degree describes an elastic behavior and when it is 90 degrees is typical for s viscous material. So it results that a specimen with $\delta < 45$ degrees tends to be more elastic in nature, whereas those displaying $\delta > 45$ degrees are highly viscous. The shape of the dependency of phase angle on angular frequency is relevant for describing the amount of the emulsion viscoelasticity. The bigger value of phase angle denotes that emulsions have a prevalent liquid character, but smaller levels of phase angle show that emulsions tend to be more solid [35].

Emulsion samples in the dilute range are fluids where the concentration of the dispersed internal components is significantly reduced [36,37]. The droplets are very much distanced that they cannot interact with each other. Each of them is behaving as if it were embedded in an immense matrix of fluid. The main benefit is that one may consider solely the single-droplet mechanics for proposing the relations describing the bulk rheology of such samples. As the concentration further increases, the adjacent droplets gradually begin to interact with each other hydrodynamically [32]. In any case, the volumetric amount of the dispersed phase could somehow be limited, thus the droplets are tightly packed up to considerable deformation. So, in moderately concentrated systems droplets interact up to a maximum packing volume at which they remain undeformed. An additional increase of concentration from this point leads to emulsion gels, where the droplets change their spherical shape into a polyhedron one. As a result, such materials present yield stress, but also prevalent elastic features when placed under the action of small shear forces [38].

Blesic et al. [39] have studied the rheological properties of emulsions consisting of a hydrocarbon mixture, using surfactants of biological origin (hydrophobins—HFB). The shear flow kinetics was analyzed regarding emulsifying performance for oil recovery purposes. Interfacial shear rheology data revealed that the film produced in the synthetic seawater (SSW) and dectol (blend of

decane and toluene) is considerably strong and elastic. The strength of the sample seems to remain unaffected by variations of the salinity, the resistance being similar to that noted at water/oil and water/air interfaces. Such aspects are optimal for barrier practical uses. The emulsification of the SSW/dectol medium in combination with HFB was examined to evaluate the influence of certain parameters on the emulsification capacity. The achieved emulsification index was comparable with that remarked for some commercial products. At 0.05 mg/mL HFB and a frequency of 1 Hz, it was noted that the rheological moduli of dectol/SSW are constant in the strain range of 0%−1% and in a time interval of 0−2000 seconds. The rheology of bulk emulsions at variable HFB amounts indicated that the samples have a gel-like behavior with bigger strength regarding surfactants like Brij and sodium dodecyl sulfate. Below 0.5 mg/mL HFB the gel is weaker (lower storage modulus). The observed gel-like properties of the HFB emulsions are not entirely suitable for enhanced oil recovery or oil fluidification via emulsification. However, in the presence of a cosurfactant, the HFB samples could be useful for the appearance of a barrier at the oil/water interface.

Li et al. [40] have prepared concentrated emulsions (50 wt.% oil) that involve biosurfactants, like quillaja saponins and rhamnolipids. The amount of introduced natural emulsifiers affects particle sizes, flow behavior, and stability of the emulsion systems. It was found that rhamnolipids determine the formation of smaller droplets. Both emulsifiers enhance the stability of the samples against several sorts of environmental conditions, such as temperatures (30°C−90°C), salt amounts ($\leq$200 mM NaCl), and acidic pHs. The shear flow experiments indicated that concentrated emulsions containing quillaja saponins or rhamnolipids displayed shear-dependent viscosity and also very small consistency coefficients.

Tsibranska et al. [41] studied the importance of the quillaja and berry saponins on viscoelasticity of corresponding emulsions in aqueous systems based on hexadecane and sunflower/oil. Both biosurfactants led to solid separation shells on the sunflower oil−water interface. Therefore the prepared emulsions were made of nonspherical drops. The interfacial elasticity ranged in the interval of 2−500 mN/m. This property has is essential for the emulsion shear elasticity, and impact less the dynamic yield stress, but no influence on the viscous stress of the obtained steadily sheared systems.

Gao et al. [42] showed that when working with emulsion droplets one needs to control the droplet dimension and shape. They have prepared a "shape-memorable" microdroplet system, which can be stabilized via a protein-surfactant chemically adapted with polyethylene glycol (PEG). The droplets were proved to be stable against coalescence for more than a month and can keep the nonspherical form for hours, as a function of the surface coverage of the modified biomacromolecules. Monodispersed droplets with aspect ratios in the interval of 1.0−3.4 were achieved with a flow-focusing microfluidic instrument. The rheological features of interfacial elasticity stored in peptide/protein networks were tested. The measurements were done via a two-inlet microfluidic-based method and Miglyol oil with the role of dispersed medium and six distinct surfactant solutions as the continuous medium. The synthetic surfactant sodium dodecyl sulfate was used as a reference because is not able to lead to a specific network when at the interface of the used liquids is taking place the phenomenon of adsorption. The breakup of microfluidic oil stream as a result of distinct and ranging shearing level were examined and estimated by fitting droplet magnitude to a linear-mathematical expression. Slopes of the dependencies were collected and correlated with an elasticity-derived factor and inserted into an adapted model. The existence of a PEG material at interfaces changes the responses of the biomacromolecular layer to external force, rendering

interfacial networks with enhanced strength. Given the benefit of the extended stabilization of less spherical droplets, one may show the possibility of chemical adaptation of the interfacial properties of the droplet with some biotin units. The steadiness of microdroplet form in the presence of surface-active compounds which also assist as a possible anchor for integrating functional segments enlarges the possibility to apply this in many industries.

28.3.2 Rheology of foams and biofilms

Rheological analysis of foam-type samples is very hard given the complex structure and the nature of the components [43–45]. The response to shear deformation is the cumulative result of several factors, such as liquid characteristics (i.e., viscosity, volume fraction, viscoelasticity, surface tension), gas properties, air phase volume, bubble shape, size, and distribution within the sample. The peculiarities of the adsorbed biosurfactants have altered the features of the thin fluid film. The practical characterization by rheological analysis of the foam sample is difficult because of its inherently unstable nature. The sort of biosurfactants is affecting the surface tension and impacts the characteristics of the lamellae thin layers by changing the degree of surfactant adsorption on the interface. The slow creep under the values of the yield stress may determine the variation of foam sample structure and coarsen. As a result, one may regard this as a dynamic process taking place inside the foam. Shear oscillatory tests are describing the deformation energy retained in the foamed material subjected to shearing, which can be depicted by Eq. (28.1):

$$G_{\text{elast}} = \phi_{\text{gas}}(\phi_{\mathbf{gas}} - \phi_{\mathbf{c}}) * (\gamma / \boldsymbol{r}) \tag{28.1}$$

where G_{elast} is the elastic or storage modulus, ϕ_{gas} is the gas volume fraction, Φc is the critical gas volume fraction, γ is the surface tension, and r is the bubble radius.

When overcoming the magnitude of the Φc parameter, the spheres from the foamed sample are coming apart and the mechanical resistance of foam is lowered until is transformed into a bubbly liquid. Such kind of stability loss is named "rigidity loss transition point" [43].

The loss modulus is depicting the viscous features of the foam revealing the deformation energy involved in shearing and lost in the examined material, as shown by relation (28.2):

$$G_{\text{visc}} = \left(\phi_{\text{gas}} - \phi_{\text{c}}\right)^2 * (\gamma / r) \tag{28.2}$$

where G_{visc} is the loss or viscous modulus.

During foam flowing, one may notice that the bubbles are moving past each other and while the stress was gradually higher, the structure is affected so the plastic properties appear. When the sample is sheared, the bubbles become strained, and given the osmotic pressure, they store surface energy. This is reflected in linear viscoelastic behavior in the regime of small values of shear stress, whereas the sample flows similar to a viscous liquid when the shear stress is sufficiently high to generate bubble redistribution. Such behavior can be linked to the foamed features at the level of the bubble size. Consequently, it requires yield stress and corresponds to the class of "Bingham fluids" that present a viscosity ranging differently regarding the imposed shear. The scale of the rheological moduli, as well as the yield stress for this case, is set by the Laplace pressure of the bubbles.

Within the linear regime, the rheological moduli are impacted by Princen relation at various liquid fractions, average bubble dimensions, and surface tension [46]. In fact, elastic behavior is not

truly observed. Regardless of the suppressing of the drainage and coalescence processes, the sample tends to age owing to Oswald ripening. The latter determines a new configuration (having low energy) due to local bubble packing resulting in an induced elasticity by external stress which is partly transformed to irreversible strain. The local elastic stress present there before the redistribution stage might be relaxed in this way [47,48].

In the situation in which the strain is larger than the yield point, the bubbles are suffering irreversible topological modifications and the sample displays non-Newtonian liquid-like properties [49]. Yielding is often noticed at strains around 0.1−1, sufficiently big so that nonlinear elastic character could be expected prior to the beginning of the plastic flow. When an elastic material is not stressed enough to show considerable plastic characteristics, the generated stress goes along with uneven normal stresses. For such cases, Poynting effect [49,50] explains the connection between the strain and normal stresses difference for nondissipative samples (lacking anisotropy) with prevalent elasticity. The viscoelastic linear features, but also the magnitude of yield stress seem to be impacted by the liquid fraction and there is noted a transient relaxation zone, separating the recorded responses comprised in the storage modulus and ultimately flow of the aerated material in steady conditions. This is caused by intermittent ephemeral reduction of elasticity by coarsening-generated structural repositioning at bubble level.

Van Kempen [45] studied the rheological behavior of oligofructose fatty acid esters. It was shown that the interactions between oligosaccharide chains are not affecting very much the shear response. The bulky character of the hydrophilic group determines the occurrence of head groups interactions at a longer length scale in comparison to the situation of a disaccharide. Therefore in the system, it appears an interfacial soft glass which is reflected in the rheological properties at the interface, which in turn produces differences in macroscopic features.

Hollenbach et al. [13] investigated the foaming characteristics of tailor-made glycolipids emphasizing the effect of hydrophilic head group on the interfacial rheology properties. Head (glucose, sorbitol, glucuronic acid, and sorbose) and tail group are influencing the foam stability. The interfacial elastic moduli of samples with branched or monosaturated fatty acid segments seem to be unaffected by frequency owing to rapid absorption kinetics. In the compression and dilatation stages, there are some interface-bulk molecular exchanges and thus glycolipid concentration gradients are compensated which determines small apparent interfacial elasticities. The specimens having linear fatty acid tails displayed larger interfacial elastic moduli than branching samples. For the first case, the diffusion is much more reduced than for systems with branched or monounsaturated fatty acid parts. The biosurfactant concentration variations are reduced with increasing dilatational frequency and determine a variation with the frequency of elastic moduli. At frequency under 0.1 Hz, the interfacial viscosity is larger for sorbose, sorbitol, and glucuronic-derived samples. Unsaturation in the fatty acid tail produced a diminishment of the interfacial viscosity, while branching generated the smallest viscosity among discussed glycolipids. Beyond 0.1 Hz the changes in interfacial viscosity are no longer relevant and at the highest frequency, the equilibrium of dilatational viscosity is remarked. The foam stability is best for samples having sorbose and glucuronic acid parts, is recommended for the formulation of foamed commercial products.

Qi et al. [51] have analyzed the role of some biosurfactants on biofilm formation using rheology. More specifically, they studied how flagella, type IV pili, biosurfactants, and extracellular polysaccharides are influencing the appearance of thin layers by *Pseudomonas aeruginosa* at the interface of air with water. Distinct features are noted when studying the wild-sort and mutant

strains which lack flagella. During 72 hours, the mutant less flagella present diverse behavior in the moduli, but no peak is observed in their dependence of time plots. The delayed attachment was notable in the case of the described mutant samples by the initial flat lines in the graphs regarding the wild-sort sample. The authors remarked no consistent trend in moduli ascribed to the mutant type samples little less than the wild-sort layer. Aspects that influence adsorption and growth impacts pellicles like literature data concerning solid surface biofilms.

Rühs et al. [52] have used interfacial rheology for characterization of biofilm properties under various external factors. The types of bacterial species led to specific viscoelastic growth profiles that in turn were impacted by the utilized growth media. One may diminish biofilm appearance by the introduction of surfactants, but also by modifying the pH, which can modulate the viscoelastic behavior of the sample. All these aspects were reflected in variations of viscosity, elasticity, and surface tension, under fixed and variable external conditions. Rheological moduli are affected by cell density and network occurrence. Via specific interactions occurring in the adsorbed biological medium, the interface turns into a viscoelastic one. An enhancement in the shear moduli is consequently an indication of higher cell adsorption, development at the interface, and network appearance via the generation of biofilm constitutive elements. In the presence of biosurfactants, a transient evolution of the elasticity is observed. After 15 hours, a sudden reduction of elasticity was noted for the *Bacillus subtilis* strain. The reported rheological data are essential for elucidating phenomena involved in the biofilm appearance, including dispersal.

28.3.3 Rheology of solutions

Literature [16,17,53] presents a few studies of rheological testing of biosurfactants in solutions. Xu and Amin [16] studied the effect of shear flow properties of rhamnolipids biosurfactant (noted CCB) on a mixed surfactants system composed of anionic sodium laureth sulfate (SLES) and zwitterionic cocamidopropyl betaine (CAPB). Under low shearing, a big decrease in viscosity was noted when inserting biosurfactants. SLES/CAPB mixture presents Maxwellian response normally remarked for entangled wormlike micelle. Upon introduction of CCB, the storage and loss moduli crossover is moved towards larger frequencies revealing a reduced relaxation time. The Cole−Cole graph shape is changed from semispherical one observed for samples Maxwellian features. The latter is remarked for in SLES, SLES/CAPB, and SLES/CAPB/CCB systems. In any case, a poorer entanglement and possible reduction in the size of micelles occurs when inserting CCB. The contour size of the wormlike micelle was lowered in the presence of biosurfactant, whilst the persistence length was not reduced that considerably. This could be interpreted as shorter more rigid rods resulting upon adding CCB regarding initial bigger size micelles in SLES/CAPB. The reduction of viscosity when inserting CCB could not be ideal for personal care products. However, this can be changed by adding salts to the system. Maxwellian kind of responses is noted at the lower pH, where the rheological moduli also present bigger values. The overlapping point at small frequencies, revealing the largest relaxation time, is shifted to higher intervals at small pH. Thus a systematic exploration of biosurfactant systems using rheology helps in the proper processing and formulation of personal care products.

Machale et al. [53] investigated xanthan gum (XG) solutions in water in the presence of a biosurfactant produced by *Eichhornia crassipes*. Rheological characterization of such a system was done for oil recovery purposes. The viscosity of aqueous XG solutions seems to increase upon the

addition of biosurfactant, while it decreases as the applied shearing is stronger. Shear oscillatory tests indicated that storage modulus is bigger than the loss one, the sample being analogous to a viscoelastic solid. There is a specific feature of the aqueous XG-based liquids when the elastic modulus is lowered with increasing strain amplitude. This is indicative of disruption of the internal structure of a sample. When adding the biosurfactant the sample has a higher elasticity and also displays a smaller relaxation mechanism. It was remarked that the loss modulus presented overshoot after 4% strain for the XG sample with biosurfactant. Considering the peculiar architecture of XG and peculiarities of the fibers of biosurfactant, the molecules were oriented and unified, resulting in a loosely type of architecture. With enhancing of strain, the material can withstand the deformation up to the point denoting the augmentation of the loss modulus. After this point, the structure was destructed by a bigger deformation exceeding the critical strain; beyond it, the macromolecules oriented along the flow field, and viscous modulus decreased. Further and gradual augmentation of strain determined disentanglement of chains and their alignment. In the linear viscoelastic range, the prepared polymer solutions present elastic nature, which is reflected in a gel-like structure. This was additionally enhanced by the presence of biosurfactants in XG samples due to strong interaction among the fibers and the helical chains. The XG fluid was degraded at higher temperatures and this limits its use for oil recovery applications. The rheological moduli of biosurfactant-containing systems initially decreased and beyond 325 K started to increase again. This is the result of the appearance of a strong and ordered network. So, the used biosurfactant leads to a highly stable and well-organized structure that is more suitable for the pursued application.

28.4 Conclusions

Bulk or interfacial rheology is an excellent tool for monitoring the properties of biosurfactants found in emulsions, solutions, or foams. The sample response to shear deformation can be quantified through shear viscosity and rheological moduli, describing the degree of microstructure alignment, consistency, elasticity, and even relaxation time. Depending on the biosurfactant structure and the ascribed system characteristic, one may use rheology to tailor the mechanical properties to obtain products with biological components and higher performance, thus opening perspectives in areas like medicine, cosmetics, environmental protection, agriculture cleaning, nanotechnology, and more.

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CHAPTER

Biosurfactants for optimal delivery of poorly soluble therapeutic agents

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29.1 Introduction

Sustainable formulations have garnered considerable interest in the pharmaceutical and cosmetic industries. This pressing need for sustainability is the driving force for the evanescing scenario from synthetic surfactants to biosurfactants, which can be a better option with several advantages [1]. The biosurfactants are amphiphilic in nature and contain hydrophilic as well as lipophilic groups. The lipophilic nature comes from the tail of biosurfactants by long hydrocarbons. While hydrophilic nature comes from the head groups because of a phosphate group, peptide group, amino acid, carbohydrates, and some other molecules [2]. The amphiphilic nature makes them effective as surface-active agents which can reduce the surface and interfacial tension between two phases and help in the solubilization of several therapeutically active compounds.

Commonly, biosurfactants are neutral or anionic compounds often synthesized by microbes, plants, and animals [3]. The biosurfactants of microbial origin are secondary metabolites that are secreted outside the microbial cell or remain glued to the microbial cell surface. These can also be produced by processes that are enzymatically controlled. The advantages of biosurfactants are lower toxicity, high foaming capacity, benign interaction with the environment, and reasonable biodegradability [4]. They have lower values of critical micelle concentration (CMS) than synthetic surfactants, which imply efficient applications [5].

Besides several potential advantages, there are some limitations or disadvantages of these biosurfactants such as higher cost of production and typical process of purification.

These biosurfactants are synthesized by various typical processes of biotechnology, which makes them more expensive and time taking [6,7]. Intensive research is being carried out to reduce the production cost, ease out availability, and identify renewable raw materials as bioresources. Biosurfactants can be utilized in formulating colored cosmetics, hair care, and skincare products. This consumer-driven industry that focuses on performance benefits, long-lastingness, pleasing sensory attributes, favorable physical stability attributes needs them to be delivered through sustainable materials [8,9]. Apart from personal care products, biosurfactants find applications in laundry detergents. Presently, surfactants used in most the laundry are chemically synthesized, these can be replaced by biosurfactant, which has no biotoxicity and contamination can also be avoided in agriculture fields [10].

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These biosurfactants have several medicinal properties, including antimicrobial and antiviral activities. They have a significant role in the development of formulation in food industries as emulsifiers and stability enhancers by virtue of their ability to lower surface tension. The critical properties of biosurfactants are lowering surface tension and interfacial tension between air or water and oil or water [11].

The chapter highlights various biosurfactants and details their potential pharmaceutical applications. We have elaborated on the diverse properties and the potential for practical applications. We will highlight the importance of biosurfactants in pharmaceutical products especially for the delivery of poorly soluble drug substances.

29.2 Biosurfactants: important component in pharmaceutical products

The use of chemically synthesized surfactants is rampant in households, agriculture, and industries but the critical environmental matters that arise due to synthetic nature and indiscriminate applications have been considered a matter of serious increasing global concern [12]. The need for environment-friendly biosurfactants which have reasonable biodegradability are mostly preferred over the past few years. The surfactant can be classified as semisynthetic, synthetic, biosurfactants (biological) based on their origin source. Among them, synthetic and semisynthetic are most widely used. The oleochemical surfactants are prepared by various chemical processes from the fats and vegetable oils, hence, they are known as semisynthetic surfactants [13]. Biosurfactants which are generated by biological systems (microbes, plants, and animals) act as natural emulsifying, foaming, wetting, the surface modifying, dispersing agents [12].

Importantly, the biosurfactants exhibit discrete pharmacological activities such as bacterial quorum sensing, biofilm formation, and cellular differentiation due to having a potential role in the cell−cell interactions. Stimulatingly, biosurfactants having low molecular weight have several significant activities. The biosurfactants (sophorolipid, iturin, and surfactin) have potent biological activities including antitumor, antiviral, antifungal, and antibacterial. These biosurfactants exhibit antimicrobial properties through cell membrane disruption [14]. The positive attributes of biosurfactants such as biodegradability, low toxicity, and distinct biological activities are major advantages for use in food, cosmetic, healthcare industries, and in the biomedical and pharmaceutical arenas. The high effectiveness of the biosurfactants under variable environments such as pH, salinity, and temperature enhanced their applications in the petroleum industry due to the higher recovery of oils [15]. On the flip side, despite various advantages of biosurfactants, they are not cost-effective for production at the commercial level which is important constraint to replace the synthetic surfactant besides potential application. Furthermore, the microbially synthesized biosurfactants have are composed of several ingredients instead of the single leading major component. Hence, isolation and purification of the major potent component have becomes one of the challenging tasks due to the likeness of their physical parameters and molecular structure. Consequently, due to lack of intricate information and interrelated physiochemical characteristics of the mixtures [16].

This is not only a constraints for their use but also does not permit optimization of their fermentation procedure for definite molecules or class of molecules. Several researchers have worked on the development of synthetic processes to get predefined molecular structure and physicochemical

properties of biosurfactants. Consequently, various biosurfactants and their derivatives with predefined molecular structure and purity have been developed through the chemical synthesis process. Furthermore, it was easy to establish a relationship between their structures, bulk aggregation profile, interfacial behavior, and precise applications [8]. A wide range of microbial strains and processes are utilized for the production of biosurfactants with exclusive chemical structures with desired interfacial properties. Hence, it can be concluded that biosurfactants may be produced to get precise requirements. The microbially derived biosurfactants may be dived into two groups, the first one having low molecular weight such as lipopeptides and glycolipids; the second one having a high molecular weight (HMV) like lipoproteins, polysaccharides, proteins, and lipopolysaccharides. HMV biosurfactants can strongly bind with the different surfaces hence they also work as bioemulsifiers [17]. For example, emulsan have two distinct portions one is hydrophobic and one is hydrophilic which stabilizes the emulsions. Rhamnolipids can be a good example of a low molecular weight biosurfactant which have acetylated disaccharides with long-chain fatty acids. These are most commonly used for lowering the interfacial and surface tension.

Several important biosurfactants (sophorolipids, surfactin, and rhamnolipids) are obtained from the microorganisms which are most commonly found in soil and water. These types of microorganisms can survive in extreme environmental conditions. The production rate of biosurfactants through microorganisms is higher than plant and animal sources [13].

The processing parameters have a significant role in the production rate and physicochemical properties of the biosurfactant. These properties depend on microbial strain, temperature, nature of the substrate, ionic concentration of culture media, culture conditions, salinity, and pH of the medium [5]. Biosurfactants can either be secreted out or can be in the cell of microorganisms. The production process of biosurfactants contains several typical enzymatic reactions, the molecules have also complex structures. The structure can be modified either by genetic engineering or through the selection of appropriate culture media.

In context to the global acceptance of biosurfactants, the worldwide commercial market value of the biosurfactant reached to US$43.6 billion in 2017 which is expected up to US$66.4 billion in 2025. The rate of registration for the new compound can be also increased up to 5.4% in 2025 [17]. Several manufacturers have taken different initiatives for the large-scale production of the biosurfactant. A few manufacturers of authentic biosurfactants include Kingorigin (China), AGAE (USA), Evonik (Germany), MG Intobio (South Korea), Jeneil Biotech (USA), Saraya (Japan), Ecover (Belgium), GlycoSurf (USA), Soliance (France; now Givaudan, Swiss), TensioGreen (USA), Rhamnolipid (USA), NatSurfact (USA), and Victex (China) [13]. The origin, synthetic process, and structure of the biosurfactants impact their physicochemical properties. It is necessary to elucidate these relationships to maximize their potential benefits by tailoring the molecules suit a specific application. The inevitable comparison with synthetic surfactants assists in understanding the relationship between surfactant structure and the physicochemical properties thereby helping in formulation development.

29.3 Potential advantages of biosurfactants

The biosurfactants act as surface-active agent and make micelles due to their self-assembling ability. They make micelles in different shapes such as wormlike, spherical, and rod-like structures and

enhance their specificity. The ability of biosurfactants to reduce the interfacial tension and surface tension makes them one of the most important ingredients of pharmaceutical products. These surfactants have more efficiency to reduce surface tension than synthetic. The CMS value of these type of surfactants are lower than the synthetic biosurfactants, hence they are mostly preferred by manufacturers [13].

This clearly shows that a lesser amount of biosurfactant is required for the maximum decrease in surface tension. Furthermore, the understanding of the structure-property attribute of the biosurfactants is necessary; therefore, considerable progress has been made on clarifying molecular structure along with their potential activity. Some important characteristics of biosurfactants like microrheology, surface activity, and bulk rheology have potential influence on the performance of foaming, cleansing, and emulsification [11]. Some potential advantages of biosurfactants over other surfactants have been depicted in Fig. 29.1.

The superior advantages of biosurfactants over synthetic surfactants have been the driving force for research activity in this arena. This has been evidenced by the notable rise in both scientific articles, as well as patents on the topic. However, it is important to record that the trend shown by patents in biosurfactants is not proportional to research activity, though is gaining momentum. Presently, the area of biosurfactants has gained attention by research scientists across the globe, publication ratio has become two times from last 5 years [5]. The biosurfactants have several

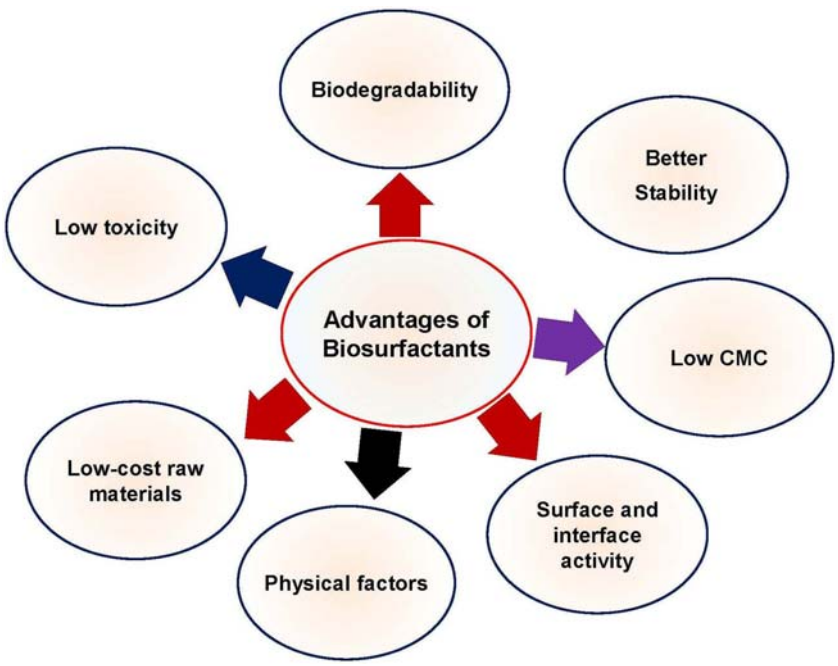

FIGURE 29.1

Significant advantages of biosurfactants.

important applications for the development of different pharmaceutical preparations some of them have been discussed hereafter.

29.3.1 Biodegradability

The impaired biodegradability of the hydrocarbon chain has been a matter of concern for chemical surfactants. These biosurfactants have better biodegradability and are ecofriendly than synthetic surfactants. Mohan et al. [18] have studied the biodegradability of biosurfactant and synthetic surfactants and found that rhamnolipids (biosurfactant) had better degradation than the Triton X-100 (chemical surfactant). Further, rhamnolipids were found to have more effective for the nitrate-reducing and sulfate-reducing ability than the Triton X-100 [8].

29.3.2 Low toxicity

Chemical surfactants have more toxicity than biosurfactant. The chemical surfactants exhibit bioaccumulation; and their production, manufacturing processes, and byproducts can be damaging to the environment. Biosurfactants have been found to have low toxicity than biodegradable. Edwards et al. [19] have performed a comparison of the toxicity profile of chemical surfactants and biosurfactants, chemical surfactants were significantly more toxic than the biosurfactants. The biosurfactants selected for the study were obtained from the microbial origin namely *Mysidopsis bahia* and *Menidia beryllina*. Many researchers observed high toxicity due to Triton X-100 (chemical surfactant) as compared to the biosurfactants [20].

29.3.3 Cost-effectiveness

Most of the biosurfactants are obtained from the acceptable and cost-effective feedstocks of microorganisms. Substrates having low cost are under investigation in long term [21]. Das and Mukherjee [22] studied potato peels for the production of biosurfactant, a lipopeptide from *B. subtilis*. Furthermore, Costa et al. [23] explored the production of rhamnolipids by using microorganisms (*Pseudomonas aeruginosa*) by using substrate from the cassava wastewater and waste cooking oil. Gurjar and Sengupta [24] produced surfactin by using substrate from the residue of rice mill polishing. Glycolipidic biosurfactant can be produced by using the residue of groundnut oil refinery and corn steep liquor [25]. The use of inexpensive substrates presents the obvious advantage of production cost reduction in addition to the lasting advantage of sustainability. The industrial waste and byproducts can be used as microbial substrates which also help in nurturing preservation of environment [26]. These substrates can not be the same as food. Hence, during the selection of substrate, we must have to consider various factors such as types of strains of microorganisms to get an optimum yield of products. Several manufacturing parameters should be also considered for the effective production rate such as pH and temperature [27]. Additionally, the quality of substrate not only affects the type of biosurfactant but also can affect the molecular structure and physicochemical properties of the product. Therefore, the research is driven toward cost-effective substrates and their effect on product, and appropriate provisions are made to achieve a quality product with maximum yield [28].

29.3.4 Temperature and pH tolerance

The biosurfactants from extremophiles have garnered considerable interest from the last decade for commercialization. Synthetic surfactants tend to be unstable with changes in temperature and pH. While most of the biosurfactants have no significant effects of environmental factors such as temperature, pH, modulation in ionic strength of the culture medium [11]. Lichenysin was produced by *Bacillus licheniformis* found to be stable at a temperature of 50°C, pH range of 4.0−9.0, and concentration of NaCl up to 50 g/L and Ca^{2+} concentration of 25 g/L [26,29]. Likewise, another microbial biosurfactant produced by *Arthrobacter protophormiae* was thermostable (30°C−100°C) and pH stable (2−12). Since, industrial processes involve the use of extreme pH, temperature, and pressure, it is extremely important to identify strains of microorganisms that can survive and function in extreme conditions [30].

29.3.5 Surface and interface activity

The surface activity, interfacial activity, and surface rheology play a significant role in formulation development. Most of the personal care emulsion-based formulations are stabilized by surfactants. Thus, a grasp of the surface-active properties of surfactants is important to confirm the stability and efficacy of the pharmaceutical product. Biosurfactants by virtue of their superior surface properties to surfactants are valuable in formulation development. The surface tension of water can be reduced by the biosurfactant from 75 to 35 mN/m. Further, interfacial tension of water/hexadecane can be lowered from 40 to 1 mN/m. Surfactin has been considered one of the important biosurfactants which can lower the surface tension of water to 25 mN/m and interfacial tension of water/hexadecane up to <1 mN/M [26,29]. The biosurfactants generated by *Bacillus salmalaya* 138SI were capable of reducing the surface tension to 27 mN/m [31].

Surfactin produced from *Bacillus subtilis* and rhamnolipid from *Pseudomonas aeruginosa* can lower the surface tension to 30 mN/m [32]. Further, biosurfactant obtained through *P. aeruginosa* can lower surface tension from 56.1 to 42 mN/m after 4 days of incubation with enhanced polycyclic aromatic hydrocarbon degradation [33]. Surfactin was used for the development of microemulsion and has lower toxicity and better physicochemical stability of formulation in comparison to chemical surfactants. The research reports affirm the replacement potential of typical surfactants with biosurfactants (rhamnolipids), in polyelectrolyte surfactant mixtures of cosmetic interest, thus paving way for the development of eco-friendly formulations [11].

29.4 Classification of biosurfactants

Biosurfactants may be divided into two classes: one having HMV and the other, with low molecular weight (LMW). The lipoproteins, lipopeptides, neutral lipids, phospholipids, and glycolipids fall under low molecular weight whereas the polymeric and particulate biosurfactants come under HMV [13]. The LMW biosurfactants are more efficacious in reducing the surface tension and interfacial tension between water/air/oils. However, HMW biosurfactants are mostly used for the stabilization of oil-in-water emulsion and are commonly known as bioemulsifiers. The molecular weight of most of the biosurfactants varies from 500 to 1500 Da [11]. Several factors that can influence

the biosurfactant characteristics include the agitation speed, temperature, types of sources for carbon and nitrogen, and pH of the media [34].

The biosurfactants are mostly used in the formulations due to having the ability to lower the surface and interfacial tension, reducing CMC value, emulsification and de-emulsification. The most widely explored biosurfactants obtained from the microbial sources are as follow emulsan (*Acinetobacter calcoaceticus*), rhamnolipids (*P. aeruginosa*), sophorolipids (*Candida bombicola*) and surfactin (*Bacillus subtilis)* [13]. However, chemically synthesized surfactants have been classified based on the polarity index. Additionally, biosurfactants are further categorized based on the following factors such as molecular weight, mode of secretion from a microorganism (extracellular and intracellular) and type of surface charge (neutral, anionic, and cationic). Biosurfactants are further categorized based on the type of moiety presents such as phospholipids, fatty acids, lipopolysaccharides, glycolipids, and lipopeptides (Fig. 29.2). Each group present on the biosurfactants has a very specific role in the molecular structure as well physiological and physicochemical properties of biosurfactants [35]. Among them glycolipids are most widely explored due to their significant yield than the rest of the classes hence, they are being produced at a large scale for commercialization. The subsequent text details the biosurfactants based on the type of moiety.

29.4.1 Glycolipids

Glycolipids are bonded with covalent bonds with carbohydrate groups which can be monosaccharides, disaccharides, or polysaccharides. Trehalose containing glycolipids are known as trehalose-lipids, sophorose containing glycolipids are known as sophorolipids, rhamnose containing glycolipids known as rhamnolipids, and so on. Among all the types of glycolipids, rhamnolipids and sophorolipids have been most widely explored [36,37]. Sophorolipids have different structural arrangements mostly explored architecture is open and cyclic arrangements. They have a major

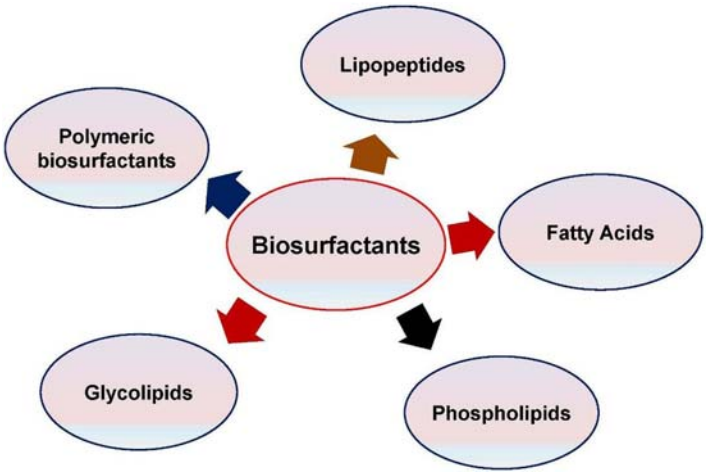

FIGURE 29.2

Types of biosurfactants.

functional group present is a carboxylic acid (−COOH) at the end of the lipophilic chain. Cyclic arrangement play role in the function of the ester which results in condensation amongst fatty acid and one hydroxyl moiety of sophorose, these cyclic esters are known as lactones [13]. Rhamnolipids have been considered as the best biosurfactants which have potential physicochemical parameters. They have been considered as most appropriate for various industrial applications due to having excellent surface activity and biological activities. Natural glycolipids may be obtained for fungi, yeasts, bacteria, and actinomycetes. Mostly the glycolipids are found in the extracellular medium of the fermentation [8].

Most of the glycolipidic microbial biosurfactants are obtained from different species of *Pseudomonas*. Glycolipidic has glycosyl head group and a fatty acid tail. Rhamnose moiety is the glycosyl head and 3-hydroxydecanoic acid is the fatty acid tail in rhamnolipids. The rhamnolipids generated by *Pseudomonas aeruginosa* have been explored widely. Further, in other rhamnolipid which are obtained from *P. fluorescens*, a methyl pentose monosaccharide microbial strain [38]. The type of rhamnolipid produced depends on the type method used for the fermentation of microorganisms. Thus, either a mono-rhamnolipid or a di-rhamnolipid varies through the number of rhamnose groups present. Rhamnolipids can also be differentiated based on the chain length, the degree of branching in the fatty acid tail, and the amount of unsaturated fatty acid chains, these parameters are deiced by fermentation conditions [5]. The bacterial strain of other families, the class, or even the has resulted in the discovery of about 60 different rhamnolipid homologes and congeners can be obtained by making changes in the concentration of *Pseudomonas* species. The major requirements for the production of rhamnolipids are sugar moiety which comes from glucose and alkanoic acid comes from fatty acids. These major components are sufficiently found in most bacteria. But additionally, some specific enzymes are required for the production of rhamnolipids which are only found in some selected species of *P. aeruginosa* and *Burkholderia* [5,8].

29.4.2 Lipopeptides

Lipopeptides are also one of the important classes of biosurfactants made by attachment of lipid and polypeptide chain [39]. Lipopeptides have potential antimicrobial properties such as antibacterial and antifungal properties. Iturin a potential lipopeptides biosurfactant produced by *Bacillus subtilis* can survive after autoclave at pH 5−11. Iturin has a long duration of shelf-life of 6 months at −18°C [40]. Further, surfactin is another important lipopeptides biosurfactant that is made up of seven amino-acid rings linked with the fatty-acid chain via lactone linkage. Surfactin is obtained *B. subtilis* and has the ability to lower both the interfacial and surface tension of water. It is also capable of inactivation of herpes and retrovirus. Structural modulation can lead to alteration in their physicochemical properties. Structural modification can be done either in fatty acid chain length or changes in the amino acids [5]. Liu et al. [41] have reported that surfactins, putisolvins I and II can be profoundly obtained from Gram-positive endospore of *B. subtilis* BS-37. Commercial application of surfactins is limited by its low yield valve and high cost of production. Various researches are going on the cost-effective production of surfactins [34]. Micelles formed by most of the synthetic lipopeptides are one-dimensional like nanofibers and helical nanoribbon in aqueous solution at the higher concentration of critical aggregation concentration, while surfactin forms the spherical shape. Further, surfactin was produced by a new strain *B. nealsonii* S2MT. The source for the carbon and nitrogen was used as 2% glycerol and 0.1% NH_4NO_3, respectively. The developed

biosurfactant was able to lower the surface tension up to 34.15 ± 0.6 mN/m with excellent emulsifying properties [42].

29.4.3 Fatty acids

Fatty acids are a significant class of biosurfactants that has been used in the development of several pharmaceutical products. Various bacteria and yeast produce large quantities of fatty acids and phospholipid biosurfactants when grown on n-alkanes. A clear microemulsion of alkanes in water can be produced by using phosphatidyl ethanolamine which can be obtained from different species of *Acinetobacter* [30]. Gautam and Tyagi [43] have studied infants and found that respiration failure was due to a lack of phospholipid protein in premature infants. They have suggested that there is a need to be cloning and isolation of genes that are responsible for the production of such biosurfactants. Further, the production of such biosurfactants necessitates fermentation methods [34].

29.4.4 Polymeric biosurfactants

Polymeric biosurfactants have been explored exponentially in the last decades. There are various effective polymeric biosurfactants that have been evolved such as lipomannan, emulsan, alasan, liposan, and many other which are mainly based on polysaccharides and proteins. Among them, emulsan is the most efficient which prominent emulsifying properties. Emulsan have good efficacy because they have significant activities at the concentration of 0.001%−0.01% [44]. Further, liposan (water soluble) is a potential emulsifier which was produced by *Candida lipolytica*. The major components of liposan are proteins (17%) and carbohydrates (83%). These polymeric biosurfactants can be used in cosmetic and food industries as an emulsifier [30].

29.4.5 Phospholipid

The phospholipids are most commonly found in plants, the cell wall of microorganisms, and animals. They have a significant role in maintaining cellular integrity and transport of cellular constituents by making semipermeable membranes [45]. Phospholipids are composed of glycerol which plays the role of backbone along with phosphoric acid groups and fatty acids. These fatty acid groups make the lipophilic tail and phosphoric acid groups have a role to make the emulsifier polar head hydrophilic. These phospholipids are amphiphilic in nature due to having polar and nonpolar regions hence can be used for the solubilization and stabilization of lipid droplets [46]. These phospholipids work as an emulsifier by adsorbing in the tail of the water−oil interface of fatty acids and polar head groups surrounding the aqueous phase. Sometimes these phospholipids make monolayers around oils; these may also make multiple bilayers which have a significant role in the stability of emulsion [47].

Lecithin has been considered as a most explored biosurfactant as an emulsifier for the development of several formulations for commercialization. The most common sources of lecithin are milk, cottonseed, soybeans, sunflower, eggs, canola seed, etc. Liu et al. [48]. Lecithin is composed of several phospholipids and lipophilic components such as sterols, triglycerides, and glycolipids, further they can be modified to get more effective [49]. The major phospholipids found in lecithin are phosphatidylinositol, phosphatidylcholine, phosphatidic acid, and phosphatidyletanolamine [50].

The head groups of these phospholipids have a charge of either zwitterionic or anionic which will depend on the pH of the environment. We can do the modifications for the required hydrophilicity and lipophilicity by modifications [51].

29.5 Biosurfactants for delivery of poorly soluble drugs

Solubility of drug substances has poised as an enormous challenge for the pharmaceutical industry, which has triggered pharmaceutical scientists to explore novel technologies to deliver the poorly soluble drug candidates [52–58]. Improved dissolution velocity of drugs having poor aqueous solubility may improve their bioavailability [55,59–61]. The pharmaceutical sector has exploited biosurfactants to the fullest possible extent due to their diverse properties suitable for industrial applications [62].

Basically, emulsions are thermodynamically unstable bi-phasic systems comprising of two nonmiscible parts, in which one part (dispersed) is distributed in another part (continuous) as fine droplets in the presence of a surface-active stabilizer called an emulsifier or emulsifying agent [53]. These emulsifiers are deposited at the interface of the two district phases and stabilize the thermodynamically unstable system and biosurfactants have been extensively deployed in the pharmaceutical/cosmetic/nutraceutical industries as effective emulsifying agents with proven desirable features like lower toxicity profile and better biodegradability. HMV biosurfactants are often deployed as effective stabilizers/emulsifiers in the pharmaceutical emulsification process.

Macro-, micro-, and nanoemulsification technologies have been widely adopted by formulation scientists to improve the solubility of poorly soluble active pharmaceutical ingredients and thereby offer optimal delivery strategies for these challenging drug molecules [59,63–65].

The literature reveals diverse groups of surfactants employed as emulsifiers in the stabilization of pharmaceutical emulsions. Mannosylerythritol lipids (MELs) have shown a much higher emulsifying activity with soybean oil and tetradecane than polysorbate 80 [66]. The emulsifier has the functionality to form stable W/O microemulsions without the addition of a cosurfactant or salt [67]. Reportedly, a mixture of rhamnolipids, sophorolipids, and lecithins can be used to develop microemulsions wherein the phases are not affected by any thermal variations, hence rendering these biosurfactants appropriate to design cosmetic or drug formulations. Rhamnolipids have been used in the fabrication of liposomal vesicles for the delivery of cargo molecules. Moreover, rhamnolipids were found safe as absorption enhancing agents for orally administered active pharmaceutical ingredients. Rhamnolipids were found very safe in Caco-2 cells studies and erythrocytes [68]. Onaizi and collaborators [69] explored the efficiency of a mixture containing surfactin and confirmed the emulsion stability. In another study, surfactin was used to improve the oral bioavailability of insulin in experimental animals [70].

Biosurfactants from the microbial origin are found to have many desirable features like (1) long-term stability, (2) ease of preparation, and (3) excellent solubilization potential and hence are suitable for drug-delivery applications [9,71].

Formulating drugs as nanoparticles has been an interesting avenue to enhance drug solubility and solubility depended on drug bioavailability [54,58,72]. Biosurfactants have been used extensively exploited in the fabrication of drug nanoparticles [73,74]. Rhamnolipid nanoparticles loaded

with pheophorbide a (Pba), a hydrophobic photosensitizer, resulted in excellent water solubility without aggregation for one month [74]. Synthetic surfactants like Polysorbate 80 and/or Span have been in use by formulation scientists for the delivery of lipophilic drug substances to overcome their insolubility issues. However, these surfactants are found to be immunogenic and hence are not safe for clinical applications [75]. Superior drug solubilization potential of hydrophobins has been established for drug delivery applications [76–78]. Intriguingly, fungal hydrophobin SC3 was used to develop suspensions of poorly soluble drugs like nifedipine and cyclosporin A [79]. The fungal hydrophobin SC3 improved the bioavailability of both the cargo molecules compared to the pure drug powder. In another study, hydrophobin HFBII produced a twofold improvement in the efficacy of a lipophilic cytotoxic agent [80]. Hydrophobin-coated niosomes loaded with doxorubicin have substantially enhanced the anticancer efficacy of the drug with improved cancer cell selectivity [81]. The efficacy of the microbial hydrophobins can be attributed to their amphiphilic nature which may form self-assembled monolayers on lipophilic materials/drugs and can coat nanoparticles for efficient drug delivery purposes [82]. Taken together, hydrophobins are appealing candidates to formulate poorly soluble drug substances.

29.6 Concluding remarks

A huge quantity of surface-active agents is manufactured annually for consumption in various sectors like food processing industries, drug, and cosmetic industries. Biosurfactants are at the forefront as a part of the "green revolution" in the pharmaceutical and chemical industries. There has been a pressing need for cost-effective production of surfactants apart from the inclination of the world for a greener product development. This synergistically has triggered researchers to develop new biosurfactants which are "green" and produced at a lower cost. The possibility of manipulation of the structure of core biosurfactants will open a new avenue for tailored, smart, and stimuli-responsive delivery of drug substances. More extensive studies may be conducted in the future to explore newer possibilities to deliver challenging drug candidates using biosurfactants.

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CHAPTER

Role of surfactants in pulmonary drug delivery

30

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30.1 Introduction

We take our breathing and our pulmonary health for granted, but the lung is a vital organ that is susceptible to airborne infection and injury [1]. Pulmonary diseases are one of the primary sources of mortality and morbidity across the earth. Pulmonary disease is an umbrella term utilized to portray local (e.g., asthma, bronchitis, cystic fibrosis, emphysema, and tuberculosis) as well as systemic (e.g., cancer, diabetes, and pulmonary hypertension) diseases [2]. Approximately 251 million people report the occurrence of chronic obstructive pulmonary disease (COPD) with 3.17 million fatalities every year, creating it the third running cause of global deaths. About 339 million citizens undergo from asthma, the prevalent chronic disease among children affecting 14% global pediatric population. Above 10 million people suffer from tuberculosis, making it one of the most common infectious diseases while lung cancer kills more than 1.76 million people every year proving to be the most lethal cancer [1,2]. Briefly, pulmonary diseases disturb not only the quality of life of the individual patients but also their family members [3]. Therefore, pulmonary diseases are the leading universal public health concern follow-on in serious socio-economic and healthcare trouble [1–3]. Fig. 30.1 showed various key facts and numbers regarding life-threatening pulmonary diseases.

The most important factor leading to the development of pulmonary diseases are tobacco smoke (including passive exposure), outdoor and indoor air pollution (e.g., biomass fuels, heating machines, vapors from paints, building materials, and furniture), pollen grains/allergens, and occupational dust, compound and chemicals [4]. The pulmonary airways are also susceptible to constant exposure to bacterial, fungal, viral attacks, and infection [5]. In addition to these conditions, systemic metabolic diseases such as obesity and sepsis are also equally responsible for pulmonary airway diseases [6]. These metabolic diseases and other comorbidities, cause a rise in lung-specific and systemic biomarkers of oxidative stress (e.g., 8-isoprostanes and malondialdehyde) and reduce glutathione levels in the bronchoalveolar fluid. Additionally, it exacerbates various pulmonary conditions, for example, hypoxia, lessening in lung volume, dyspnea, and augmented bronchial sensitivity. Besides, environmental particulate matter such as coarse particles ($\leq 10\ \mu m$) enter the peripheral airways and are evacuated by the upper airways and nose. However, fines ($\leq 2.5\ \mu m$) or ultrafine ($\sim 0.1\ \mu m$) and gases pierce the distal airways probably produces inflammatory responses and injuries. Particularly, the ultrafine particles have a distinct pathology [4,7]. Numerous in vitro

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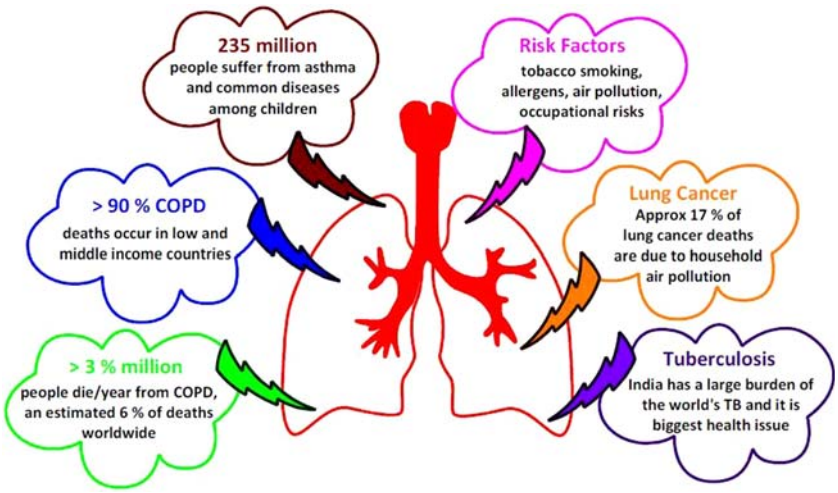

FIGURE 30.1

Various key facts and numbers regarding life-threatening pulmonary diseases.

and clinical investigations have confirmed that these ultrafine particles can reach deep within the airways to source localized swelling. They may also cross the protective surfactant coat of the alveolar sac to exert immediate intracellular actions in airways and/or penetrate the pulmonary vasculature [4,7]. The anatomy and pathophysiology of pulmonary airways allow for a range of options from nonspecific to site-specific targeted drug delivery systems. The pressurized metered-dose inhalers (pMDIs), nebulizers dry powder inhalers and soft mist inhalers are the dosage form options in the treatment of pulmonary diseases [8–10]. While, dry powder coating [11], jet milling [12,13], spray drying [14], extrinsic lactose fines [15,16], Technosphere, PulmoSphere, inhaled small particles easily respirable and emittable (ISPERSE), inkjet-printed aerogel particles, hot-melt extrusion, particle replication in nonwetting templates [17] and electrospinning/electrospraying [18,19] are the key platform technologies involved in the development of powders and particle suitable for pulmonary drug delivery. Understanding these aspects, various key therapeutic approaches, and molecules for the management of life-threatening pulmonary diseases are discussed in the next section.

30.2 Pulmonary diseases management: therapies and interventions

The existing therapies used to control pulmonary diseases mainly involve short-acting bronchodilators, for example, short-acting beta-agonists and short-acting muscarinic antagonists, long-acting bronchodilators, for example, long-acting beta-2 agonists and long-acting muscarinic antagonists, inhaled corticosteroids (e.g., fluticasone propionate and beclomethasone dipropionate), and tobramycin (bacteriostatic amino cyclitol glycoside) [20]. Additionally, molecules prescribed in the management of pulmonary disorders come in a variety of novel mono-, dual-, and triple-combinations [21]. Besides, the Global Initiative for Chronic Obstructive Lung Disease guideline recommends

tiotropium bromide for the treatment of COPD as it is believed to be the gold standard owing to its strong safety and clinical summary [22]. Fig. 30.2 showed the molecular structure of commonly prescribed molecules while Table 30.1 enlists the various marketed short and long-acting bronchodilators and steroids. Most of the listed molecules are prescribed to manage the unusual mucus secretion and swelling to limit the pulmonary tissue injure. Airway mucus hypersecretion is also the main feature of respiratory diseases such as COPD, asthma, and cystic fibrosis [23]. Therefore, various active researchers and clinicians also worked on numerous mucoactive agents intending to alleviate airways mucus hypersecretion. Mucoactive agents mainly contain:

- *Mucolytics* (reduces the viscosity of mucus), for example, cysteine, *N*-acetylcysteine, nacystelyn, thiopronine, ethyl-cysteine, nesosteine, dithiothreitol, usherdex-4 (a low-molecular-weight form of dextran), and peptide mucolytics (e.g., bromelain, leucine amino peptidase and dornase alfa) [23].
- *Expectorants/mucokinetics* (increases volume and hydration of secretions/enhances "kinesis" of mucus), for example, ambroxol, bromhexine, guaifenesin, inorganic iodides, sodium citrate, and YM-40461 (surfactant secretagogue) [23].

Among the mucoactive agents, *N*-acetylcysteine is one of the most frequently explored in the treatment of respiratory situations [23]. It is reported in the COPD course of action of both the American Thoracic Society and the European Respiratory Society. The aerosolized inhaled

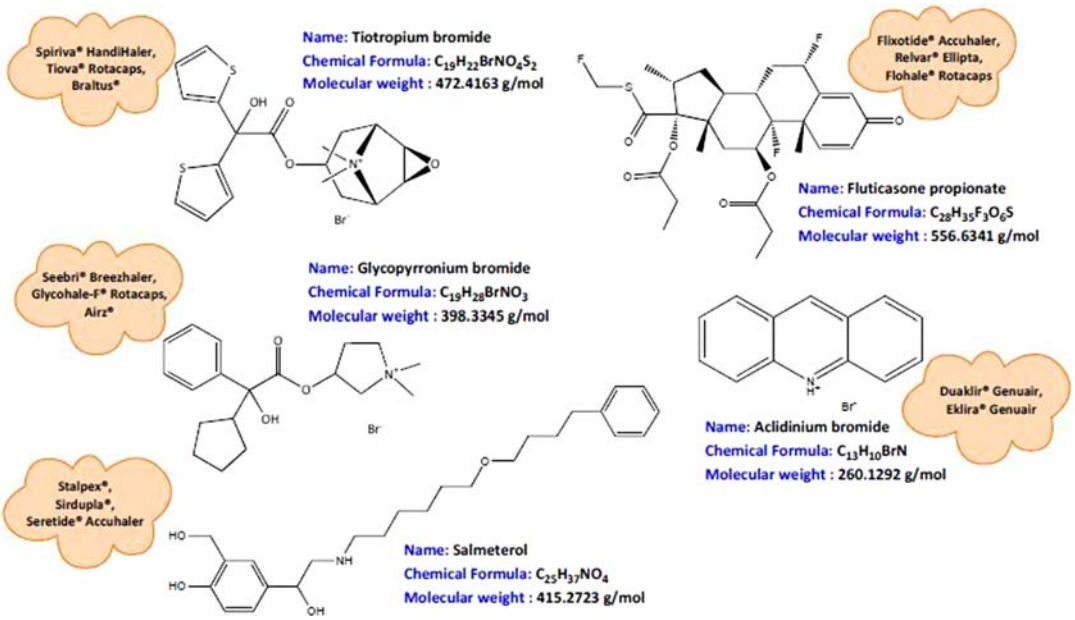

FIGURE 30.2

The molecular structure of commonly prescribed molecules in the treatment of pulmonary disease.

Table 30.1 Marketed short/long-acting bronchodilators and steroids with their combinations.

Active agent	Proprietary name	Device details	Company
Short acting beta agonist (SABA)			
Salbutamol sulfate	Salbulin MDPI Novolizer	Multidose device	Meda Pharmaceuticals Ltd., UK
Salbutamol sulfate	Easyhaler salbutamol sulfate	Multidose device	Orion Pharma Ltd., UK
Long-acting muscarinic antagonists (LAMA)			
Tiotropium bromide	Spiriva Handihaler	Capsule-based device	Boehringer Ingelheim GmbH, Germany
Tiotropium bromide	Braltus Zonda inhaler	Capsule-based device	Teva Ltd., UK
Glycopyrronium bromide	Seebri Breezhaler	Capsule-based device	Novartis Pharmaceuticals Ltd., UK
Umeclidinium bromide	Incruse Ellipta	Multidose device	Glaxo Smith Kline Ltd., Ireland
Aclidinium bromide	Eklira Genuair	Multidose device	Astra Zeneca Ltd., UK
Long-acting beta-2- agonists (LABA)			
Indacaterol maleate	Onbrez Breezhaler	Capsule-based device	Novartis Pharmaceuticals Ltd., UK
Formoterol fumarate dihydrate	Foradil	Capsule-based device	Novartis Pharmaceuticals Ltd., UK
LABA /LAMA combination			
Indacaterol maleate and glycopyrronium bromide	Ultibro Breezhaler	Capsule-based device	Novartis Pharmaceuticals Ltd., UK
Formoterol fumarate dihydrate and aclidinium bromide	Duaklir Genuair	Multidose device	Astra Zeneca Ltd., UK
Formoterol fumarate dihydrate and glycopyrronium bromide	Glycohale-F Rotacaps	Capsule-based device	Cipla Ltd., Mumbai, India
LABA/ inhaled corticosteroid combination			
Salmeterol and fluticasone propionate	AirFluSal Forspiro	Multidose device	Sandoz Ltd., UK
Salmeterol and fluticasone propionate	Sereflo	Multidose device	Cipla Ltd., Mumbai, India
Salmeterol and fluticasone propionate	Seretide Accuhaler	Multidose device	Glaxo Wellcome Ltd., UK
Salmeterol and fluticasone propionate	Stalpex	Multidose device	Glenmark Pharmaceuticals Ltd., UK

N-acetylcysteine disconnects mucin disulfide bonds to decrease the mucous viscosity. It also reduces the capacity of bacteria to stick to the pulmonary epithelial cells and reduce airway bacterial load. Additionally, it contains free "thiol" groups responsible for antioxidant properties [23].

In a recent article Rogers and Cismowski [4] showed that reactive oxygen species (e.g., superoxide, nitric oxide, and hydrogen peroxide) are responsible for direct injury to pulmonary epithelial cells. Thus, enhancement of biological antioxidant levels using a suitable pharmacological intervention and/or a nutritional regimen might be a fit alternative in the management of the pulmonary disease. Antioxidant, antibacterial, and antiinflammatory activities of herbal extracts and isolated natural compounds are well-reported [24–26]. Amid the natural antioxidants, careful deliberation has been directed on phyto-molecules such as flavonoids (e.g., naringin, baicalein, and resveratrol), lignans (e.g., honokiol), diterpenoids (e.g., andrographolide), alkaloids (e.g., atropine), and cannabinoids (e.g., dronabinol) [7,27]. Most of the studied phytoconstituents containing aerosols showed tremendous therapeutic potential in preclinical trials and early clinical trial phases [7,27]. Additionally, several complementary and alternative medicine and therapies are well-reported for the long-term management of pulmonary diseases [28–30]. Besides bronchodilators, corticosteroids, natural antioxidants, and mucoactive agents, surfactant therapy is also considered an important management pathway for the cure of airway diseases. Pulmonary surfactant therapy is discussed in the subsequent segment.

The pulmonary airways are seldom measured as a lipid metabolic organ [31]. Yet, it does maintain dynamic lipid metabolism, particularly in the alveoli sacs, where surfactant homeostasis is neatly controlled to guarantee the continuous working of each respiratory sequence. Pulmonary surfactant regulates surface pressure during inspiration and controls alveoli collapse at the base of expiration. The alveolar epithelium type 2 cells (T2C) are mainly responsible for the effective management of surfactant formation, recycling, and secretion in diverse physiologic conditions [31]. Airway surfactant is a phospholipo protein complex (90% lipid and 10% proteins), mainly including phospholipids and is essential for effective gas exchange. Phosphatidylcholine (PC) and dipalmitoylphosphatidylcholine (DPPC) are the main lipid moieties accountable for the surface tension regulation activities of the surfactant mixture. Four surfactant proteins, that is, SP-A, SP-B, SP-C, and SP-D have so far been documented in pulmonary lipoprotein complex. The SP-A and SP-D proteins are hydrophilic and contribute to the immune activities of surfactants since they can fasten exogenous pathogens and ease their clearance through phagocytes [31,32]. The SP-B, SP-C proteins are small hydrophobic polypeptides and extremely important for surfactant film dynamics such as interfacial adsorption, film stability, and surfactant respreading activities. They are also playing a key role in the formation and stabilization of pulmonary surfactants. Briefly, beyond their vital functions such as gas exchange, energy storage, alveolar film dynamics, and immune activities undoubtedly surfactants also play various important functions during normal physiological and pathophysiological processes [31,32]. Additionally, the pulmonary surfactant layer is the primary lining of the pulmonary airways that come in contact with external elements [33]. Therefore, lipid changes and surfactant insufficiency are mainly ground for various respiratory distress. Table 30.2 enlists the lipid changes and surfactant insufficiency in respiratory diseases. By knowing the impact of the surfactant profile on pulmonary health, surfactant therapy remains one of the milestones in the successful management of respiratory diseases. Therefore, in the succeeding section, basic aspects of surfactants and biosurfactant are explained.

Table 30.2 Overview of lipid profile in respiratory diseases [31].

Respiratory diseases	Lipid profile
Acute respiratory distress syndrome	Surfactant lipid deficiency, neutral lipid buildup, increased phospholipid mediated fibrin polymerization, and defensive function of sphingolipid signaling
Acute lung injury	T2C impairment, surfactant lipid differences, uncontrolled lipid transfer, and defensive function of sphingolipid signaling
Chronic obstructive pulmonary disease	T2C impairment, surfactant lipid insufficiency, disturbed reverse lipid transport, interrupted alveolar structure, and damaged alveolar macrophage sphingolipid signaling
Infant respiratory distress syndrome	Surfactant insufficiency
Idiopathic pulmonary fibrosis	Downregulated T2C lipid metabolism, disturbed alveolar macrophage lipid metabolism, and eicosanoid production, surfactant lipid variations, damaged sphingolipid signaling, and abridged alveolar surface area
Influenza	Lipid-dependent host protection and host-pathogen lipid interaction
Pulmonary alveolar proteinosis	Luminal surfactant buildup and alveolar macrophage cholesterol growth
Pneumonia	Host-pathogen lipid interaction, surfactant lipid modifications, disturbed lipid transport, and alveolar cellular injure
Severe acute respiratory syndrome and Severe acute respiratory syndrome Coronavirus 2	Diffuse alveolar injure and T2C hyperplasia
Tuberculosis	Host-pathogen lipid interaction, host eicosanoid differentially affect pathogenesis
Vaping-associated lung injury	Intracellular/luminal lipid accumulation and dysregulated alveolar macrophage lipid metabolism

30.3 Surfactants: properties and applications

Surfactants, agents that adhere to various interfaces (e.g., solid−liquid, liquid−gas, water−oil, and/or −gas) and lower their surface energy are called surface-active agents [34]. The efficiency of a surfactant is concluded by its effectiveness to lessen the surface tension. A good surfactant can drop the surface tension of water from 72 to 30 mN/m. Additionally, the effectiveness of surfactants also can be concluded from the critical micelle concentration (CMC) value. More effective surfactants have a lower CMC value [35]. The CMC is described as the concentration of surfactants beyond which micelles form and all surfactants consequently included in the system become micelles [34]. Briefly, the amphipathic molecules having the capability to shape micelles are called surfactants [33,34]. Surfactants have plentiful applications in various important sectors such as medicines, cosmetics, personal care products, foodstuffs, cleaners, toiletries, coatings, automotive fluids, paints, and other processing applications [34,35].

Particularly, in pharmaceutical preparations surfactants plays several roles such as enhancing drug dissolution kinetics, improving wetting and deaggregation of drug particles in tablet and capsule formulations, vehicles (e.g., nonionic surfactants) for suppositories, the solubilizing agent in

oral-liquid formulations, suspending agent for suspension formulations of pMDIs to enhance the solubility of active pharmaceutical ingredients/adjuvants in organic solvents, etc. to ease the design and development of the pharmaceutical dosage forms [33–36]. They are also used for several cosmetic preparations due to their distinctive stabilizing, solubilizing, wetting, foaming, detergent, and penetration enhancing properties [37]. Sodium lauryl sulfate, quaternary ammonium chloride, polyol esters, poloxamers, fatty acid esters of sorbitan (Spans), and their ethoxylated by-product (Tweens) are the few most frequently utilized surfactant in pharmaceutical and cosmetic formulations. Anionic surfactants like alcohol ether sulfates, alkylbenzene sulfonates, secondary alkane sulfonates, and alcohol sulfates show prominent detergency performance and are key additives of recent cleaning products [38].

Recently published articles highlighted the growing environmental risk linked to synthetic surfactants [38]. Synthetic surfactant's environmental risk assessment is typically executed by comparing the (predicted) environmental concentration (PEC) with the (predicted) no-effect concentration (PNEC). The PEC/PNEC ratio is called a risk characterization ratio (RCR). If RCR < 1 then it is accomplished that surfactant revealing a similar ecological profile and highlighting its safety for the organisms living in this ecosystem [38]. Most synthetic surfactants have several key environmental and biological issues. Synthetic surfactants have shown a vital impact on ecological compartments, that is, water and soil [38]. Therefore presently, the most attentiveness is toward the alternative ecological process and agents for the manufacturing of various types of natural surfactants.

30.4 Biosurfactants: source, properties, and purpose

Surfactants derived from living cells are normally called as biosurfactants [39]. Biosurfactant can be synthesized from renewable sources, that is, plant, animal fat, and microorganisms [39–41]. Biosurfactant is occasionally interchangeably used to denote to surfactants obtained from natural sources. However more precisely, biosurfactant refers to the surfactants synthesized directly by microorganisms that naturally consist of proteins, lipids, and carbohydrate moieties and are often allied with cell membranes or walls [39–41]. They are organically synthesized extracellularly by bacteria, yeast, and/or fungi using a variety of substrates for example sugars, oils, alkanes, and organic wastes [42]. *Acinetobacter* sp., *Arthrobacter* sp., *Bacillus* sp., *Enterobacter* sp., *Halomonas* sp., *Lactobacillus* sp., *Pseudomonas* sp. and *Rhodococcus* sp., are key cases of biosurfactant generating bacteria [43]. Several fungal strains produce biosurfactant, for example, *Aspergillus* sp., *Candida* sp., *Trichosporo* sp., and *Ustilago* sp. while *Kluyveromyces* sp., *Kurtzmanomyces* sp., *Pseudozyma* sp., and *Torulopsis* sp. are the main causes of yeasts biosurfactant producers [43]. The microorganism used, substrates type, and processing conditions present biosurfactants with several physicochemical properties, adequate for multiple specific applications. Generally, the molecular mass and CMCs of the biosurfactants vary from 500 to 1500 Daltons and 1 to 200 mg/L, respectively. The biosurfactants are more competent and effectual with their CMC value approximately 10–40 times smaller than that of artificial surfactants. They are stable at different pH and temperature conditions [33,34]. Biosurfactant can be divided into four groups: fatty acid-type (e.g., fatty acid soaps and phospholipids), lipopeptide-type (e.g., surfactin), glycolipid-type (e.g., sophorolipids, mannosylerythritol, and rhamnolipids), and polymer-type (e.g., emulsan, liposan, and alasan) [33,39,41].

Increase attention on biosurfactants is attributed to their ecological character, chemical multiplicity, the feasibility of far-reaching manufacturing, feat under the critical situation, selectivity, and their prime importance in ecological enrichment [39,41]. They can be called "green" owing to their biodegradability, low toxicity, and better stability under a broad series of physicochemical and biological milieu [44]. In latest years, biosurfactants have been drawing the attention of dynamic clinicians, active scientific and industrial society. The chemical diversity of biosurfactants supports them for their imminent uses in the domain of medicine, food, cosmetics, agriculture, and petroleum [44]. They are also useful in limiting the greenhouse consequences by regulating the production of carbon dioxide. Resembling synthetic surfactants, biosurfactants have also shown several applications related to emulsification, wetting, foaming, phase separation, cleansing, and surface activity [44]. These all diverse physical and chemical properties of biosurfactants are particularly helpful for the pharmaceutical, cosmetics, and food industry (Fig. 30.3).

In the case of pulmonary disease management, biosurfactants are commonly used for several functions. Curosurf (poractant alfa), Survanta (beractant), and Infasurf (calfactant) are the three most commonly used surfactants. Curosurf is a white to creamy white suspension manufactured by Chiesi Farmaceutici, Italy. Curosurf is an extract of natural porcine lung surfactant. Curosurf

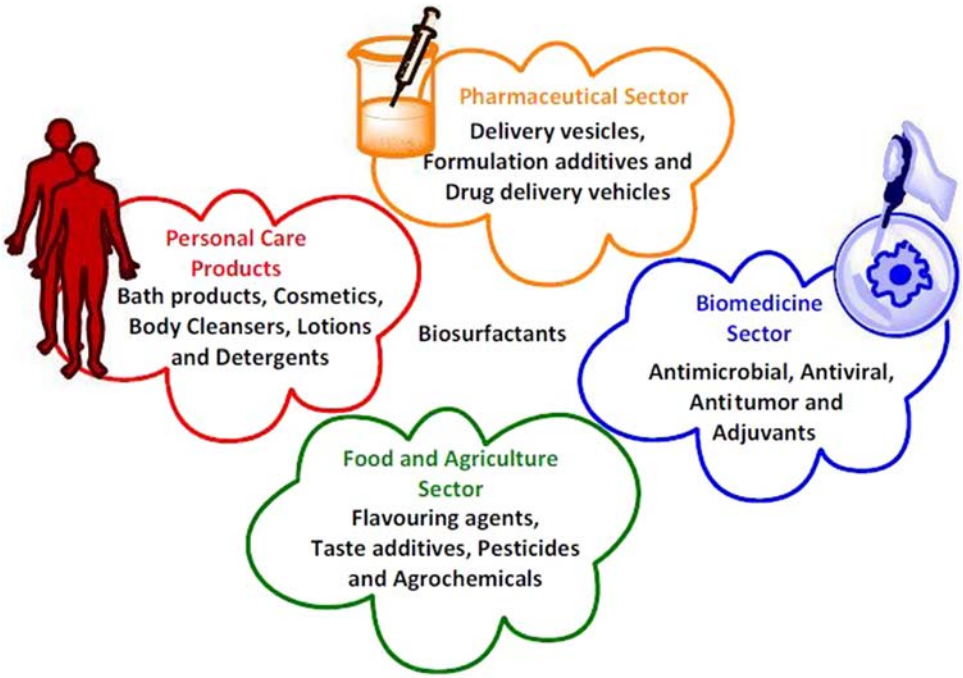

FIGURE 30.3

Applications of biosurfactants.

contains 80 mg surfactant extract (poractant alfa) that contains phospholipids (76 mg) and protein (1 mg; 0.45 mg SP-B and 0.59 mg SP-C). Curosurf is designated for the rescue therapy of respiratory distress syndrome (RDS) in premature infants. Curosurf is a sterile, nonpyrogenic, intratracheal suspension available in 1.5 and 3 mL vials with 120 and 240 mg surfactant extract, respectively [45]. Survanta (beractant) is an off-white to light brown liquid manufactured by AbbVie Inc., USA. Survanta is a natural bovine lung extract composed of neutral lipids, phospholipids, fatty acids, and proteins to which dipalmitoylphosphatidylcholine, tripalmitin, and palmitic acid are inserted to normalize the preparation and to imitate the surface-tension dropping functionalities of biological airway surfactant. Survanta composition provides phospholipids (25 mg/mL), triglycerides (0.5−1.75 mg/mL), free fatty acids (1.4−3.5 mg/mL) and protein (1.0 mg/mL). Survanta is a sterile, nonpyrogenic, intratracheal formulation without preservatives, indicated for the RDS in premature infants. Survanta is available in 4 and 8 mL vials with 100 and 200 mg phospholipids, respectively [46]. Infasurf (calfactant) is an off-white suspension manufactured by ONY Biotech Inc. USA. Infasurf is an extract of natural calf lung surfactant intended for intratracheal instillation. It contains neutral lipids, phospholipids and hydrophobic proteins SP-B and SP-C. Infasurf is available in 3 mL vial and indicated for the RDS in premature infants [47]. Apart from these surfactants, bovine surfactant Newfantan is also well explored for pulmonary surfactant therapy. It is also an animal-derived, that is, minced bovine lung extract-based surfactant manufactured by Yuhan Corporation, Korea [48]. Table 30.3 summarizes the key surfactant preparations available for pulmonary disease management. In the next segment, an attempt has been made to review and explain the applications of various biosurfactants in pulmonary drug delivery systems.

Table 30.3 Surfactant preparations available for pulmonary diseases management [45−48].

Surfactant	Surfactant trade name	Extraction	Composition	Manufacturer
Calfactant	Infasurf	Animal-derived (bovine lung lavage)	SP-B, SP-C (proteins) and DPPC, PG	ONY Biotech Inc. USA.
Beractant	Survanta	Animal-derived (bovine lung extract)	SP-B, SP-C (proteins) and DPPC, PG	AbbVie Inc., USA.
Surfactant TA	Surfactin	Animal-derived (minced bovine lung extract)	SP-B, SP-C (proteins) and DPPC, PG	Mitsubishi Tanabe Pharma Corporation, Japan
Poractant alpha	Curosurf	Animal-derived (minced porcine lung extract)	SP-B, SP-C (proteins) and DPPC, PG	Chiesi Farmaceutici, Parma, Italy
Bovine surfactant	Newfantan	Animal-derived (minced bovine lung extract)	SP-B, SP-C (proteins) and DPPC, PG	Yuhan Corporation, Korea

SP, *Surfactant protein;* DPPC, *dipalmitoylphosphatidylcholine;* PG, *phosphatidyl glycerol.*

30.5 Applications of biosurfactants in pulmonary diseases

Various active researchers, dynamic clinicians, and material scientists have thoroughly studied and scrutinized the biosurfactants for drug delivery [49], to understand the interactions between surfactant monolayer, surfactant composition, and inhaled drug particles [50]. This involves analysis of particle morphology on surfactant monolayer [51] using a wide range of tools and techniques such as coarse-grained molecular dynamics [51], surface pressure-area isotherms [50], surface potential-area isotherms [50], small-angle X-ray scattering [52], atomic force microscopy [53], quartz crystal microbalance equipped with dissipation monitor [54] and different biological assays [55]. Furthermore, different formulation systems such as carbon nanotubes [55], single-walled carbon nanotubes [56,57], silver nano-wires (70 nm diameter and 1.5 μm length) [58], graphene nano-sheets [56], fluorescent silica particles (40 nm) [59], polymer nanoparticles (NPs) stickers (100 nm) [60], hydrophilic NPs decorated with DPPC lipid molecules [61] and leucine modified spray-dried voriconazole microparticles [62] are well investigated through several biophysical analysis to probe role and functions of pulmonary surfactants as well as natural surfactant preparations. Most of these experimental and computational investigations showed that the natural surfactant preparations can be used as a spreading agent or carrier for long-term pulmonary therapy. The basis of utilizing a natural surfactant as a delivery channel for therapeutic moieties contains three important benefits [53]. Primary, natural surfactant preparation possesses biodegradability and biocompatibility as it can be evacuated from the alveolar sacs by endocytosis back into T2C or engaged by the phagocytes. Also, natural surfactant preparation can augment the water solubility of aquaphobic molecules using the drug solubilization method [63]. Lastly, the natural surfactant preparations can be dispersed involuntarily along with the inner lining of the airways, the fact recognized as the Gibbs-Marangoni effect [64–66]. Natural surfactant preparations permit to delivery of drugs at the distal, peripheral, and alveolar regions after intratracheal administration [53]. Some of the interesting natural surfactant preparations applications in pulmonary therapy are explained underneath.

Yeh et al. [67] showed that early postnatal intratracheal administration of budesonide (BUD) using a Survanta (bovine lung surfactant) as a carrier considerably enhanced the mutual outcome of death and chronic respiratory diseases in very premature infants without causing instant side effects. Dani et al. [68] showed that intratracheal delivery of beclomethasone dipropionate (BDP) with a Curosurf (porcine lung surfactant) successfully controlled the lung oxidative stress and enhanced the respiratory function in a preterm lamb model. Likewise, Wang et al. [53] studied the possibility of surfactant preparation, Infasurf (calf lung surfactant) as a carrier for two frequently prescribed corticosteroids specifically BUD and BDP. The lateral layer arrangement assessed by atomic force microscopy and surface movement studied by the Langmuir balance recommends that when Infasurf is explored as a vehicle, the BDP potency up to 10 wt.% or BUD potency ≥ 1 wt.% of surfactant has no significant impact on the biophysical characteristics of Infasurf, therefore being reasonable for pulmonary delivery. Rising BUD or BDP potency beyond this range causes the rapid fall down of the surfactant layer owing to amplified film fluidization [53]. The surfactant thin film formation and spreadability are important aspects of pulmonary surfactant therapy [69]. Therefore, in the latest study, Hermans et al. [69] investigated the mechanisms underneath the surfactant thin film stability and spreadability using drainage run flows from a hemispherical field. The commercially available three airway surfactants, that is, Curosurf, Survanta, Infasurf, and phospholipid

DPPC were used in the present study. The facade of the hemispherical field was carefully enclosed with human alveolar epithelial cells and all trials were performed at the physiological temperature. During thin film drainage analysis, drainage is delayed owing to the company of all the diverse airway surfactants and thus the thin films demonstrated improved stability. Yet, during scaling, analysis merged with the imaging system verified that several mechanisms are included. Survanta was found to be rheologically active while Curosurf and Infasurf followed the Gibbs-Marangoni phenomenon to impart stability. Finally, in the case of DPPC, the dilatational properties played a key function. Briefly, the surface viscoelasticity is an important aspect to allocate the airway surfactant effectively, while retaining film constancy against fast drainage [69].

The micro or nano particle-phase transition process is an important progression at the air-water interface during the normal breathing process. Therefore, different investigators studied the particle phase transition process using molecular dynamics simulations. Few attractive molecular dynamics case studies are discussed at this juncture. Lin et al. [70] studied the interactions between NPs and DPPC single-layer to probe the inhaled NPs-based pulmonary drug carrier systems. Particularly, Martini force field coarse-grained molecular modeling was used to analyze the interactions between hydrophilic as well as hydrophobic NPs and DPPC single-layer at the air-water edge. Hydrophilic NPs were easily translocated into DPPC single-layer with little influence on the monolayer while hydrophobic NPs disrupted the DPPC single-layer and therefore obstructed the standard phase changeover of the DPPC single-layer upon film condensation. However, the inhibitory result of hydrophobic NPs can be controlled using PEGylation. Briefly, results demonstrated that the DPPC layered NPs can effortlessly soak up onto the pulmonary surfactant and ease the drug delivery process [70]. This same research group was involved in performing coarse-grained molecular dynamics simulations to explore the relations of polyamidoamine (PAMAM) dendrimers with a DPPC single-layer at the air-water interface during the end-expiration progression. Dendrimers are one of the emerging drug delivery systems for pulmonary drug delivery [71,72]. Three different PAMAM dendrimers (<10 nm) generations, that is, G3, G5, and G7 were explored in the present study. Simulations study demonstrated that different generations have distinct effects on the DPPC monolayer. PAMAM G3 dendrimers had a small effect on the DPPC single-layer arrangement while higher generations PAMAM dendrimers (G5 and G7) largely deformed the DPPC single-layer. Thus, they inhibit and reverse the normal phase transition of the DPPC single-layer upon film compression. These structural transformations are highly energy-favorable with modest involvement from the PAMAM dendrimer's elasticity [71]. In a few words, simulation results pointed out that the particle transition phase is very important and highly related to the toxicity as well as the delivery potential of the dosage form [71,73].

Shah and Banerjee [74] developed dexamethasone disodium phosphate (DXP) loaded surface-active liposomes using D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphoglycerol ammonium salt, and DPPC for lung surfactant replacement therapy. TPGS (25 wt.%) with a low concentration of DXP (10−12.5 wt.%) helped to maintain low surface tension <2 mN/m. Developed liposomes were adsorbed more rapidly at the air-liquid rim than the control surfactant preparation. Particle size analysis and electron microscopy showed particle size <300 nm with unilamellar nature. The high airway patency ($>98\%$), low viscosity, and shear thinning property with submicron size are suitable for effective nebulization. During in vitro aerodynamic analysis using twin stage impinger, nebulized liposomes exhibited a 1.91-fold upgrading in DXP deposition as evaluated beside the DXP solution. Additionally, TPGS (as an

antioxidant) may play a valuable part in easing airway inflammation in chronic lung conditions. This study suggests that TPGS, DPPC containing liposomes may offer a hopeful policy for treating the state of surfactant dysfunction and chronic pulmonary inflammation [74]. In another study, Altube et al. [75] developed multifunctional macrophage-targeted-pH-sensitive nanovesicles and nebulized using NE-U22 vibrating mesh Omron nebulizer on immortalized murine Balb/c macrophages (J774A.1) cells roofed by Prosurf single-layer. The 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt/p-xylene-bis-pyridinium bromide and Lissamine rhodamine B 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt double labeled macrophage-targeted pH-sensitive nanovesicles (archaeosomes) were developed using dioleoyl-sn-glycero-3-phosphoethanolamine: archaeolipids: cholesteryl hemisuccinate [4.2:2.8:3]. Specifically, total polar archaeolipids were obtained from the hyperhalophile archaebacteria *Halorubrum teben-quichense*. Developed unilamellar archaeosomes showed particle size and zeta potential of 174 nm and −30 mV, respectively. The portion of nebulized archaeosomes remained dimensionally stable after surpassing the pulmonary surfactant single-layer depicting successful cytoplasmic delivery. These nebulized archaeosomes were found to be more stable for cytoplasmic drug delivery as compared to archaeolipids lacking nanovesicles. Additionally, archaeosomes did not provoke any biophysical changes causing pulmonary surfactant inactivation [75]. In brief, the archaeosomes-based delivery systems showed vast potential in the cure of pulmonary diseases [75]. Recently, in biophysical analysis, Cimato et al. [76] showed glucocorticoids incorporated in pulmonary surfactants (Prosurf) as a different option to enhance drug delivery efficiency with clearing harmful effects. Specifically, formulations containing Prosurf (1−2 mg/mL) and BDP, BUD, and fluticasone (10 mg/mL) remained intact as crystals in the water phase without changing the biophysical nature of the surfactant [77]. Additionally, Cimato et al. [77] reported that the greater the proportion of glucocorticoids incorporated in Prosurf, the better is the effectiveness of this pulmonary glucocorticoid system.

The small interfering RNA (siRNA) has a great prospective for the prevention or treatment of several respiratory disorders. Once the RNA molecules have reached the predetermined cells, they could restrain the emergence of specific gene sequences via the RNA interference means and produce curative consequences. However, the chief hurdle in turning siRNA treatment from lab to clinic is an apt delivery strategy [78]. Therefore, different molecular researchers also explored pulmonary surfactants as a delivery channel for improved siRNA delivery. Backer et al. [79] developed hybrid NPs containing small interfering RNA (siRNA)-loaded dextran nanogel core and Curosurf surfactant outer shell for improved pulmonary action. Additionally, hybrid NPs were decorated with folate (targeting ligand) to decrease cellular dose and stimulate receptor-mediated endocytosis. Uniform and spherical hybrid NPs showed a particle size of approximately 100 nm. The surfactant shell improved the colloidal stability with controlled siRNA release in the biological media while targeting ligand improved both gene silencing potential and cellular uptake. Further, the addition of 10 wt.% PEGylated lipids do not weaken hybrid NPs delivery potential [79]. Taken together, Backer et al. [80] also studied the surfactant-coated hybrid NPs for the release of siRNA to resident alveolar macrophages following pharyngeal delivery in female BALB/c mice. Surfactant-layered hybrid NPs (1 mg/kg siRNA dose) significantly downregulate the target mRNA levels with a 70% knockdown. The mild to acute pro-inflammatory cytokine and chemokine reactions with moderate neutrophil infiltration was observed after pharyngeal aspiration. However, the latter inflammatory and infiltration issues were significantly decreased by the elimination of surplus surfactant from the final preparation [80].

With this understanding of in vitro and in vivo potential, the same research group performed a series of biological experiments to identify the key molecular constituents of pulmonary surfactant accountable for the improved siRNA delivery. In biological experiments the surfactant protein, that is, SP-B was found to be a potent siRNA delivery booster. Thus, SP-B was reconstituted in surfactant-coated hybrid NPs. The developed SP-B coated hybrid NPs showed depleted in vivo toxicity with superior ingestion by resident phagocytes. Additionally, SP-B is also important for hybrid NPs to attain a major silencing of tumor necrosis factor-α in a murine lipopolysaccharide provoked acute lung injury model [81]. Similar findings were also reported by Cohen et al. [82]. They successfully formulated SP-D-loaded poly (lactic acid-*co*-glycolic acid) PLGA NPs and studied them for biological and toxicological activities. Spherical shape NPs showed 105 nm size, −36 mV zeta potential, and 59% encapsulation efficacy. Fabricated NPs were not toxic to adenocarcinomic human alveolar basal epithelial cells and did not provoke any inflammatory action in C57bl/6 mice lung for short (2 weeks) and long-term (4 weeks) periods. Briefly, SP-D protein-loaded PLGA NPs were found less invasive in the treatment of lung injuries [82].

30.6 Clinical trial perspective

Successfully translating pulmonary surfactant therapies to critically ill patients is a high priority of clinicians and scientists working in the discipline of pulmonary drug delivery. These clinicians and scientists are actively working on traditional (e.g., animal models) as well as new experimental models (e.g., biological simulations) for the better preclinical understanding of these pulmonary surfactants. Although the preclinical trials have demonstrated positive therapeutic outcomes, there are still safety concerns in human beings. Therefore, it is obligatory to perform a clinical trial and comprehend how pulmonary surfactants interact with the pulmonary air-liquid interface. Clinical trials serve as the gold standard to assess the safety and efficacy of drugs, medical devices, and biologicals before marketing approval [83]. Therefore, in the present section, we have discussed the pulmonary surfactants that have been studied for clinical trials.

In the past few years, several leading research institutes and pharmaceutical companies have studied pulmonary surfactants in various clinical phases. Clinical trials associated with pulmonary surfactants have been cited and are simply accessed on the Clinical Trials.gov by US FDA and Clinical Trials Register by European FDA. Clinical assessment with pulmonary surfactants have been performed for RDS, neonatal respiratory disease syndrome, preterm birth, chronic lung disease, skin and connective tissue diseases, severe respiratory failure allied with human COVID-19, meconium aspiration syndrome, etc. Few notable clinical trials are listed in Table 30.4. Optima Pharmazeutische GmbH (Germany) is a well-known pharmaceutical organization for the design and development of novel drug delivery systems. Optima is actively connected to the progress of different pulmonary surfactant-based delivery platforms. Optima's liposomal phospholipids (LipoAerosol©) are specially designed for reinforcing the pulmonary airways surfaces fluid film. It is a nonprescription liposomal throat spray. It offers warming, moistening, and cleaning of the pulmonary airways that supports the innate moistening of the coat in pulmonary disorders [84]. All this progress signifies an upgrading for the clinical development of pulmonary surfactant-based

Table 30.4 A summary of ongoing clinical trials for pulmonary surfactants.

Intervention/treatment	Condition or disease	Phase	Identifier	Sponsor
Curosurf	Severe acute respiratory syndrome associated with COVID-19	II	NCT04502433	Chiesi Farmaceutici S.p.A.
Curosurf	Respiratory distress syndrome in newborn	III	NCT02772081	Chiesi Farmaceutici S.p.A.
Curosurf 80 mg/mL intratracheal suspension	Bronchiolitis, Viral	III	NCT03959384	Azienda Ospedaliera Universitaria Integrata Verona
Budesonide inhalation suspension with Curosurf intratracheal suspension	Bronchopulmonary dysplasia and respiratory distress syndrome	IV	NCT03521063	Hospital Central "Dr. Ignacio Morones Prieto"
Curosurf	Meconium aspiration syndrome	IV	NCT02041546	Zekai Tahir Burak Women's Health Research and Education Hospital
Survanta© 100 mg/kg	Hyaline membrane disease	II	NCT01615016	Hamilton Health Sciences Corporation
Survanta©	Bronchopulmonary dysplasia	II and III	NCT01203358	NICHD Neonatal Research Network
Survanta©	Pulmonary hemorrhage	–	NCT01860014	Dr. Sami Ulus Children's Hospital
Aerosolized Infasurf©	Bronchiolitis	I	NCT03748173	ONY Inc., USA
Infasurf© Aero	Neonatal respiratory distress	–	NCT03582930	ONY Inc., USA
Infasurf©	Respiratory distress syndrome and Bronchopulmonary dysplasia	–	NCT00208039	Children's Hospital of Philadelphia

products. Still, many clinical trials of pulmonary surfactant-based products have to be executed for a broad series of pulmonary disorders.

30.7 Conclusion

Inventive green chemistry ideas, ecofriendly nature, and sustainability have made biosurfactants, the center of research activities in the past few years. These versatile amphiphilic molecules have proved numerous applications in several sectors ranging from medicine to agriculture. Undoubtedly biosurfactants are multifunctional. In terms of pulmonary interventions, many researchers and clinicians are genuinely focused on the most effective way of drug delivery to make biosurfactants economically more viable. Apparently, in the upcoming period, extensive active computational and biological efforts are highly required to optimize and understand the in-depth mechanism of biosurfactants for better pulmonary therapy.

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CHAPTER

Antioxidant activity of biogenic surfactants

31

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Surfactants are a kind of amphiphilic molecules also known as surface-active agents. Generally, all surfactants are comprised of two distinct parts; a head group with charged functionality like COO^-, SO_3^-, etc. attached to nonpolar hydrocarbon chain called tail group (Fig. 31.1).

Owing to the presence of charged head group and nonpolar hydrocarbon chain attached to the same molecule, surfactants display both hydrophobic as well as hydrophilic characteristics. They can accumulate at the interface of two partially immiscible liquid pairs. This feature makes biosurfactants capable of decreasing surface tension (ST) of liquid and interfacial tension (IFT) of binary liquid pair with differential polarity. Broadly, surfactants are grouped into two classes based on the source of their origin: synthetic surfactant and microbial or biosurfactant. Synthetic surfactants are derived from petrochemical sources [1] and have been broadly developed for different industrial processes primarily for their detergent-like properties and widespread application in surface cleaning actions. However, currently growing concerns for environmental degradation caused by industrial processes and hence demand of sustainable development are generating a greater thrust for biosurfactant research as a potential alternative.

31.1 Biosurfactants

Microbial surfactants or biosurfactants are amphiphilic molecules of microbial origin. These molecules are generated as a product of various metabolic processes that occurred in diverse microorganisms [2]. The vast majority of biosurfactants are produced by aerophilic aquatic microorganisms mostly of bacterial origin (*Bacillus, Pseudomonas*, and *Acinetobacter*), fungal origin (*Fusarium* and *Aspergillus*), and yeast (*Candida* and *Pseudozyma*) using a source of carbon like fats, carbohydrates, crude oil, etc. [3]. The chemical structure, properties, techniques used for characterization of various biosurfactants, and their application in diverse fields have been extensively reviewed and discussed in some excellent review articles [4–8].

31.2 Properties of biosurfactants

Some major advantageous characteristic of biosurfactants over synthetic ones are their high surface activity, low toxicity, tolerance to intense conditions like pH, temperature, salinity, derived from

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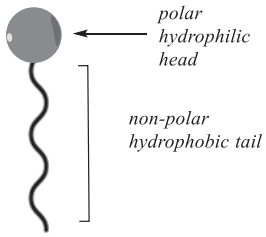

FIGURE 31.1

Representative structure of surfactant.

renewable resources, and lastly biodegradability. These advantages of biosurfactants make them an obvious choice in various synthetic and analytical fields. The characteristic properties of biosurfactants that make them superior to synthetic surfactants are discussed in the next section.

31.2.1 Critical micelle concentration

Surfactants with their amphiphilic nature tend to aggregate in solution and such polymeric aggregations are called micelles. For each surfactant, there is a certain minimum concentration at which micellization occurs and that particular concentration is called critical micelle concentration (CMC). Typically efficient biosurfactants have low CMC values than synthetic surfactants [9].

31.2.2 Surface and interfacial properties

Surfactants can lower ST and IFT of a mutually immiscible binary liquid pair at their interface. An efficient biosurfactant is capable of lowering ST and IFT of a liquid at relatively lower concentrations owing to their low CMC value compared to synthetic surfactants. Surfactin, a lipopeptide biosurfactant, for instance, can suppress ST of water to 25 mN/m and the IFT of water and hexadecane pair to <1 mN/m. Similarly, sophorolipid derived from *T. bombicola* can lower the ST of water to 33 mN/m and the IFT of water: hexadecane to 5 mN/m.

31.2.3 Temperature and pH tolerance

Some biosurfactants are less sensitive to harsh environmental conditions like high or very low temperature, extreme pH value, etc. These surfactants are often obtained from extremophiles. Biosurfactants are known to withstand fivefold higher salt concentrations than the salt concentration (2%) that deactivates synthetic surfactants [10]. For instance, some lipopeptides of bacterial origin (*B. subtilis*) are known to tolerate various intense conditions without losing their surface activity. They can withstand autoclaving conditions at 121°C/15 minutes, storage at −18°C for 6 months, variation of pH between 5 and 11, and salt concentration as high as 20% of NaCl [5]. Since industrial procedures require efficiency under a wide range of

temperature and pH, it is compelling to select the promising biosurfactants which operate under such extreme conditions.

31.2.4 Biodegradability and low toxicity

Biosurfactants can easily be degraded compared to synthetic surfactants and, therefore, are an ideal candidate for biosorption, bioremediation, and waste management [11,12]. Very little study on the toxicity of biosurfactants is available. They are typically considered nontoxic and hence, stand out to be a good candidate for use in the medicinal industry [5].

31.2.5 Emulsification

Biosurfactants are capable of forming and breaking emulsion and hence they are considered as effective bioemulsifier (that brings about emulsification). An emulsion is actually a fine dispersion of one liquid in another where both the liquids are immiscible (Fig. 31.2).

Emulsions are categorized as oil in water (o/w) and water in oil (w/o). Emulsions are usually not very stable but efficient emulsifiers like biosurfactants can enhance the stability of an emulsion for a considerable period [13].

The stability of emulsions is driven by the following factors:

- hydrocarbon
- salt concentration
- pH
- temperature

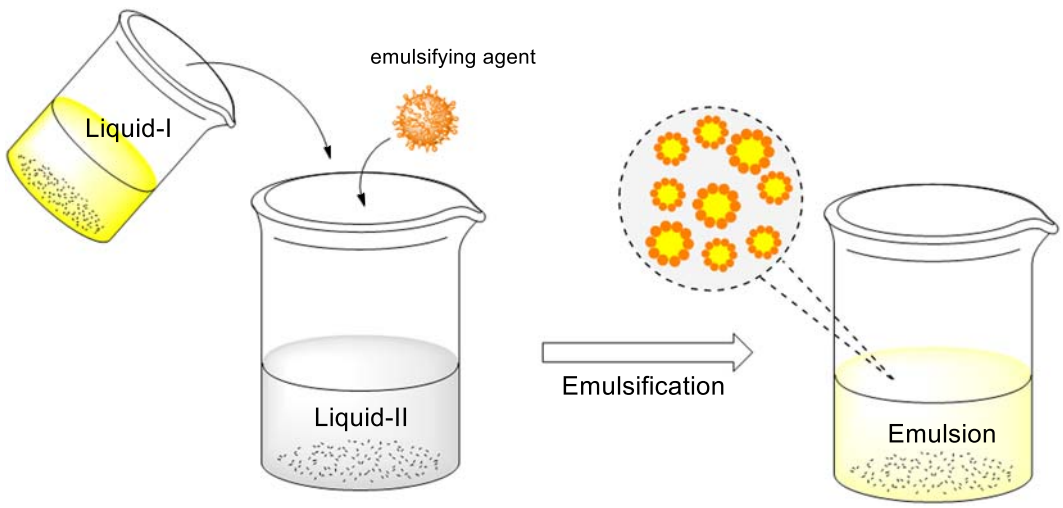

FIGURE 31.2

Schematic representation of emulsification.

31.3 Classification and chemical nature of biosurfactants

Biosurfactants can be classified based on their origin and chemical structure as high molecular weight biosurfactants and low molecular weight biosurfactants. The low molecular weight biosurfactant includes glycolipid, phospholipid, lipopeptides, lipoproteins, and fatty acids and the high molecular weight biosurfactant includes polymeric and particulate surfactants [14].

31.3.1 Glycolipids

Glycolipids are one of the most studied biosurfactants. They generally comprise carbohydrates that are connected with hydroxyl fatty acids through the ether or ester group. The carbohydrate part generally is composed of glucose, xylose, galactose, or rhamnose moieties. Rhamnolipids, trehalolipids, and sophorolipids are the most studied subclass of glycolipids.

31.3.1.1 Rhamnolipids

Rhamnolipids are glycolipids with rhamnose sugar molecules linked with hydroxy fatty acid through glycosidic linkage occurring between its OH group and reducing the end of a rhamnose sugar. Another fatty acid is attached to the molecule by ester bond formation through free OH group as shown in Fig. 31.3 [1,15].

The length of the hydroxyl fatty acid chain usually ranges from 8 to 16 carbon atoms [16].

31.3.1.2 Trehalose lipids

Trehalose lipids are nonreducing sugar and framework of two glucose units connected with an α,α-1,1-glycosidic bond (Fig. 31.4). Microorganisms like *Mycobacterium* sp., *Nocardia* sp., and *Rhodococcus* sp., are known to produce trehalose lipids [17,18].

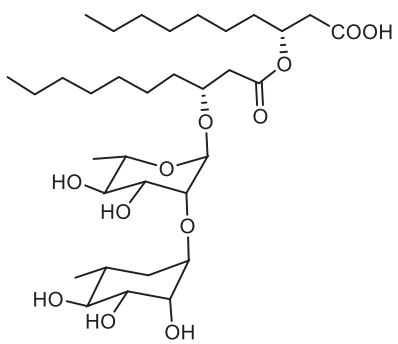

FIGURE 31.3

Chemical structure of representative rhamnolipid.

Courtesy of Pubchem CID 5458394.

31.3.1.3 Sophorolipids

Sophorolipids are glycolipids produced by yeasts particularly *Candida bombicola* and *C. torulopsis* [19]. Structurally, they are made of the disaccharide sophorose, covalently bound to a long-chain hydroxyl fatty acid. Sophorolipids exist in two different forms of sophorose; lactonic and acid form (Fig. 31.5) [20].

Sophorolipids are of particular importance as

1. Antibiotic, antifungal, and spermicidal agents in cosmetic formulation.
2. It can lower ST and IFT.
3. It exerts resistance against microorganisms and aid carbon storage.

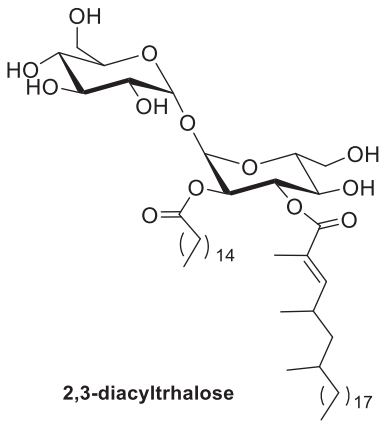

FIGURE 31.4

Chemical structure of trehalose lipids.

Courtesy of http://www.lipidhome.co.uk/lipids/simple/rhamno/index.html.

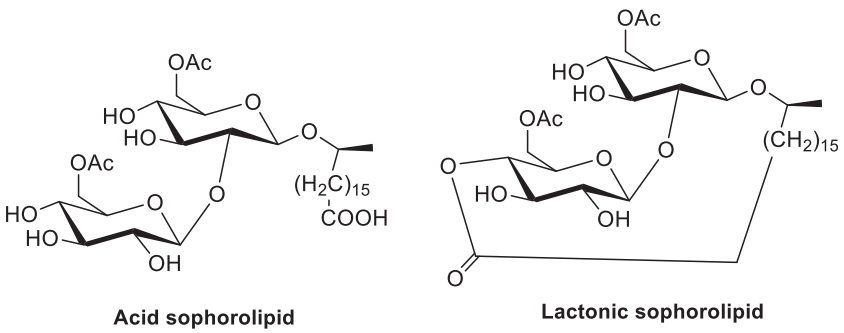

FIGURE 31.5

Chemical structure of acid sophorolipid and lactonic sophorolipid.

Courtesy of http://lipidlibrary.aocs.org/Primer/content.cfm?ItemNumber=39361.

31.3.2 Lipopeptides and lipoproteins

This class of biosurfactant usually contains cyclic peptides attached to the fatty acid. They possess a higher degree of surface activity and hence are drawing major attention relative to other biosurfactants. Surfactin is the most valued biosurfactant of the lipopeptide category among the group produced by *Bacillus subtilis*.

Surfactin structure consists of seven amino-corrosive ring structures attached to an unsaturated fat chain (Fig. 31.6). Other than surfactin, important examples of lipoprotein include viscosin, lichenysin, serrawettin, polymyxin, etc.

31.3.3 Polymeric and particulate biosurfactants

Few extensively studied biosurfactants include emulsan (Fig. 31.7), liposan, mannoprotein, and some other lipopolysaccharide and polysaccharide–protein complexes. *Acinetobacter calcoaceticus* is the best-studied microorganism that produces polymeric biosurfactants containing heteropolysaccharide as a backbone covalently linked to fatty acids.

Vesicles of extracellular membrane origin are particulate biosurfactants which through partitioning hydrocarbons produce microemulsions. These microemulsions can assist alkane metabolism by microbial cells. Vesicles of *Acinetobacter* sp. were known to possess protein, phospholipids, and lipopolysaccharide.

31.3.4 Fatty acid, phospholipids, and neutral lipids

Several bacteria and yeasts produce this kind of biosurfactants during their inoculation on n-alkenes as a carbon source. In *Acinetobacter* spp. phosphatidylethanolamine containing vesicles are produced and

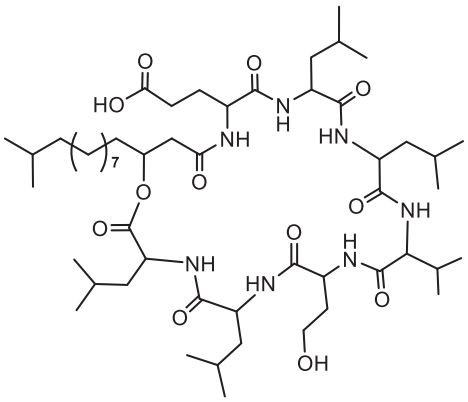

FIGURE 31.6

Structure of surfactin [21].

Courtesy of A. Perfumo, T.J.P. Smyth, R. Marchant, I.M. Banat, Production and roles of biosurfactants and bioemulsifiers in accessing hydrophobic substrates. in: Handbook of Hydrocarbon and Lipid Microbiology, Springer, Heidelberg, Berlin, 2010, pp. 1501–1512.

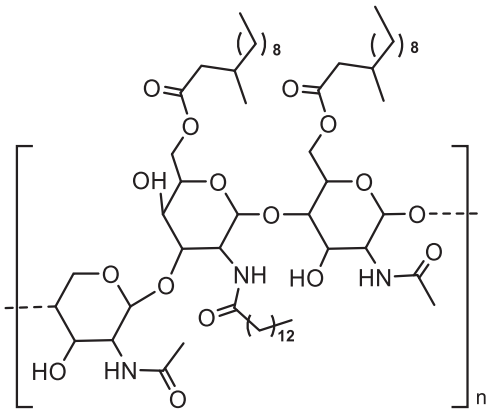

FIGURE 31.7

Structure of emulsan: a polymeric surfactant.

Courtesy of http://homepages.rpi.edu/~grossr/research/research017.html.

they produce clear oil in water-type microemulsions. Phosphatidylethanolamine obtained from *R. erythropolis* decreases the IFT of hexadecane, water pair to less than 1 mN/m [22].

31.4 Biosurfactant production

Bacteria are the chief producer of surface-active biosurfactant molecules [23]. Microorganisms that grow in seawater can produce biosurfactants under drastic environmental conditions affected by prolonged exposure to UV light, alteration in salinity, pH and temperature, and presence of limited nutrients [24,25]. A list of biosurfactants produced by various nonpathogenic microbes is shown below [4–6] in Table 31.1.

Typically, Bushnell Haas broth is employed as the standard medium for biosurfactant production. The broth is inoculated with bacterial culture (24–48 hours old) prepared in Nutrient broth medium or fungal culture (144–168 hours old) in potato dextrose. Next, the broth medium is subjected to shaking conditions under ambient temperature and consequently inoculated culture is allowed to cultivate under suitable conditions for 7–10 days. Further, centrifugation of the culture broth (10,000 rpm for 15 minutes) allows removal of the cells to acquire clear sterile supernatant of the biosurfactant [26].

31.4.1 Substrates used for commercial biosurfactant production [27]

31.4.1.1 Agricultural waste

Different aqueous extracts derived from agricultural waste materials like peels of orange, potato, banana, and baggage after proper treatment have been used for the effective production of biosurfactants.

Table 31.1 Biosurfactants from nonpathogenic organisms.

Serial No	Biosurfactant type	Microorganisms
1	Rhamnolipid	*Streptococcus mutans* NS
2		*Pseudomonas florescene*
3		*Pseudomonas putida* BD2
4		*Burkholderia thailandesis*
5	Glycolipid	*Streptococcus thermophilus*
6		*Streptococcus thermophilus* A
7		*Lactobacillus plantarum*
8		*Lactobacillus pentosus*
9		*Lactobacillus casei* MRTL3
10		*Leuconostoc mesenteroides*
11	Lipopeptides	*Bacillus subtilis* DM-03
12		*Bacillus licheniformis*
13		*Brevibacterium aureum* MSA13
14	Glycolipopeptide	*Lactobacillus pentosus*
15	Xylolipids	*Enterococcus faecium* MRTL9
16		*Lactococcus lactis*
17	Glycoprotein	*Lactobacillus acidophilus*
18		*Lactobacillus casei*

31.4.1.2 Dairy industry whey

Curd whey after the removal of casein is used for biosurfactant production.

31.4.1.3 Industrial waste

Various industrial waste and effluents are used as a production medium for biosurfactants that also improve production costs. The bacteria *Corynebacterium aquaticum* showed promising production of biosurfactant with fish and bagasse residues used as carbon source.

31.4.1.4 Vegetable oils

Vegetable oils are widely used as an inexpensive source for biosurfactant production.

31.4.2 Factors affecting the production of biosurfactants

31.4.2.1 Nutrient sources and salt concentration

Carbon sources used in the production of biosurfactants have a major influence on the quantity as well as the quality of biosurfactants produced [28]. Glucose, sucrose, crude oil, etc. are considered as a good choice of carbon sources in biosurfactants' production.

Nitrogen is a crucial factor in the production of biosurfactants. Nitrogen being a limiting nutrient, a medium containing nitrogen is vital for the growth of microbes, enzymes, and protein syntheses. Yeast, various ammonium salts like ammonium nitrate, ammonium sulfate are some common

sources of nitrogen [29]. Phosphate which is usually supplied in triphosphate form is also an important factor in the growth of microorganisms. Growing Gram-negative bacteria on ethanol with low phosphate content affects the maximum concentration of rhamnolipids [30]. Cellular activities are usually affected by salt concentration and therefore, it has some role to play in the production of biosurfactants [31]. The study made on biosurfactant production from *P. aeruginosa* suggested concentration of NaCl in the range of 1%−10% affects maximum production of rhamnolipids. However, some biosurfactants are insensitive to salt concentration although a slight decrease in their CMC takes place in that case.

31.4.2.2 Environmental factors

Environmental factors like temperature, pH, agitation speed, and aeration are influential regarding the determination of the quality and characteristics of biosurfactants. For instance, biosurfactant growth is excellent within the temperature bracket 25°C−37°C [32] and pH of 8 [33]. With some exceptions, bacteria are observed to produce best in basic pH however production of biosurfactants by yeast and fungi is observed to be the best in acidic conditions. The incubation period, aeration, and agitation do play a vital role in biosurfactant production.

31.4.3 Extraction of biosurfactants

Selected methods for extraction of biosurfactants are shown in Table 31.2.

Table 31.2 Various methods adopted for extraction of biosurfactant.

Method	Description	References
Cold acetone precipitation method	Crude biosurfactant solution is treated with chilled acetone and allowed to stand at 4°C for 10 h to cause precipitation. Precipitate is collected after centrifugation (10,000 rpm for 20 min) and evaporated to remove acetone and subsequently a solution is made in sterile water.	[34]
Acid precipitation method	In this method precipitation of biosurfactant is caused by lowering the pH to 6 using 6 N HCl and storing it at 4°C for overnight. Centrifugation (15 min at 8000 rpm) at 20°C provides pellets of pure biosurfactant that is dissolved in distilled water. pH is maintained at 8.0 by adding 1 N NaOH for additional use.	[35]
Chloroform: methanol precipitation method	Crude biosurfactant is acidified to pH 2 with HCl followed by extraction with a solution of equal volume of chloroform: methanol	[36]
Ammonium sulfate precipitation	This process is employed for high molecular weight biosurfactants wherein ammonium sulfate precipitation is used for precipitation of biosurfactants such as emulsan, biodispersion (protein rich compounds) etc. The After precipitation dialysis and lyophilisation are performed for further purification.	[37]
Ethanol precipitation	Here ethanol is used to get crude extract of biosurfactant from the microbes' supernatant of the culture. Broth culture is then removed by centrifugation for 20 min at 11,000 rpm and 4°C. Consequently precipitation was achieved from the supernatant by using cold ethanol.	[38]

31.4.4 Purification of biosurfactants

Several methods are available for purification of biosurfactants. Among the various methods the frequently used ones are discussed below

31.4.4.1 Thin-layer chromatography

It is employed primarily for the initial characterization of biosurfactants. In this method, crude biosurfactant is first eluted on silica get coated glass plate using a mixture of chloroform: methanol: water (10: 10: 0.5 v/v/v). The biosurfactant is then characterized by the use of a solvent system that develops colored spots, for example, red spots appear on the application of ninhydrin to the chromatographic plate which indicates the presence of lipopeptide.

31.4.4.2 Dialysis

This is an inexpensive and easy method used for the purification of biosurfactants that uses cellulose dialysis bags. A solution of the precipitate obtained by the extraction process is prepared in a small amount of distilled sterilized water which is used for dialysis against double distilled water for 2 days at 10°C [39].

31.4.4.3 Isoelectric focusing

It is one of the newest methods for the purification of biosurfactants. The setup for isoelectric focusing comprises a column equipped with solutions of the variable density gradient, electrolyte, and nonion conducting polymers. Under the combined influence of pH, applied electricity, and density gradient, the ampholyte moves in the column till it attains neutral pH. The column allows the separation of various fractions depending on differential pH. After complete separation, electrofocusing is stopped and the purity is an evaluation by comparing the activity of pure biosurfactant with its crude form [39].

31.5 Characterization of biosurfactants

There are many chromatographic and spectroscopic techniques available for characterization of biological surfactants. The chromatographic methods for characterization of biosurfactants include thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) while spectroscopic technique comprises nuclear magnetic resonance (NMR), Fourier transformation infra-red spectroscopy (FT-IR), liquid chromatography-mass spectrometry (LC-MS), and gas chromatography-mass spectrometry (GC-MS). Among these techniques, LC-MS is the most frequently used one.

NMR technique is based on transitions in atoms with a magnetic moment in presence of an applied external magnetic field. This is an important technique in detecting functional groups and their position of the attachment in biomolecules. Similarly, FT-IR gives important information about available functional groups in unknown molecules. Electrospray ionization-mass spectrometry (ESI-MS) is a soft ionization method generating gas-phase ions of biomolecules with high molecular weight. The method alone can be used in determining the molecular weight of a sample and the flexibility of this method allows it coupled with other chromatographic techniques like LC, GC, or HPLC to gain the deeper insight into various biomolecules [40].

Similarly, chromatographic techniques are useful in different aspects. The LC-MS method is well suited for the separation, purification, and quantification of lipopolysaccharides. GC-MS, on the other hand, is useful in determining the molecular weight of biosurfactants wherein hydrolytic cleavage of the peptide-protein or carbohydrate-lipid portions is required. Furthermore, HPLC finds usage in the separation, identification, and quantification of a mixture of biosurfactants [41].

31.6 Applications of biosurfactants

31.6.1 Application in cosmetic industry

In the cosmetic industry, synthetic surfactants are encouraged to replace with biosurfactants to ensure maximum compatibility with nature. Biosurfactants possess a wide variety of applications in the cosmetic industry for their foaming, water binding, emulsion forming, wetting, and spreading properties [12].

31.6.2 Application in laundry industry

Laundry industries typically use synthetic surfactants that cause serious hazards to aquatic life. Recently, consciousness about environmental pollution has greatly increased the popularity of natural alternatives to synthetic surfactants for the laundry industry. Cyclic lipopeptides show excellent tolerance to high temperature, wide pH range (7−10), forms a good emulsion with vegetable oils, and most importantly they show promising stability as well as compatibility with industrial-grade detergents. These features facilitate their potential inclusion in laundry detergent formulation [42].

31.6.3 Application in petroleum

Biosurfactants are effective emulsifiers that are widely explored as biocatalysts within oil reservoirs for biofouling degradation and biocorrosion of hydrocarbons [43]. Biosurfactants have a significant role to play in petrochemical manufacturing, petroleum extraction, refining, and upgradation.

31.6.4 Application in microbial enhanced oil recovery

Microbial surfactants utilize their metabolic process to acidify solid phases that enhance the production of oil-producing reservoirs. Some microbes, for example, *P. aeruginosa, B. subtilis*, etc. are known for cleaning up oil spilling [42].

31.6.5 Application in food processing industry

Biosurfactants with their ability to lower ST and IFT enhance the formation and stability of the emulsion. They also find application in preventing agglomerization of fats, improving consistency and texture of fat-based food products, rheological properties of wheat dough, etc. [44].

31.6.6 Application in agriculture

Biogenic surfactants are known for their antimicrobial action and hence they are useful in agriculture for pathogenic microbes that affect harvest growth. These are also used in controlling pollutants that deplete the quality of the soil. The selected number of bacteria produces lipopeptide that works as an insecticide against fruit fly *Drosophila melanogaster* [45] and therefore suitable for application as a biopesticide.

31.6.7 Pharmaceutical applications

Biosurfactants are associated with a wide variety of pharmacological activities. Many biosurfactants display prominent antibacterial, antifungal, anticancer, and antivirus action. For example, biosurfactants produced by *B. circulans* show antibacterial activity against both Gram-negative and Gram-positive strains [17]. Similarly, some microbial extracellular glycolipids help prompt cell separation in the human promyelocytic leukemia cell line and hence act as anticancer agents. Furthermore, many surfactants isolated from various microorganisms are reported to demonstrate excellent antioxidant properties. Therefore the next few sections of this chapter are dedicated to the antioxidants, their classification, various assays performed for antioxidant study, and primarily antioxidant property of biogenic surfactants.

31.7 Antioxidants

In general, antioxidants are chemical compounds that can retard the speed of oxidation of lipids in different food items. From biochemistry and medicinal point of view, it can be defined as organic substances or enzymes that can neutralize harmful effects of oxidation of animal tissues. Biologists define antioxidants as natural or synthetic compounds which are added to the final products to slow down the oxidation reactions [46,47].

31.7.1 Source of antioxidants

Fruits and vegetables are the main sources of antioxidants. Moreover, other types of food such as nuts, grains, some meats, poultry, and fish also contain varying amounts of antioxidants. For example, sweet potatoes, carrots, squashes, apricots, pumpkins, mangoes, etc. contain β-carotene. Lutein which is required for healthy eyes is abundant in collard greens, spinach, and kale. Another antioxidant lycopene is present in tomato, watermelon, guava, papaya, apricot, pink grapefruit, blood orange, etc. [48].

31.7.2 Types of antioxidants

Antioxidants are divided into two categories- natural antioxidants and synthetic antioxidants. Natural antioxidants are also known as primary antioxidants react with lipid radicals to break the chain of oxidants and convert them into more stable products. Some common examples are vitamin B, vitamin C, vitamin E, phytochemicals, etc. On the other hand, synthetic or secondary antioxidants are phenolic

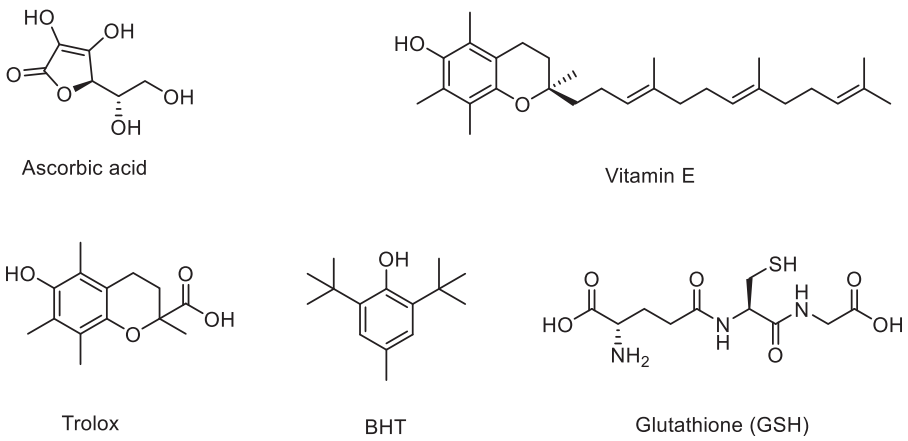

FIGURE 31.8

Chemical structure of some common Antioxidants.

compounds and their function is to inhibit the production of free radicals by reacting with them. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), metal chelating agent (EDTA), etc. are some widespread synthetic antioxidants [49].

31.7.3 Classification

Depending on their nature of action antioxidants are divided into two classes - enzymatic and nonenzymatic. Enzymatic antioxidants work by breaking down the hazardous oxidative products into small molecules by multistep process or by removing free radicals. Examples include superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, etc. On the other hand, free radical chain reactions are interrupted by nonenzymatic antioxidants. Vitamin C (ascorbic acid), vitamin E, plant polyphenols, carotenoids, Se, and glutathione (GSH) are some familiar examples of none-enzymatic antioxidants. Chemical structures of some widespread antioxidants are depicted in Fig. 31.8 [49].

31.8 Methods for evaluation of antioxidant activity

There are numbers of methods available in the literature to evaluate the antioxidant activity of the testing samples. But none of the methods is fully comparable to one another. Some of the common models are discussed in the present chapter which is used to determine the antioxidant activity of biosurfactants.

31.8.1 1-Diphenyl-2-picryl hydrazyl scavenging activity

This is the most simple and broadly used model to determine the antioxidant activity of chemical compounds. When 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical having deep violet color is

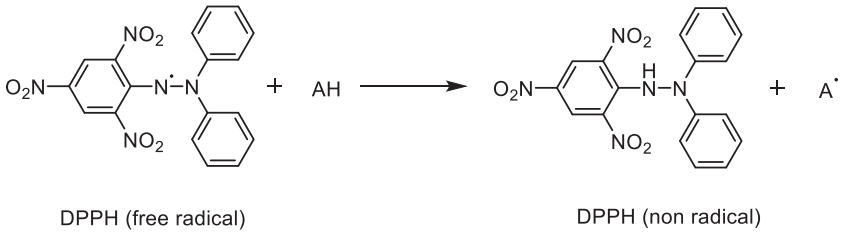

FIGURE 31.9

Interaction of 1-diphenyl-2-picryl hydrazyl radical with sample tested.

added to the test sample (AH), it gives the reduced form of DPPH by the donation of a hydrogen atom from the AH (Fig. 31.9) and then the violet color disappears [50].

By monitoring the optical density variation of DPPH radicals, the antioxidant activity of the test samples is evaluated. To the ethanolic solution of DPPH, different concentrations of the test samples are added and the absorbance is measured at 517 nm after 30 minutes. Sample solutions in ethanol at different concentrations are used as a blank sample. The percentage of the DPPH radical scavenging is determined using the following formula as depicted below:

$$Scavenging\ \ ability\ \ (\%) = \left[(A_{br} - A_{ar})/A_{br}\right] \times 100$$

where A_{br} and A_{ar} are the absorbances before and after reaction, respectively.

31.8.2 Trolox equivalent antioxidant capacity method/ABTS radical cation decolorization assay

In this method, the color of the blue-green chromophore $ABTS^{\bullet+}$ (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) disappears when an antioxidant is added to it. A diode-array spectrometer is used in this particular antioxidant model. $ABTS^{\bullet+}$ is reduced by the antioxidant to ABTS and therefore the color disappears. According to the process described by Seeram's group in the year 2006 [51], by adding MnO_2 (80 mg) to an aqueous solution of ABTS (5 mM), ABTS radical cation is prepared. Here Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), an analog of vitamin E is used as a standard antioxidant which is soluble in water. At first, a standard calibration curve is constructed for Trolox at different concentrations such as 0, 50, 100, 150, 200, 250, 300, and 350 μM. The test samples are diluted and added to 200 μL solution of $ABTS^{\bullet+}$ in 96-well plates and absorbance is measured at 750 nm for 5 minutes in a microplate reader. From the Trolox standard curve, the values of TEAC can be calculated which is expressed as Trolox equivalents (in mM).

31.8.3 Hydrogen peroxide scavenging assay

This particular method was described by Ruch et al. in 1989 [52]. In this particular method H_2O_2 solution is prepared in phosphate buffer of pH 7.4 and using a spectrophotometer, the concentration of H_2O_2 solution is measured at 230 nm. Afterward, different concentrations of the test sample

solution in distilled water are added to the solution of H_2O_2, and absorbance at 230 nm is determined after 10 minutes against a blank solution having only phosphate buffer. By using the formula given below the percentage of H_2O_2 scavenging is calculated.

$$Scavenging\ \ ability\ \ (\%) = \left[(A_i - A_t)/A_i\right] \times 100$$

where A_i and A_t are the absorbances of control and test samples, respectively.

31.8.4 Ferric reducing antioxidant power assay

The capacity of antioxidants to reduce Fe(III) was measured by FRAP method. The basis of this method is that at low pH, reduction occurs from Fe(III) complex and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) to Fe(II). Using a diode-array spectrophotometer, this reduction is observed by measuring the variation of absorption at 593 nm. According to this method given by Benzie and Strain (1999) [53], 3 mL of prepared FRAP is added to a dilute solution of the test sample (100 mL) and the absorbance at 593 nm is measured after incubation of 30 minutes at the temperature of 37°C. By comparing the absorption change in the test sample and those obtained from increasing the concentrations of Fe(III), the FRAP values can be calculated which is expressed as mM of Fe(II) equivalents per kg for solid food and per liter for samples of the beverage.

31.8.5 Reducing power method

The basic principle of this method is that it is dependent on the absorbance of the reaction mixtures. The antioxidant activity of the sample is directly proportional to the absorbance of the mixtures. Here, the antioxidant compound forms a colored complex with potassium ferricyanide, trichloroacetic acid, and ferric chloride and the absorbance of the complex is calculated at 700 nm. The reducing power of the test samples increases as the absorbance of the samples increases. In this model developed by Oyaizu in 1986 [54], the test sample solution (in water) is mixed with phosphate buffer of pH 6.6 (2.5 mL solution of 0.2 M) and 1% w/v solution of $K_3Fe(CN)_6$ (2.5 mL). Afterward, the mixture is incubated for a period of 30 minutes at 50°C and then 10% w/v solution of trichloroacetic acid (2.5 mL) is added to this mixture and centrifuged for 10 minutes at 3000 rpm. After centrifugation, the upper layer is collected (2.5 mL) and 2.5 mL of distilled water and 0.1% w/v solution of $FeCl_3$ (0.5 mL) is added to it. Finally, the absorbance of the mixture is carefully measured against a blank sample at 700 nm.

31.8.6 Superoxide radical scavenging activity

It is well known that the superoxide anion is a weak oxidant, but it produces very strong and dangerous hydroxyl radicals and singlet oxygen. These two radicals finally contribute to oxidative stress. The method was developed by Robak and Gryglewski in 1988 [55]. According to the method, when the sample is mixed with 0.5 mL solution of each of 0.3 mM nitroblue tetrazolium (NBT), 0.936 mM NADH, and 16 mM Tris−HCl buffer of pH 8, superoxide anion radicals is produced. By adding 0.12 mM solution of phenazine methosulfate (PMS) (0.5 mL) the reaction is

started and then the mixture is incubated for 5 minutes at the temperature of 25°C. At last, the absorbance of the sample solution is measured and compared with the blank sample at 560 nm.

31.8.7 Ferric thiocyanate method

This antioxidant assay was developed by Kikuzaki et al. in the year of 1991 [56]. In this model, ethanolic solution of the test sample (4 mg in 4 mL) is mixed with 2.51% ethanolic solution of linoleic acid (4.1 mL), phosphate buffer of pH 7 (0.02 M, 8 mL), and 3.9 mL of distilled water in a screw cap vial and then place the vial in an oven at 40 °C in absence of light. After that 0.1 mL of the above solution is transferred to a test tube and 75% (v/v) aqueous ethanol (9.7 mL) is added to it. Then, 30% aqueous ammonium thiocyanate (0.1 mL) and 0.1 mL of $FeCl_2$ (0.02 M in 3.5% HCl) solutions are added to the above mixture. The absorbance of the mixture is measured at 500 nm after 3 minutes of the addition of $FeCl_2$ solution. The absorbance is measured for a time gap of 24 hours until reached the maximum absorbance value of the control. In this model standard antioxidant is used as positive and the blank sample is used as a negative control.

31.8.8 Phosphomolybdenum method

With the help of this model, the total antioxidant capacity (TAC) of the samples is calculated. The basic principle is that the test sample reduces Mo(VI) to Mo(V) and forms the phosphate−molybdate complex at an acidic medium. According to this method [57], 0.1 mL of sample (100 μg) solution is mixed with 1 mL of reagent consisting of sulfuric acid (0.6 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM). Then the mixture is incubated in a water bath at 95°C for a period of 1.5 hours. Then the sample is cooled to room temperature and after that, the absorbance of the sample is measured with the help of a UV spectrophotometer at 695 nm against the blank. The antioxidant capacity of any samples can be expressed as equivalents of ascorbic acid.

$$Active\ \ antioxidant\ \ (\%) = \left[(A_t - A_i)/A_i - A_A\right] \times 100$$

where A_i, A_t, and A_A are the absorbances of the test sample, control, and ascorbic acid, respectively. Here ascorbic acid was considered to have 100% antioxidant activity.

31.8.9 Hydroxyl radical scavenging activity

In the biological system, it is a powerful reactive oxygen species that damages the cell by reacting with polyunsaturated fatty acid moieties of cell membrane phospholipids. The hydroxyl radical scavenging activity is determined by the method described by Kunchandy and Rao in 1990 [58]. The reaction mixture (1 mL) is prepared by adding 500 μL sample solution to 100 μL of 2-deoxy-D-ribose (28 mM in 20 mM buffer solution of KH_2PO_4-KOH with pH 7.4), 1.04 mM solution of EDTA (200 μL), 1:1 v/v $FeCl_3$ solution (200 μM), 1 mM solution of H_2O_2 (100 μL) and 1 mM solution of ascorbic acid (100 μL). Then the solution is incubated for 60 minutes at the temperature of 37°C. After that, 1 mL each of 1% solution of thiobarbituric acid and 2.8% solution of trichloroacetic acid are added to the above solution and once again incubated for 20 minutes at 100°C. The

solution is then cooled and measured the absorbance of the solution at 532 nm against the blank sample.

31.8.10 Metal chelating activity

A red color chelating complex is formed by ferrozine with ferrous ions. The presence of other chelate-forming ions can hamper this reaction and hence the red color of Fe(II)-ferrozine complex decreases. The chelating ability of a sample is determined by measuring the color reduction of the complex. In this method described by Dinis et al. in 1994 [59], the chelating power of ferrous ions is determined. At first, the sample (0.1 mL) is mixed with 0.2 mM solution of $FeCl_2$ (0.5 mL). Then, 5 mM ferrozine (0.2 mL) is added to the above mixture and incubated the solution at room temperature for 10 minutes. Finally, the absorbance of the solution mixture is measured at 562 nm. In this method, EDTA or citric acid is used as a positive control.

31.8.11 ß-carotene linoleic acid method/conjugated diene assay

The basis of this method is that with the help of reactive oxygen species (ROS) which is formed by oxygenated water, linoleic acid gets oxidized. Oxidation of ß-carotene is done by the oxidized products which are formed in the first step. Due to the oxidation of ß-carotene, the color gets disappeared. Antioxidants can reduce the rate of discoloration which is estimated by determining the absorbance at 434 nm. From the absorbance reading, antioxidant activity can be calculated. According to the method developed by Kabouche et al. [60], ß-carotene solution is prepared in chloroform (0.5 mg/mL) and added to an emulsified mixture of linoleic acid (25 μL) and Tween-80 (200 mg). Then, from the above mixture, chloroform is evaporated and to the remaining mixture, distilled water is gradually added and the solution is strongly agitated to form a stable emulsion. After that, 4 mL of the above solution is mixed with different concentrations of the test sample (200 μL methanol solution in each case). After the addition of emulsified solution to the sample solution, the absorbance is measured at 470 nm. Then the final solutions (different concentrations) are incubated for 120 minutes at 50°C. In this particular model, ascorbic acid is used as a standard antioxidant. With reference to the control, the antioxidant potency of the sample is determined as follows:

$$Percentage\ \ of\ \ inhibition\ \ (I\%) = \left[1 - (A_i - A_{120})/(A_c - A_{c120})\right]$$

where A_i is initial absorbance, A_{120} is absorbance of the sample at 120 minutes, A_c and A_{c120} are the initial and final absorbance (at 120 minutes) of the control respectively.

31.9 Biosurfactants and their antioxidant property

There are massive number of biogenic surfactants available in the literature that have numerous applications in the field of biological and pharmaceutical sciences. Among the biosurfactants available in the literature some of them showed good to excellent antioxidant activity. In this chapter, we are trying to cover all the biosurfactants that have mild to excellent antioxidant activity. In

2010 Emine Yalcm and Kultigin Cavusoglu [61] studied the antioxidant behavior of biosurfactants produced by *B. subtilis* RW-I which was isolated from refinery wastewater. At first, the biosurfactant was isolated, purified, and then characterized by spectroscopic analyses which confirmed that it had a lipopeptide structure. The antioxidant potency of the biosurfactant formed from *B. subtilis* RW-I was evaluated by using three different widely used models. The reducing power of the antioxidant was fully dependent on the concentration of the biosurfactant. Upon increasing the concentration of the biosurfactant, the reducing power increased, indicating the presence of electron donors in the biosurfactant structure that could change free radicals to more stable products. The antioxidant activity of the biosurfactant was also investigated with DPPH assay and found that at 1 mg/mL concentration the maximum scavenging activity of biosurfactant was in the range of 75%−80%. The IC_{50} value was also evaluated and compared with BHT which was lower than BHT (IC_{50} of BHT 0.09 and biosurfactant 0.25 mg/mL, respectively). The presence of free hydroxyl moieties was responsible for the DPPH scavenging effect of the biosurfactant. In the FRAP assay, the Fe(III) ion scavenging activity of the biosurfactant was found to be 87.2% at the concentration of 2 mg/mL. Similar to reducing power, the scavenging effect of ferrous ions was also concentration-dependent. But the activity of the biosurfactant was lower than that of the EDTA which was used as standard (98.9%). As compared to BHT and EDTA, biosurfactants discussed here have low antioxidant activity which could be due to the lower contents of hydroxyl groups in the biosurfactant.

Two years later, Tabbene et al. [62] investigated the antioxidant behavior of three Bacillomycin D-like lipopeptides (biosurfactants) such as C14, C15, and C16 produced by B38 strain. They studied the antioxidant potency of these lipopeptides with the help of six commonly used antioxidant assays. All the biosurfactants showed concentration-dependent antioxidant activity in the DPPH method. The IC_{50} values of all the biosurfactants were almost identical, 93.88, 95.47, and 90.08 μM for lipopeptides C14, C15, and C16, respectively. These biosurfactants showed less potency than ascorbic acid and Trolox but they showed more activity than BHT. The ferric reducing antioxidant power of these biosurfactants was enhanced with increased concentration. The reducing capacity of C14, C15, and C16 biosurfactants occurred at 44.36, 46.01, and 44.7 μM, respectively, which showed identical activity of these compounds. As compared to standard antioxidant ascorbic acid, these compounds exhibited more reducing power. The presence of acidic and hydrophobic residues (Glu and Tyr) in these molecules and their cyclic structure and acyl chain of beta-amino fatty acid could be responsible for the excellent reducing power of these biosurfactants. No significant ferrous chelating power of these lipopeptides was observed which was evaluated by the ferrozine method. The EC_{50} values of these biosurfactants were determined and found EC_{50} values of 172.93, 172.27, and 171.47 μM for C14, C15, and C16 lipopeptides respectively. 80% metal chelation was observed for all these compounds at the concentration of 1 mg/mL. The presence of the carboxyl group of the side-chain of the Glutamic acid residue of these biosurfactants was mainly responsible for the metal chelating activity. These lipopeptides also showed strong inhibition of lipid peroxidation in the linoleic acid emulsion system with IC_{50} values of 46.17, 53.21, and 50.94 μM for C14, C15, and C16 biosurfactants respectively. They showed activity twice that of standard antioxidant BHT which was used as a positive control in the current study. Further, the superoxide radical scavenging capacity of these lipopeptides was evaluated, and found that they showed strong activity which was slightly lower than ascorbic acid and Trolox. The EC_{50} values of C14, C15, and C16 biosurfactants were evaluated as 103.46, 109.37, and 99.65 μM, respectively.

The presence of hydrophobic residues as Tyr and Pro in the biosurfactants structure could be responsible for their strong superoxide radical scavenging activity. Similarly, the biosurfactants showed excellent hydroxyl scavenging activity with IC_{50} values of 88.74, 88.95, and 84.02 μM for biosurfactants C14, C15, and C16, respectively. They showed lower activity as compared to BHT and Trolox with IC_{50} values of 54.45 and 2 μM respectively but they had higher potency than carnosine and glutathione with IC_{50} values of 654 and 661 μM, respectively.

In the same year, Kitamoto et al. [63] investigated the antioxidant potential of different Mannosylerythrithol lipid (MEL) derivatives which were known as biosurfactants. The antioxidant activity of MEL derivatives viz., MEL A, B, and C were evaluated by using DPPH and the superoxide anion scavenging model. In the DPPH assay, the biosurfactants exhibited dose-dependent strong activity, but they were less active as compared to arbutin (standard antioxidant) used as a positive control. Among these biosurfactants, MEL C showed the highest antioxidant activity of 50.3% at the concentration of 10 mg/mL. Further, the antioxidant property of MELs was examined through superoxide anion scavenging in which MEL C again showed the highest activity as compared to MEL A and B. MEL A also showed a lower level of antioxidant activity while MEL B did not show activity at any concentrations. At the concentration of 2 mg/mL, biosurfactant MEL C derived from soybean oil showed the highest activity of 60%. Further in vitro antioxidant potency of MEL C was investigated by using cultured skin fibroblasts (NB1RGB cells) under H_2O_2 induced oxidative stress and found that it had higher protective power against oxidative stress as compared to arbutin. The biosurfactant MEL C had a protective ability of 30.3% which was higher than that of arbutin (the protective ability of 13%) at the concentration of 10 mg/mL.

Ayed et al. in 2015 [64] studied the antioxidant potency of biosurfactant produced by *B. mojavensis* A21 with the help of four different commonly used models. In the DPPH assay, different concentration of biosurfactant *B. mojavensis* A21 was investigated for their antioxidant activity and observed that it was dependent on concentrations. The biosurfactant showed 65% scavenging power even at 1 mg/mL, but showed less potency than BHT (standard antioxidant) at the same concentrations. They also studied the antioxidant activity through ferric reducing power assay. From the results, it can be concluded that the reducing power of the lipopeptide biosurfactant was concentration-dependent and the highest activity was observed at the concentration of 10 mg/mL. The reducing ability was lower than that of BHT which could be due to the existence of lower contents of OH groups in the biosurfactant compared to BHT. The biosurfactant *B. mojavensis* A21 also showed strong inhibition of ß-carotene bleaching which was also dose-dependent. The inhibition rate was increased from 61% to 72% with increased concentrations of biosurfactant from 5 to 10 mg/mL. Further the IC_{50} value was also calculated and found to be 3.7 mg/mL which was higher than that of BHT (0.7 mg/mL). The antioxidant activity of the biosurfactant *B. mojavensis* A21 was also evaluated through linoleic acid autoxidation assay which was comparable with the α-tocopherol used as standard. The acyl chain present in the beta-amino fatty acids may improve the interaction between linoleic acid and A21 lipopeptides. This could be the possible reason for the excellent antioxidant activity.

Zouari et al. [65] evaluated the antioxidant property of the lipopeptide biosurfactant produced by *B. subtilis* SPB1 by using different types of antioxidant assays. At first, the authors studied the antioxidant activity of the biosurfactant through DPPH assay and found that the antioxidant activity was dependent on the concentration of the biosurfactant. They determined the activity at different concentrations of the biosurfactant and the highest activity was exhibited at the concentration of 10 mg/mL. Even at the concentration of 1 mg/mL, the biosurfactant showed good scavenging

activity of 70.4%. Further, the IC_{50} value of the biosurfactant was also determined and compared with standard antioxidant BHT (0.048 mg/mL) which was found to be 0.55 mg/mL. The excellent activity of the biosurfactant was due to the presence of some active residues such as the hydrocarbon fatty acid chain in the peptide ring of the lipopeptide biosurfactant *B. subtilis* SPB1. The reducing ability of the biosurfactant was evaluated by using a ferric reducing power model and compared with BHT. The reducing power also depends on concentrations and the highest value was obtained at 10 mg/mL. But the reducing power was lower than that of BHT at all concentrations which may be due to the presence of less number of hydroxyl groups (responsible for reducing power) compared to BHT in the lipopeptide structure. In the ß-carotene bleaching assay, with increasing concentrations of biosurfactant from 3.5 to 10 mg/mL, the inhibition of ß-carotene bleaching was increased from 57.09% to 77.72%. They also evaluated the inhibition concentration IC_{50} of the biosurfactant *B. subtilis* SPB1 which was found to be 2.26 mg/mL. Similarly, the biosurfactant showed dose-dependent scavenging effect on ferrous ion which was increased from the concentrations of 1 to 10 mg/mL, and the highest activity was found to be 80.32%. The IC_{50} value of the biosurfactant was estimated and found to be 0.62 mg/mL.

In 2017 Jemil et al. [66] examined the antioxidant activity of DCS1 lipopeptides produced by *B. methylotrophicus* DCS1 strain with the help of five different assays. The study was initiated by the most widely used DPPH assay and significant antioxidant activity was observed which was dependent on concentrations of the surfactant. At the concentration of 1 mg/mL biosurfactant showed significant scavenging potency of 80.6%. But they had a slightly lower scavenging effect as compared to BHT, a synthetic antioxidant. This excellent activity was attributed due to the presence of a hydrocarbon fatty acid chain and some active residues in the peptide ring that had a good hydrogen atom or an electron donor capacity and could react with free radicals of DPPH. Further, the antioxidant power was evaluated through ferric reducing assay in which biosurfactant DCS1 showed dose-dependent antioxidant potency, and the highest value of 3.0 ($OD_{700\ nm}$) was obtained at 2.0 mg/mL. The presence of hydroxyl groups in the biosurfactant molecules could be responsible for the reductive power of the biosurfactant DCS1. The ferrous ion chelating activity of the biosurfactant was also investigated and showed strong ferrous chelating activity at the concentration of 4 mg/mL and chelated almost 80% Fe^{2+} ion. But the result was quite lower than that of EDTA, a common and widely used metal chelating ligand. In the β-carotene bleaching assay, the biosurfactant showed concentration-dependent antioxidant activity which was lower than that of BHT at all concentrations tested in this study. The IC_{50} value of the biosurfactant DCS1 was found to be 42 μg/mL. The biosurfactant DCS1 also inhibited 60.22% lipid peroxidation after 3 days of the incubation period, whereas increasing the incubation time from 3 to 9 days, the biosurfactant showed 76.8% lipid peroxidation inhibition.

Gargouri et al. [67] isolated biosurfactant derived from *Stenotrophomonas* sp. B-2 strain and characterization was performed with different spectroscopic methods. The authors studied the antioxidant activity of the biosurfactant by using a widely used DPPH radical scavenging assay. The biosurfactant showed moderate to good activity at different concentrations, but it had lower potency compared to BHT used as standard. The IC_{50} values of the surfactant and the standard BHT were also examined and a comparable IC_{50} value 27.3 μg/L was obtained for the biosurfactant (IC_{50} of BHT 17 μg/L).

Merghni et al. [68] studied the antioxidant activity of the biosurfactants isolated from *Lactobacillus casei* by using a DPPH scavenging assay. They isolated two cell-associated

biosurfactants BS-Bl and BS-Z9 and their antioxidant activity was tested at different concentrations using ascorbic acid as standard. Both of them showed good antioxidant activity of 74.6% and 77.3% for BS-Bl and BS-Z9, respectively at the concentration of 5 mg/mL. But the activity of the biosurfactants was slightly lower than that of ascorbic acid (96.2%) used in this study as standard. From their study, it was observed that the antioxidant potency of the biosurfactants increased with an increase in concentrations of biosurfactants. Further, IC_{50} values of the biosurfactants were evaluated and compared with ascorbic acid (IC_{50} of 1.35 mg/mL) and found that IC_{50} values of BS-LBl and BS-LZ9 were 2.09 and 2.16 mg/mL respectively.

Kiran et al. [69] reported the production of lipopeptide biosurfactant which was isolated from marine sponge *Fasciospongia cavernosa*. The biosurfactant was then purified and characterized with different physicochemical methods. The biosurfactant was tested for their antioxidant activity with the help of the DPPH model using BHT as a standard antioxidant for reference. From the DPPH assay, it was found the antioxidant activity of the biosurfactant isolated in this study was completely concentration-dependent and maximum activity (65%) was observed at 6 mg/mL. The antioxidant activity of the biosurfactant MSA31 was due to the presence of unsaturated fatty acid in the biosurfactant.

Ramrajan et al. [70] studied the isolation and characterization of biosurfactants derived from the novel marine bacterium *Streptomyces* sp. N11 and then evaluated the total antioxidant activity of the biosurfactant. It was observed that the total antioxidant potency of the biogenic surfactant was dose-dependent. With increasing the biosurfactant concentration from 100 to 300 μg/mL, the antioxidant potency increased from 78.83 to 84.23%. It had lower antioxidant potency with respect to standard antioxidant vitamin C having antioxidant activity of 96.2% at the concentration of 300 μg/mL.

In 2018, Madiha Basit et al. [71] developed the production of biosurfactants from native strain *B. cereus* and evaluated for their antioxidant activity. The isolated biosurfactant-producing strains were characterized as *B. cereus* MMIC 1, MMIC 2, and MMIC 3, all of them belonged to the lipopeptide class. In the FRAP method, the maximum antioxidant activity was obtained at the concentration of 2 mg/mL. On the other hand, like other antioxidant assays developed by research groups of different areas, the DPPH scavenging activity of the biosurfactants discussed in this study was also dose-dependent. With increasing the concentrations from 0.5 to 2 mg/mL, the antioxidant power of the biosurfactants was increased from 27% to 63%.

Giri et al. in 2019 [72] investigated the antioxidant potential of biosurfactants isolated from *B. subtilis* VSG4 and *B. licheniformis* VS16 through two important antioxidant models. In the DPPH assay, biosurfactant *B. subtilis* VSG4 showed maximum antioxidant activity of 69.1% at the concentration of 5 mg/mL whereas *B. licheniformis* VS16 showed slightly better activity of 73.5% at the same concentration. From the above assay concentration-dependent antioxidant behavior was observed for both the biosurfactants used in this study. Hydroxyl radical scavenging activity was also evaluated for both the surfactants and found that *B. subtilis* VSG4 and *B. licheniformis* VS16 exhibited scavenging activity of 62.3% and 68.9%, respectively at 5 mg/mL.

In 2020 Nasrin Samadi's group [73] evaluated the antioxidant activity of biosurfactants derived from *B. amyloliquefaciens* NS6 (surfactin), and *Pseudomonas aeruginosa* MN1 (rhamnolipids). At first, they isolated the biosurfactants and characterized them with the help of different spectroscopic analyses, and then the antioxidant power was calculated by using three different antioxidant assays. In the FRAP models, the reducing power of the biosurfactants was compared with vitamin C which was used as standard. For both the surfactants the reducing power was lower than that of vitamin

C. Literature revealed [61] that presence of hydroxyl group in the biosurfactant structure may be responsible for the higher reduction potency of the biosurfactant. Since hydroxyl group contents in rhamnolipids surfactant are lower so it had the lower reducing ability. On the other hand, the presence of hydrophobic, acidic, and sulfur containing amino acids increased the reducing potency of a biosurfactant isolated from *B. amyloliquefaciens* NS6. The antioxidant potency of both the surfactants was also calculated with the help of the DPPH assay. The IC_{50} values of both the surfactants were evaluated and compared with vitamin E and BHT used as standard. Both the surfactants showed lower antioxidant potency (IC_{50} value of surfactain and rhamnolipids were 2.73 and 4.15 mM, respectively) in comparison with vitamin E with IC_{50} value of 0.036 mM. On the other hand, the antioxidant activity of surfactain was comparable with another standard antioxidant BHT with IC_{50} value of 2.86 mM. But rhamnolipids derived from *Pseudomonas aeruginosa* MN1 had lower antioxidant activity than BHT. The DPPH scavenging activity of the biosurfactants was mainly due to the presence of some active residues in the peptide ring including tyrosine residue via its phenolic hydroxyl group and proline residue from its pyrrolidine ring. In the FTC model, the lipid peroxidation inhibition activities of surfactin and rhamnolipids were evaluated and compared with vitamin E and BHT. Both had higher IC_{50} values 1.65 and 4.6 mM for surfactin and rhamnolipids, respectively than vitamin E (IC_{50} value of 0.04 mM), but surfactin had a similar activity with BHT and rhamnolipids had lower activity. The lipid peroxidation inhibition activity of both the surfactants is directly proportional to the concentrations of the surfactants.

Mouafo et al. [74] isolated biosurfactants from *Lactobacillus casei* and characterized them with different physicochemical and spectroscopic methods (Fig. 31.10). The antioxidant power of the isolated biosurfactants was determined by three models such as DPPH radical scavenging assay, ABTS radical cation assay, and Ferrous ion chelating assay, and by using phosphomolybdenum method, the total antioxidant activity of the biosurfactants was determined. The biosurfactants isolated in this study showed a maximum scavenging effect at the concentration of 3.5 mg/mL (72% for DPPH and 80% for ABTS). Excellent antioxidant activity of the biosurfactants isolated in the present study was due to the presence of active amino acid residues of peptide moieties and free hydroxyl groups of sugar moieties present in the biosurfactants. The free OH groups of the sugar moieties acted as hydrogen atom donors or electron donors which reacted with the free radicals. In the ferrous ion chelating assay, biosurfactants showed 82.29% potency at the concentration of 3.5 mg/mL. The presence of the carboxyl group in the biosurfactants structure could be responsible for the excellent chelating activity of the biosurfactants which can bind to iron.

Haque et al. [25] isolated a biosurfactant produced by *Marinobacter litoralis* MB15 and from the preliminary characterization of the biosurfactant confirmed that it had glycolipid molecules in

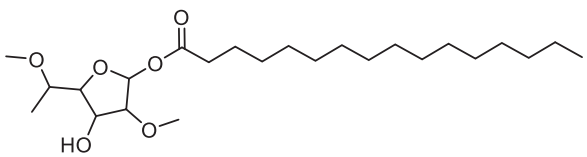

FIGURE 31.10

Chemical structure of 2,5-O-methyl-rhamnofuranosyl-palmitate representing the active fraction T-1 of biosurfactants from *Lactobacillus casei* subsp. *casei* TM1B.

the structure. Further, characterization of the biosurfactant was done thoroughly with the help of different spectroscopic analyses and found that it contains a mixture of mono and di-rhamnolipids homologs. The antioxidant activity of the biosurfactant was evaluated by using DPPH free radical assay in which ascorbic acid was used as control. It was found that the antioxidant activity of the biosurfactant was dependent on concentrations and maximum activity was observed at the concentration of 5 mg/mL. The biosurfactant showed antioxidant potency of 72.6% at 5 mg/mL which was higher than that of mannosyl erythritol lipid (50.3% at 10 mg/mL).

Recently, Silva et al. [75] isolated a biosurfactant from *Candida bombicola* URM 3718 and examined its different applications including antioxidant activity in different food items. They characterized the biosurfactant produced from *C. bombicola* URM 3718 with different physicochemical methods and then the antioxidant activity was determined with three different assays. In the DPPH assay, the antioxidant activity of the biosurfactant was dependent on concentrations and the highest activity (52.42%) was observed at 1000 μg/mL. Similarly, the total antioxidant activity of the biosurfactant was also concentration-dependent but it had lower activity of 25.47% at 2000 μg/mL. The biosurfactant showed very low sequestering activity of $ABTS^{\bullet+}$ radical cation (24.27%) at the concentration of 5000 μg/mL as compared to the standard Trolox (84.58%) at the same concentration.

The results summarized in the present chapter indicate that the biogenic surfactants which had good to excellent antioxidant potential can be used as alternatives to widespread chemical antioxidants in different fields such as the food industry, therapeutic and pharmaceutical purposes, as cosmetic, etc. A comparative analysis (Table 31.3) of the DPPH radical scavenging potential of some

Table 31.3 1-diphenyl-2-picryl hydrazyl radical scavenging activity of biogenic surfactants in comparison with standard antioxidants.

Sl. No.	Biosurfactant-producing microorganism	DPPH scavenging activity (%)	IC_{50} (mg/mL)	Concentration (mg/mL)	References
1	*Bacillus subtilis* RW-I	75–80	0.25	1	[61]
2	*Bacillus subtilis* B38	~80	0.095	0.5	[62]
3	*Bacillus mojavensis* A21	65.0	–	1	[64]
4	*Bacillus subtilis* SPB1	70.4	0.55	1	[65]
5	*Bacillus methylotrophicus* DCS1	80.6	0.357	1	[66]
6	*Stenotrophomonas* sp. B-2	–	2.73×10^{-5}	–	[67]
7	*Lactobacillus casei* BS-Z9	77.3	–	5	[68]
8	*Nesterenkonia* sp. MSA31	65.0	–	6	[69]
9	*Bacillus cereus*	63.0	–	2	[71]
10	*Bacillus subtilis* VSG4	69.1	–	5	[72]
11	*Bacillus licheniformis* VS16.	73.5	–	5	[72]
12	*Bacillus amyloliquefaciens*	–	2.73 mM	–	[73]
13	*Pseudomonas aeruginosa*	–	4.15 mM	–	[73]
14	*Lactobacillus casei* subsp. casei TM1B	72.0	0.97	3.5	[74]
15	*Marinobacter litoralis* MB15	72.6	–	5	[25]
16	*Candida bombicola* URM 3718	52.4	–	1	[75]
17	BHT	91.4	1.25×10^{-5}	0.06	[76]
18	Ascorbic acid	96.2	0.06	0.1	[77]

of the biosurfactants discussed in this chapter with standard antioxidants such as ascorbic acid and BHT is done which shows that the existing synthetic antioxidants can be substituted by biogenic surfactants as natural antioxidants in the upcoming days.

31.10 Conclusion

In the last few decades, biosurfactant molecules gained numerous attention among the scientific community due to their diverse applications in pharmaceutical and biological fields. Additionally, they can replace the widespread synthetic surfactants used in our daily lives which have many side effects. Antioxidants are substances that can inhibit the oxidation reaction, mainly in stored food items. The most commonly used synthetic antioxidants in different fields of natural sciences have some drawbacks such as along with healthy cells they can also protect some harmful cells. Nowadays, biosurfactants can be used as alternatives to common synthetic antioxidants since they have good to excellent antioxidant potency. This particular chapter covers the antioxidant potential of all the biosurfactants produced by different microorganisms by using commonly used antioxidant assays. This chapter is expected to provide useful information towards the discovery and development of novel biosurfactant molecules as antioxidants.

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CHAPTER

Recent advances in biosurfactant as antiadhesion/antibiofilm agents

32

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32.1 Introduction

Biofilms (BFs) are the colonization of microorganisms on the solid surface of substratum or may form complex aggregates without adhering to the surface [1]. They are mostly formed on the biotic or abiotic surface as single or complex communities of microorganisms. The development of BF formation on medical instruments, insertional implants plays a vital part in the problems of many nosocomial and health-related diseases.

The pathogenic BF, a cluster of bacterial cells secured by a self-developed polymer matrix (PM) is considered as a global threat, making them highly resistant to antibiotics and the host immune system [2]. There is an urgent requirement for a novel antibiofilm (ABF)/antiadhesive (AAD) agent to prevent or control pathogenic BF-forming microorganisms. The latest therapeutic techniques are developed against BF and growing attention is currently paid to biosurfactants (BSs). Most of the BSs are well known to display antimicrobial, AAD, and ABF properties [3].

BSs are surface-active agents that are produced by various microorganisms and capable of reducing the surface and interfacial tension between different fluid phases. They are well known for many years and have potential applications in a wide array of sectors including food, medical, agriculture, cosmetic, pharmaceutical, and petroleum industries [4]. BS has several advantages over chemical surfactants due to their low toxicity, higher biodegradability, highly stable at extreme temperature, pH, and eco-friendly nature [5]. Several areas of research have shown that under certain test conditions, BS could be very efficient than many standard BF prevention and/ or disruption techniques [6]. The sophorolipid-based amphotericin was assumed to interfere with gene expression, downregulating hyphae-specific genes. The importance of BS in the field of biomedical has been identified due to its antibacterial, antifungal, antiviral, inhibition of BF formation, and their AAD properties against various pathogenic microorganisms. The present book chapter discusses the potential application of BS as AAD and ABF agents.

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32.2 Microbial biofilm formation

The extracellular polymeric matrix is the part of the BF which consists of extracellular polysaccharides, proteins, lipids, and extracellular [7]. They also consist of glycoprotein, glycolipids, lipoteichoic acid, BS, and outer membrane vesicles [8]. All the matrix components are necessary for the adhesion of the cell, accumulation, and BF maturation process. Polysaccharide is also found in the matrix are chiefly consists of neutral sugar and possess a molecular mass of $0.5-2 \times 10^6$ Da [9]. Matrix protein could be obtained from active and dead cells of BF and transferred via vesicles [10]. Matrix proteins are macromolecules with a weight of 10−200 kD and contain hydrophobic amino acids [11].

The BF formation involves four steps (i) adhesion, (ii) microcolony formation, (iii) BF maturation, and (iv) detachment of bacteria. The first step in the adhesion process is the attachment of the planktonic cells to the solid surface (SF). Further, led by the adsorption of protein to the SF and forms the film which promotes bacterial adhesion. The bacteria are called the primary colonizers as they are the first to attach to the surface; their occurrence lets the secondary colonizers (other microbial species) attach or interrupt this process.

Araujo et al. [12] described the mechanism that facilitates the interaction of bacteria with the surface include Brownian Pedesis, the interaction of polar molecule, Lifshitz-van der Waals forces, convective mass transport, and active transport. The adhesion process is one of the important stages in the BF. The factors involved in the adhesion process are hydrophobicity, bacterial cell charges, surface charge, roughness, and microtopography. According to Araujo et al. [12] one of the most key factors for the adhesion process is surface hydrophobicity. An incredibly vital function is also achieved by culture medium wherein the process is carried. The attachment of the bacteria could be affected due to the charge of the bacteria cell and its hydrophobicity which may be the effective strategy to combat the microbial BFs [13]. Environmental signals like nitric oxide, proteinase K, or molecules essential for cell signaling can also influence the detachment of the cells (in the first contact) on the solid surface [14]. The reversal of the primary adherence is known as desorption.

The continuous growth of bacterial cells leads to the development of mature BFs containing millions of pillar and mushroom-shaped masses which act as the primitive circulatory system for the exchange of nutrients and waste products. Several microenvironments differ for pH, oxygen concentration, availability of nutrients in the BF colony. The final stage of BF development is the detachment of the cells from the BF colony. This process also involves numerous environmental signals, signal transduction pathways, and effectors [15].

Microbes in the BF formation can also communicate with cells of the same or other species which is commonly known as quorum sensing (QS), controls and regulates the activity of bacteria in the environment for example formation of BFs, synthesis of antibiotics, bioluminescence [16]. QS takes place based on chemical signaling molecules (SMs) such as autoinducers and Gram-negative bacteria mainly use signal molecule homoserine lactones and Gram-positive bacteria use oligopeptides. Once a minimum threshold stimulatory concentration of the molecules is reached, the microbes must recognize and react jointly for example by varying the gene expression [17]. These properties of the sessile bacteria mentioned above make it exceedingly hard to eliminate BF from the medical device or implants used in the medical field.

32.3 Biosurfactant as antiadhesive agent

The role of BS in microbial adhesion and desorption has been widely known. The adsorption to the solid surface can involve an effective method to prevent microbial adhesion and combat pathogenic organisms colonization, not just in the biomedical field, but also in other areas like the food industry [18,19]. Swarming motion and BF formation plays a key role in the colonization of the surface by several bacteria and increase the chance of hospital-acquired infection. BSs are well known to inhibit the adhesion of pathogenic microorganisms to solid surfaces or the site of infection.

The precoating the vinyl urethral catheters by running the surfactin solution through them before inoculation with media reduced the BF formation by *Salmonella typhimurium*, *S. enterica*, *Escherichia coli*, and *Proteus mirabilis* [20]. *Salmonella* species most often cause urinary tract infections (UTIs). AIDs patients have been shown to have a higher risk of *Salmonella* infection including UTI [21], these findings have great potential for practical applications.

Various BsS display antibacterial, antifungal, and antiviral properties, which makes them important biological molecules for the use in combating against several infections and diseases. BSs produced from *L.paracasei* are shown to decrease the adhesion of pathogenic and nonpathogenic microbes [22,23].

Another beneficial application of BS is its use as AAD property against pathogens. Rufino et al. [24] reported the antimicrobial and AAD properties of Rufisan BS isolated from *Candida lipolytica* UCP 0988, a growth medium containing groundnut refinery residues. Apart from the antimicrobial activity (AA), the AAD activity of BS was assessed against various microorganisms (*Lactobacillus* sp, *E. coli*, *Streptococcus* sp, *P. aeruginosa, S. aureus, S. apidermis, C. albicans*). The BS showed AAD activity against all the tested organisms, but the AAD effects rely upon the concentration of the compound and the microbes tested. The AAD activity was tested at the concentration of 0.75 mg/L against all the microorganisms. The AAD activity was proportional to the concentration of BS. Their findings suggested that 91% and 99% (at a minimum concentration of BS) of AAD properties were observed against *L. casei* and very low inhibition was observed for *S. epidermidis* (27%) and *E. coli* (21%) at a maximum concentration of BS, respectively.

Luna et al. [25] demonstrated the antimicrobial and AAD properties of BS Lunasan isolated from *Candia sphaerica* UCP 0995. The isolated *C. sphaerica* UCP 09995 was grown in low-cost media with 9% refinery residue of soybean oil and 9% corn steep liquor and achieved a BS yield of 9 g/L. The BS at the concentration of 10 mg/mL displayed AA against *S. oralis*, *C. albicans*, and *S. epidermidis*. The AAD activity of BS at the concentration of 10 mg/mL showed complete inhibition of adhesion against *P. aeruginosa* (100%), *Staphylococcus aureus* (100%), *S. oralis* (97%), and very low inhibition was achieved from *S. mutans* HG 985(50%) and *S. epidermidis* GB (22%). Their study suggested that BS can be used in the medical field for applications against several microbes that cause infections like urinary, vaginal, and gastrointestinal tracts. Moreover, due to their AAD properties, BS can be used in various medical equipment (as coating agents), but this application field is yet to explore for BS derived from yeast.

Lactic acid bacteria is widely known as normal microflora in human gastrointestinal and genitourinary tracts, which play a key role in the maintenance of homeostasis and prevents the colonization of pathogenic organisms. The BS derived from LAB is considered as safe and has been used in the food industry. Gudina et al. [26] for the first time studied the BS production *L. agilis*

CCUG31450. Three different *Lactobacillu*s strains were studied on conventional MRS medium. The strain *L. agilis* CCUG31450 produced cell-bound surfactant and was characterized as Glycoprotein BS. They found an increase in the production of BS when cheese whey was used as a culture medium and achieved a higher production of 960 mg/L. The BS exhibited AA against *S. aureus*, *S. agalactiae*, and *P. aeruginosa*. The BS showed AD property against *S. aureus* even at a lower concentration (1 mg/L). The study suggested that BS could be utilized to reduce the *S. aureus* adherence on various solid substrates and inhibits the BF formation and colonization of microbes on medical instruments.

The effect of BS on the adhesion of BF is due to changes in the microbial surface interaction, such as variations in charges present in the bacterial cell wall or modification in the substrate properties that influence the adherence and dissociation of microbes. Moreover, the exact mechanism of this activity is still not been completely described and an intricate process may be involved.

The preceding adhesion of BS on solid surfaces could involve a novel and efficient means of inhibiting the colonization of pathogenic microbes. Meylheuc et al. [27] reported that BS produced from *P. fluorescens* inhibited the adhesion of *Listeria monoctogenes* LO28 to polytertrafluoroethylene (PTFE) and AISI 304 stainless surfaces.

The BS produced by *Lactobacillus* strains are known to inhibit the primary adhesion of uropathogenic *E. faecalis* strains to the amphiphilic substrate in the presence of phosphate buffered saline (PBS) or urine, when observed in a parallel-plate flow chamber(PPFC) [28,29]. Velrades et al. [30] investigated the ability of surfactin BS from *L. acidophilus* RC14 to inhibit the primary adhesion of uropathogenic bacteria and yeast strains on silicon rubber. Heinemann et al. [31] reported the surface-binding protein released from *L. fermentum* RC-14, inhibited the adherence of *E. faecalis*.

The antiadhesive potential of lipopeptide (LP) BS derived from marine origin was reported by Das et al. [32]. The antiadhesive activity of the BS was tested against a *P. vulgaris, S. typhimurium, S. marcescens, A. faeclais, M. flavus*, and the AAD activity was concentration-dependent and organism tested. The BS at a concentration of 10 g/L inhibited the microbial adhesion up to 89% and hence suggested the BS potential on biomedical applications and in surgical instruments. Rivardo et al. [33] reported the antiadhesion activity of *B. subtilis* and *B. licheniformis*. Their investigation reported that both the strains produced BS up to 10% NaCl. The strains produced lipopeptide BS and exhibited AAD activity against *E. coli* CFT073 and *S. aureus* ATCC 29213. They also found that and BF formation was reduced to 97% (*E. coli* CFT073) and 90% (*S. aureus* ATCC 29213), respectively.

The *Candida* spp. can form BFs. The study of Bulgasem et al. [34] reported the antiadhesion activity of LAB from honey against five *Candida* sp. BF (*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis*, and *C. krusei*). The antiadhesion activity of LAB culture-free supernatant (CFS) was tested against *Candida* sp. was carried out in precoating (PC) and coincubation experiments. The *L. curvatus* HH significantly reduced the formation of BFs of *C. glabrata* ATCC2001 (79.4%) and *C. albicans* (61.1%) in the precoating (PC) method. The antiadhesion activity of LAB CSF was stable after the heat treatment. The antiadhesion was stable at pH 3 and at pH 7 the adhesion was lost. The strain *L. curvatus* HH was most effective (at pH7) against *C. glabrata* ATCC2001 and *C. alcicans* ATCC 14953, respectively.

The prevention of *C. albicans* BF is the main problem in the medical practice and preventive medicine and new technology have to be developed. The microbial BSs are the potential of disrupting the membrane and affecting the adhesion of microorganisms or cells [35]. Biniarz et al. [36]

reported that adhesion of the BS to a substrate layer alters hydrophobicity, interrupts the microbial adhesion and desorption process. The PC catheters and other implantable medical devices with BS may serve as prevention techniques to suppress the BF formation of pathogens and thereby limiting the usage of antibiotics and pharmaceuticals [19].

Ceresa et al. [37] demonstrated the inhibition of *C. albican* attachment and BF on medical-grade silicone elastomeric disks (SEDs) by the BS derived from *L. brevis* (CV8LAC). The BS (200 μg/mL) reduced the formation of BF on SEDs by 89%, 90%, and 90% (after 24, 48, and 72 hours) in the coincubation condition. The fungal adhesion and BF formation on precoated medical-grade silicone elastomeric disks were decreased by 62%, 53%, 50%, and 44%. The authors found that the antiadhesive properties of CV8LAC BS had a potential role in the prevention of fungal; infection associated with silicone medical devices. Another study by Ceresa et al. [38] reported the activity of lipopeptide from *B. subtilis* AC7 against the BF formation and adherence of *C. albicans* on SEDs. The coincubation and precoating of strain AC7 (2 mg/mL) considerably lowered the adherence and BF formation of three *C. albicans* strains on SED in a range of 67%−695% and 56%−57%, respectively. The fungal adhesion and BF formation was reduced at the range of 57%−62% and 46%−47%, respectively. The findings suggested a promising function of AC7 *B. subtilis* coatings for inhibition of infection caused by fungi related to medical-grade silicone devices.

Janek et al. [39] reported the pseudofactin II lipopeptide (LP) BS isolated from *P. fluorescens* BD5 and tested the AAD activity against different materials such as polystyrene glass and silicon surface against *E. coli, E. faecalis, E. hirae, S. epidermidis, P. mirabilis*, and two *C. albicans* strains. The pseudofactin II pretreated on polystyrene surface with 0.5 mg/mL inhibited the microbial adhesion (36%−90%) and *C. albicans* (92%−99%). The pretreated Peusdofactin II on urethral silicone catheters and incorporation of BS in the growth medium resulted in the effective reduction of *C. albicans* BF. The LP BS Pseudofactin II and Surfactin were capable to lessen the adherence of fungi to polystyrene both in preincubation and PC conditions [40].

De Gregorio et al. [41] characterized the nonhomogenous lipopeptide BS from vaginal *L. crispatus* BC1 with CMC of 2 mg/mL and capable to inhibit *Candida* sp adhesion. Their investigation suggested that BS has the potential to reduce mucosal damage caused by *Candida* sp during vulvovaginal candidiasis.

32.4 Biosurfactant as antibiofilm agent

The glycolipid biosurfactant was derived from *L. fusiformis* S9 and effectively inhibited the BF formation of *E. coli*, and *S. mutans*. The bacteria inhibited the BF formation on the surface like glass and catheter tubing and proved to be promising in biomedical applications [42]. The antimicrobial properties and ABF activity was reported by Rienzo et al. [43] using sophorolipid biosurfactant against Gram-positive and Gram-negative strains. The BS concentration at 5% inhibited the *Cupriavidus necator* ATCC 17699 and *Bacillus subtilis* BBK006 growth. The sophorolipid BS at the concentration of 5% inhibited the BF formation by single and mixed culture of *B. subtilis* BBK006 and *S. aureus* ATCC 9144 strains.

De Rienzo et al. [44] employed mono-rhamnolipid (Rha-C_{10}-C_{10}) obtained from *P. aeruginosa* ATCC 9027 and di-rhamnolipid (Rha-Rha-C_{14}-C_{14}) derived from *Burkholderia thailandensis* E264

to inhibit the BF of *B. subtilis* BBK006. The BF produced by the strain BBK006 was more sensitive to di-rhamnolipid at 0.4 g/L derived from *B. thailandensis* E264 than the mono-rhamnolipid.

The application of sophorolipids BS in the pharmaceutical industries has gained attention due to their antimicrobial, AAD, and ABF properties. Haque et al. [45] used low-cost mediums such as rice bran and cottonseed oil for the higher production of sophorolipids. Additionally, sophorolipid was niosomal formulated with potent antifungal drug ex. amphoterix B and was evaluated for ABF activity against *C. albicans*. The prepared sophorolipids-based amphotericin was characterized by microscopic methods and the mean entrapment efficiency was 23.20% ± 3.86%. The studied BS was found to downregulate the gene responsible for the formation of hyphae.

Balan et al. [46] identified a new marine bacterium *Pontobacter korlensis* SBK-47 which produced a lipopeptide BS. Based on the spectroscopic analysis the structure of the BS was elucidated as Plamitic acid-Ser-Asp-Ser-Ser. The isolated lipopeptide was named Pontifactin. The maximum ABF activity was achieved at 2 mg/mL against *B. sutilis*, *S. aureus*, *S. typhi*, and *V. choleae*.

The LP 6−2 BS derived from *B. amyloliquefaciens* anti-CA effectively disrupted the BF formation and preformed BFs of *P. aeruginosa* PA01 and *B. cereus* 1A06374. The study also revealed the strong inhibition of pslC gene expression (involved in the exopolysaccharide formation in PA01 strain and maintaining the BF structure) by the LP 6−2 [47].

A lipopeptide named coryxin produced by *Corynebacterium xerosis* was reported by Dalili et al. [48]. The corynix lipopeptide was structure illustrated as 3-hydroxydecanoic acid containing a heptapeptide part with Asn-Arg-Gln-Pro-Asn-Ser. The BS disrupted preformed BF of *S. aureus, S. mutants*, *E. coli*, and *P. aeruginosa* (percentage of inhibition of 82.5%, 80%, 66%, and 30%, respectively).

Nalini et al. [49] reported a lipopeptide from *B. cereus* SNAU01 and based on the spectroscopic analysis the lipopeptide BS was predicted by the presence of octadecanoic acid methyl ester and Pro-Leu-Gly-Gly short peptide sequence. The SNAU01 lipopeptide disrupted the BF at the concentration of 250 μg/mL against the pathogenic strains *P. aeruginosa* MTCC 2453 (72%) and *E. coli* MTCC 2939 (69%) and confirmed the removal of BF on the glass surface by confocal laser microscopy analysis.

Coronel-Leon et al. [50] reported the lichenysin production from *B. licheniformis* effectively disrupted the BF formation of methicillin-resistant *S. aureus* (MRSA) (69.73%) and *C. albicans* (74.35%) with effective dose 50 value of 8.3 and 17.2 μg/mL, respectively. The Lichenysin BS was also effective in surface posttreatment to remove BFs of MRSA (55.74%) and *Yersinia enterocolitica* (51.51%) with an effective dose 50 of 2.79 and 4.09 μg/mL respectively.

Padmavathi et al. [51] for the first time reported a BS production from coral-associated bacteria from the mucus of coral *Acropora digitifera*. Six coral-associated bacterial namely U7, U9, U10, U13, and U16 were found to be BS producers. The isolated *P. rettgeri* (U7), *Psychrobacter* sp (U9) effectively inhibited the BF formation of *P. aeruoginosa* ATCC 100145 (% of inhibition up to 76% and 77%, respectively). The strain maintained the ABF activity even at a high temperature of about 100ºC.

The BF formed by *P. aeruginosa* and *S. aureus* is the hazards BF in the clinical wastes/industries. The efforts have been made to control the surface growing pathogenic microbes which are antibiotic-resistant, as they are developing as a health issue worldwide. The BS coated with nanoparticles can be a useful aspect in blocking the hazards BF. Khalid et al. [52] described the facile process for the production of RLs coated with Ag and Fe_2O_3 nanoparticle and for their synergistic

antibacterial, AAD, and ABF activity against BF formation by *P. aeruginosa* and *S. aureus*. The rhamnolipid-coated nanoparticles displayed higher AAD and ABF activity against *P. aeruginosa* (88%) and *S. aureus* (91%). The RLs coated with silver or iron oxide may be used as efficient drug carriers and dressings for the treatment of wounds.

The glycolipid producer *Serratia marcescens* was isolated from hard coral, *Symphyllia* sp. The BS was characterized based on spectroscopic analysis and the glycolipid consists of glucose and palmitic acid. The glycolipid BS prevented the BF formation and also the preformed BFs in microtitre plate and on the glass surface of *C. albicans* BH, *P. aeruginosa* PA01, and *B. pumilis* TiO1. The BF formatin in polystyrene microliter was disrupted at the concentration of 50 μg/mL up to 55%, 62%, and 55%, respectively. The BS concentration at 100 μg/mL effectively disrupted the preformed BF up to 70%−80% and the disruption of BF was confirmed by confocal laser microscopic and electron microscopy on glass surface [53].

Borah et al. [54] reported that *P. aeruginosa* SS14 utilized rice-based distillers dried grains with solubles (RBDS) as a carbon source for the production of RLs. In the present investigation, response surface methodology was used for the optimization of BS and yielded 14.87 g/L of rhamnolipid. The rhamnolipid RBDS produced mono and di-rhamnolipid congeners. The rhamnolipid BS effectively disrupted the BF (70% reduction of BF) of *C. tropicalis* at the concentration of 250 μg/mL and the higher concentration of 1000 μg/mL, 95% reduction of BF was observed. The authors visualized the changes in the cell morphology with field emission scanning electron microscopy (FE-SEM) analysis. The untreated BF showed a complex structure encased by extracellular polymeric substances (EPSs) in FE-SEM analysis. The mechanism involved in the inhibition of EPS production by RLs depends upon the capability to prevent β-1,3-glucan synthase activity, which damages the synthesis of EPS of the β-1, 3-glucans, a key factor of extracellular polymeric substances in *C. tropicalis*. Eventually, leads to its elimination. The elimination of EPS leads to affect the ingression of RL leading to damage of cell membrane resulting in the lysis of DNA, protein, and electrolytes. The effect of rhamnolipid on preformed BF of *B. pumilus* was disrupted competently at subminimal inhibitory concentration suggesting the disruption potential of BS. The lower concentration of rhamnolipid inhibited the attachment of *B. pumilus* (46%−49%). The rhamnolipid concentration of 100 mM disrupted up to 93% of the performed BFs of *B. Pumilus* in polystyrene microtitre plate [55].

Yan et al. [56] investigated the antimicrobial, AAD, and ABF potential of BS isolated from *P. acidilactici* and *L. plantarum* against *staphylococcus aureus* CMCC26003. The BS isolated from *Pedicoccus acidilactici* significantly affected the expression of ICA A gene and the release of signaling molecules (Al-2) in the QS system. The gene expression level of agrA and sarA genes were considerably downregulated when *S. aureus* was grown in the presence of 50 mg/mL of the two types of BS. The results from their investigation indicated that different kinds of BS pose different effects on the expression of BF-related genes.

Kiran et al. [57] 2010 reported the ABF activity of glycolipid BS isolated from the marine actinobacterium *Brevibacterium casei* MSA19 against BF forming pathogenic bacteria (*V. parahaemolytics* MTCC451, *V. harveyi* MTCC3438, *V. alginolytics* MTCC 4439, *V. alcaligenes* MTCC 4442, *V. vulnificus* MTCC 1145, *P. aeruginosa* MTCC 2453, and *E. coli* MTCC 2339). The BS significantly inhibited the BF-forming capability of both mixed and individual strains at 30 μg/mL. The MSA19 glycolipid could prevent the commencement of the adhesion mediated by flagella and pili.

Ohadi et al. [58] described a new lipopeptide BS produced by *Acinetobacter junii*. The antimicrobial, ABF and antiproliferative activities of lipopeptide BS were investigated. The obtained

results revealed the BS disruption of the BF formation at a concentration of 1250 μg/mL against *S. aureus* (35%) *P. mirabilis* (10%), and *P. areuginosa* (32%), respectively. The *A. junii* concentration at 2500 μg/mL disrupted the BF formation of *S. aureus* (52%), *P. mirabilis* (31%), and *P. areuginosa* (70%), respectively. The study suggested isolated BS has potential application in the biomedical field.

32.5 Conclusion and future prospects

BF formation processes are very complex, requires various steps and almost all the surface are vulnerable to colonization. Microbial colonization and resulting indwelling BF formation may lead to contagion with serious economic and medical implications. Several surface-active agents BS exhibit antimicrobial properties as well as ADD that disrupts the BF. Thus BS is a promising agent in biomedical application and healthcare. A wide range of BS applications as ABF and antiadhesive agents needs to overcome the issues with higher production cost and very limited information about the mechanism involved for the ABF and AAD activities and the toxicity of BS towards humans. The development of BS as coating agents can be performed to develop a biomaterial with a capacity to decline the initial attachment of microbes. Further consideration should be given to investigating the ABF ability of BS combined with other molecules such as antibiotics, metal nanoparticles, and enzymes. More attention has to been paid to BF formed by multidrug-resistant microbes and by microbial consortium. BS is a fascinating approach because it could be feasible to alter the surface properties to make it an AAD agent and also control/disrupt the BF formation.

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CHAPTER

33 Current trends in the application of biosurfactant in the synthesis of nanobiosurfactant such as engineered biomolecules from various biosurfactant derived from diverse sources, nanoparticles, and nanorobots

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33.1 Introduction

Nanotechnology and nanoscience have emerged as one of the most challenging fields in multidisciplinary research. In the past few decades, the research in nanostructured materials synthesis has grown massively due to their size, morphology, crystalline nature and chemical components are properties and characteristic dependants [1]. Synthesis by low cost with high yield is a technical challenge in technology development and depends on the ability to synthesis wide applications [2]. There are three major methods are exist viz physical [3], chemical [4], and biologicals [5,6]. However, the methods are limited in production and applications due to their contaminations and toxicity. The synthesis of nanoparticles by biological method reduces the complications and biomediated synthesis using microbes has been involved as a promising substitute for conventional synthesis methods [7]. Microbial synthesis of nanoparticles (NPs) interconnects microbial biotechnology with nanotechnology and microbes reported the production of nanoparticles by enzymatic activity such as reducing and stabilizing agents [8,9]. Metal nanoparticles are synthesized biologically when microbes seize target ions from salt solutions consist environment and then turn into metal ions into metal by enzymes synthesized by microbial cell activity.

Microorganisms serve as nano factories for low cost and eco-friendly synthesis of nanomaterials such as gold, silver copper, and metal oxides such as titanium oxide, zinc oxide, etc., exist in different shapes and sizes including nanorod [10], nanowires [11], and nanoparticles [12]. This green approach exploits biological creatures such as bacteria, fungi, algae, yeast, viruses, and actinomycetes for bioproduction. The bio route provides low cost, nontoxic way to produce with unique

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properties. Natural products from microorganisms are structurally diverse and represent a rich source for the production of active compounds for biomedical applications.

Biosurfactants (BSs) are biological surface-active compounds mainly derived from microbial sources that are cationic or neutrals produced from intracellular or extracellularly [13]. Most BS is produced by microbes by a complex mixture of BS at the growth phase under water-immiscible substrates and a better alternative to chemical surfactants. The amphiphilic compounds containing both hydrophilic and hydrophobic portions referred to as head and tail facilitate to decrease the interfacial and surface tension of liquid−liquid, solid−liquid, or air−liquid medium [14]. The hydrophilic moieties can be alcohol or carboxylic acid, phosphate, cyclic peptide or proteins, amino acid, and mono-, oligo- or polysaccharides and hydrophobics are α-alkyl β-hydroxy fatty acid, saturated, unsaturated fatty acid, which are nontoxic biomolecules that are biodegradable, active at extreme temperatures, pH and salinity as well [15,16].

The properties of BS are determined by the size and location of their functional groups and divided as low molecular weight agents (biosurfactants), which are glycolipids, lipopeptides, and phospholipids. Particularly BS exhibits strong emulsification of hydrophobic compounds and forms stable emulsions and higher molecular weight polymers are emulsion-stabilizing agents [17]. Multifunctional BS is produced from industrial wastages including a dairy, oily, distillery, slaughterhouse waste, sugarcane molasses, and starchy effluents [13,18−20]. The concentration and chemical structure of BS is dependent on microbial types and growth conditions [21]. BS is characterized by properties associated with charge and chemical structure, critical micelle concentration, hydrophilic−lipophilic balance. Physicochemical properties of critical micelle concentrations are changed and the value is used to measure the efficiency of BS. Low micelle concentration of BS reduces the surface tension [22]. Reduced surface and interfacial tensions, increased solubility, and bioavailability of hydrophobic organic substances are enabled by BS through micelle formation [22]. Hydrophilic−lipophilic balance is another property of BS to analyze the viscosity, density, turbidity, conductivity, and osmotic pressure, the value indicates the oil-in-water or water-in-oil emulsion of BS. The great advantages and suitability of BS have become increased attention in multifunctional utilization and applications in various industries.

33.2 Microbial synthesis of biosurfactants

Microbial BS has received more attention over synthetic surfactants due to its several significant advantageous properties vis Hydrophilicity, hydrophobicity, detergency, emulsification, gelling, flocculation, foaming, low critical micellar concentration, antimicrobial efficiency, metal sequestration [23], and enhanced bioavailability applied to a variety of industrial processes. Microbes are the main source for BS synthesis including bacteria, fungi, and yeast are the potential precursors and produce a complex mixture of BS [24].

Bacteria is a major producer for the synthesis of BS by amphiphilic molecules which are extracellular as microbial metabolites. Biosurfactants synthesized by a broad range of microorganisms are nonpathogenic and acquired increased attention due to diverse applicability, low toxicity, biodegradability, and the capability of the production from inexpensive substrates [24,25]. The number of microorganisms used to produce biosurfactants that are isolated from contaminated soils and wastewater sources is divided into several classes.

Table 33.1 A representative list of microbes used to synthesis of biosurfactants.

Biosurfactants group	Classifications	Producing microbes	References
Glycolipids	Rhamnolipids	*Pseudomonas aeruginosa*	[44]
		Burkholderia glumae	[45]
		Pseudomonas aeruginosa A10	[46]
		Pseudomonas aeruginosa P6	[47]
		Pseudomonas guariconensis LE3	[48]
		Acinetobacter calcoaceticus	[49]
		Pseudomonas putida	[50]
		Pseudomonas guguanensis	[51]
		Acinetobacter junii	[52]
		Enterobacter asburiae	[53]
		Pantoea stewartii	[54]
		Thermus aquaticus	[55]
		Meiothermus ruber	
		Serratia rubidea	[56]
	Sophorolipids	*Candida bombicola*	[57]
		Streptococcus mutans and *Lactobacillus acidophilus*	[58]
		Starmerella bombicola	[59]
		Candida albicans SC5314 and *Candida glabrata CBS138*	[60]
	Mannosylerythritol lipids	*Pseudozyma aphidis*	[61]
		Staphylococcus aureus ATCC	[62]
		Pseudozyma tsukubaensis	[63]
		Pseudozyma antarctica	[64]
		Candida antarctica	[65]

1. *Glycolipids*: Carbohydrates linked by long-chain hydroxyaliphatic acids or aliphatic acids by ester group, for example, sophorolipids, trehalolipids, rhamnolipids, mannosylerythritol lipids (Table 33.1).
2. *Lipoprotein/lipopeptides*: Mainly produced by *Bacillus* spp. which are cyclic lipopeptides with large numbers, for example, kurstakins, surfactins, fengycins, and iturins.
3. *Lipopolysaccharides*: High molecular weight compounds.
4. *Phospholipids, fatty acids, and neutral lipids*: *R. erythropolis*, *Acinetobacter* sp., and yeast are mainly produced at n-alkane conditions.
5. *Polymeric biosurfactants*: Liposan, alas an, emulsan, and other polysaccharide−protein complexes. *Acinetobacter calcoaceticus*.

Glycolipids biosurfactants are among the most popular saccharide headgroups and fatty acid tails and are compositionally distinct based on producing microbial strains (Fig. 33.1). Glycolipids are subdivided into rhamnose lipids, trehalose lipids, sophorose lipids, cellobiose lipids, mannosylerythritol

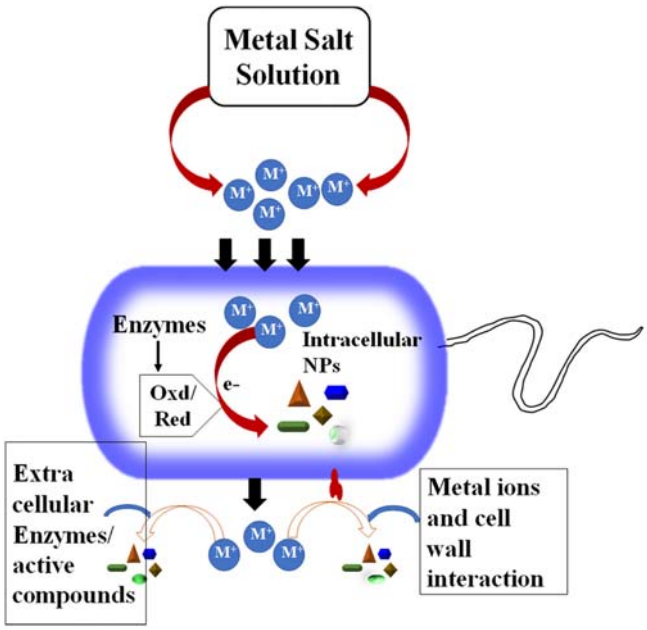

FIGURE 33.1

Pie chart for approximate distribution of microbial producers of simple biosurfactants (glycolipids).

lipids, lipomannosyl-mannitols, lipomannans and lipoarabinomannanes, glycosyl diglycerides, monoacylglycerol, and galactosyl-diglyceride. As such, this chapter will focus primarily on advances in biosynthesis, and applications of these valuable glycolipid biosurfactants in nanoparticle synthesis.

33.2.1 Applications of biosurfactants

Biosurfactants are most attractive wide applications including food production; BS exhibit useful emulsifiers properties [26], antiadhesives [27], and antimicrobial agents medicine [28] cosmetics [29] agricultural [30], pharmaceutics, and chemical industries. In food industries, BS is used as an emulsifier to reduce the surface tension between two immiscible phases at their interface and is also used as antimicrobial agents against bacteria, fungi, viruses, and yeast. Particularly, lipopeptides and others such as bacillomycins, iturin, fengycin produced by *B. subtillis* are widely used in food industries. In farm animal production, dietary and nutritional manipulation is the main direction of BS including rhamnolipid, a class of alkyl polyglucosides, supports to increase ruminant nutrition.

In biomedical applications, BS is used as drug delivery agent to induce passive immunization particularly when drug treatments are limited. Liposomes are promising candidates with wide applicability to vaccination. Glycolipid(MEL-A) is a cationic liposome that promotes gene transfection in mammalian cell culture. Surfactin from *B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*, and *B. pumilus* strains exhibit wide medical applications including antimicrobial and antitumor activities, target cell membranes, and interaction the membrane molecules. In addition to that surfactin inhibits

the formation of a fibrin clot. Inhibition of spleen cytosolic phospholipase and platelet, blocking of cyclic adenosine monophosphate (AMP) activity, increasing the formation of ion channels in the lipid membrane. Many microbial surfactants are used in environmental applications: (1) remediation of organic and metal contaminants due to the unique advantages in emulsification, deemulsification, foaming, and coating by useful physicochemical properties [31], (2) Biosurfactants used in biological remediation by hydrocarbon-degrading bacteria present in polluted soil resulting increases the bioavailability of hydrocarbon [32], (3) removal of heavy metals from aqueous solution, and (4) increased bacterial tolerance to heavy metals.

33.2.2 Role of biosurfactants in biosynthesis of nanoparticles

Synthesis of nanoparticles has received great attention in past decays due to their unique properties such as size, shape, and surface chemistry can be precisely controlled, leading to functional materials and multiple applications in health care and other industries [33,34]. Identifying the importance of developing eco-friendly methods to synthesis active and stable nanoparticles, growing awareness of biosynthesis method to overcome certain limitations over physical and chemical methods (Table 33.2). Although the biological methods are superior, complex downstream processes, generation of nanoparticles slower (reaction times between 24 and 120 hours), and extended reaction/reduction time are the limitations (Fig. 33.2). In this context, biosurfactants are emerging alternatives for nanoparticle synthesis. Here we are presenting the glycol lipid biosurfactants act as both reducing/stabilizing/capping agents (Fig. 33.3).

Bacterial rhamnolipids are used to stabilize, capping, and dispersant in the synthesis of nanoparticles. Researchers used *P. aeruginosa* to extract rhamnolipid (RL) BS for the synthesis of silver nanoparticles [35,36]. Acidified bacterial suspension allowed to precipitate rhamnolipid at 4°C and extracted by ethyl acetate. Pure rhamnolipid is used to synthesis AgNPs. Rhamnolipid along with sodium borohydride at lowest concentration used to synthesis under ice bath, then silver nitrate solution dropped, and slowly the color changes were observed by the researcher to confirm the AgNPs. Rhamnolipid acts as a stabilizer in this reaction for AgNPs synthesis. The stability of nanoparticles is an important factor considering their application in various fields. From the result of the researchers, there was a loss of stability by chemically synthesized AgNPs after 10 days, subsequently, using rhamnolipid synthesized AgNPs tested for long time storage and observed the absorbance peak of AgNPs were stable after 33 days. Based on the results, it was confirmed AgNPs were stabilized by the RL stabilizer to prevent the oxidation of nanoparticles.

Bacterial RL has recently been used for dispersant and capping agents of nanomaterials; particularly the previous reports are demonstrated that RL capped nanomaterials e highly stable to avoid oxidation which can improve the duration of storage. The capping ligands facilitate a double bond between the shell capping agent with materials is more and increase the dissolution and longer carbon chain which prevents the nanomaterials aggregations. Zinc oxide nanoparticles (ZnO NPs) synthesized by RL as capping ligands *P. aeruginosa* CEMS077 strain supplemented with glycerol at desired pH helps to synthesis RL. Hydrophilicity is another property of nanomaterial which is requisite to meet the needs of biocompatibility. *P. aeruginosa* BS01 has been used to synthesis of RL and zinc sulfide (ZnS) nanocrystals with high and uniform dispersity and hydrophilicity. RL stabilized/reduced/capped ZNS nanocrystals which are high reproducibility, consistent with size, shape, and distribution, low agglomeration [8,37].

Table 33.2 A representative list of biosurfactants synthesized by microbes for nanoparticle synthesis.

Microbial source	Biosurfactants	Types of nanoparticles	References
Pseudomonas aeruginosa PTCC 13401	Rhamnolipid	Au	[66]
Brevibacterium casei MSA19	Glycolipid	Ag	[67]
Bacillus subtilis T-1	Surfectin	Ag	[68]
Pseudomonas aeruginosa TEN01	Rhamnolipid	Ag	[69]
Pseudomonas aeruginosa	Rhamnolipid	Ag	[35]
Ustilago maydishas	Mannosylerythritol lipid	Ag	[70]
Pseudomonas aeruginosa MKVIT3	Surfectin	Ag	[71]
Bacillus vallismortis MDU6	Crude	Ag	[72]
Pseudomonas aeruginosa BS-161R	Rhamnolipid	Ag	[73]
Bacillus subtilis CN2	Lipopeptide	Ag	[74]
Bacillus subtilis ANR 88	Rhamnolipid	Ag and Au	[75]
Candida bombicola	Sophorolipid	Ag	[76]
Bacillus amyloliquifaciens KSU-109	Surfactin	Cd	[77]
Pseudomonas aeruginosa	Rhamnolipid	CuO	[78]
Stamerella bombicola	Sophorolipid	Au	[79]
Candida bombicola	Sophorolipid	Au	[42]
Ustilago maydis	Mannosylerythritol lipid	Au	[43]
Pseudomonas aeruginosa	Rhamnolipid	Au	[80]
Acinetobacter junii B6	Lipopeptide	Au	[81]
Bacillus natto TK-1	Surfectin	SPION	[82]
Pseudomonas aeruginosa SP4	Rhamnolipid	Polyaniline (PANI)	[39]
Pseudomonas aeruginosa BS01	Rhamnolipid	Poly(methylmethacrylate) (PMMA)	[83]
Pseudomonas aeruginosa CEMS077	Rhamnolipid	ZnO	[37]
Pseudomonas aeruginosa BS01	Rhamnolipids	ZnS	[8]
Pseudomonas aeruginosa	Rhamnolipids	ZnS	[84]
Starmerella bombicola	Sophorolipid	SiO_2	[40]

Preparation of NiO nanomaterials using RL as BS with high crystalline nature and the morphology of as-prepared NiO nanomaterials depends on the presence of pH in the media [38]. Two different procedures followed to synthesis NiO by microemulsion; RL and *n*-heptane mixed with $NiCl_2$ $6H_2O$ and the second method with the presence of NH_4OH as catalysis. Solution. The author observed that the pH value from low to high influences the shape changes as flaky and spherical shapes. Subsequently, the agglomeration of prepared nanoparticles is much higher at high pH values (9.3 and 9.6). RL favored the structural changes at different pH values from low (6) to high (<9) of different morphology and dimensions. Similarly, conductive polymers (CPs) have been explored in past decays due to their unique property as an alternative substance of metallic

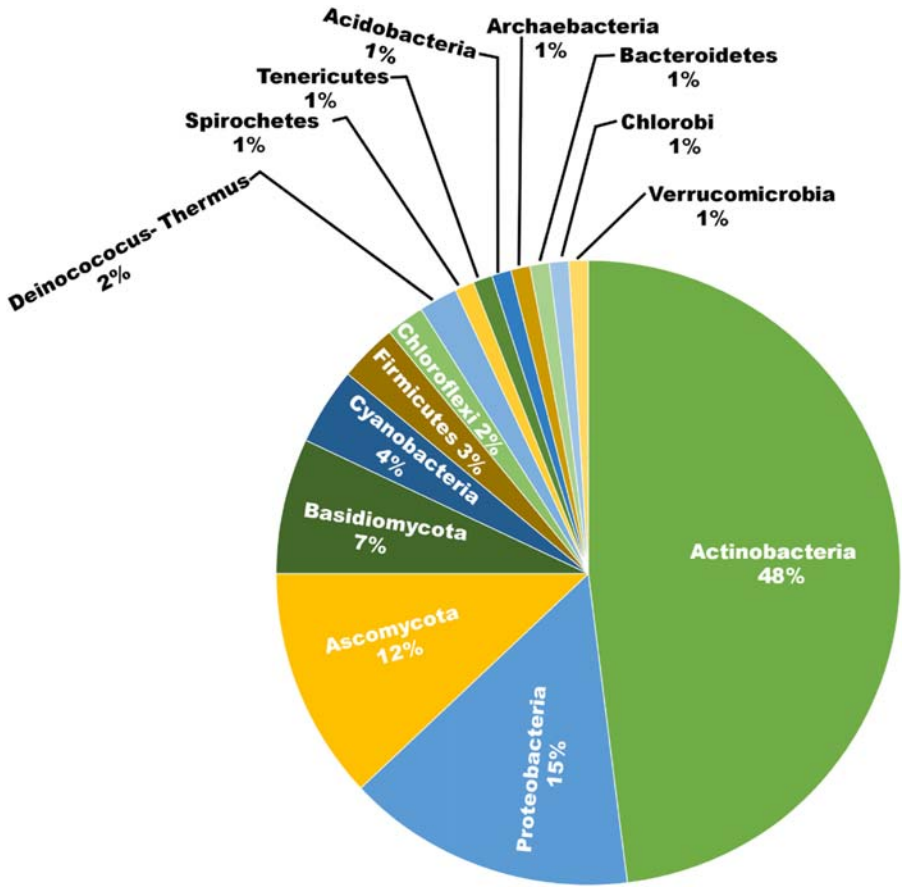

FIGURE 33.2

Schematic illustration of intracellular and extracellular mechanisms involved in the formation of nanoparticles using microbial sources.

interfaces in applications of high electroactivity in biomedical devices. Various methods are explored in the synthesis of such polymers with some limitations such as long procedures, poor reproducibility, and poor performances. Polyaniline (PANI) is one of them with high utility in all aspects [39]. PANI preparation using RL as a template by the formation of microstructures such as micelles and vesicles that helps to stabilize or entrap the organic or inorganic precursors. RL 9 the author as Rha−C10−C10 (73.5%) is a predominant composition. With a different ratio of ANI and RL were used to synthesis different shapes viz 11:1 exhibited irregular structure due to aggregation, 19:1 to 28:1 ratio showed fibrillar structure.

Sophorolipids are composed of glucose a carbon substance to provide a sophorose group, disaccharides, and fatty acids as a secondary carbon source to provide a lipid chain. Sorpholopids are widely used in the food, pharmaceutical, cosmetic, and cleaning industries. From research porous

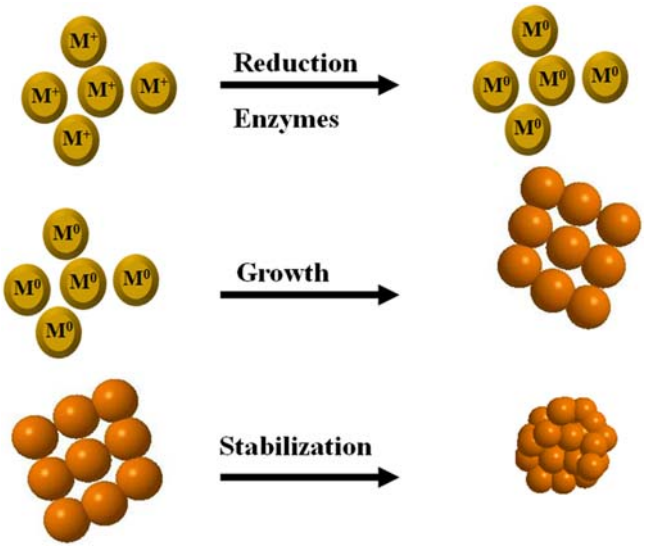

FIGURE 33.3

Sequential formation of nanoparticles using biosurfactants involves different mechanisms.

silica using sophorolipids from *Starmerella bombicola* (ATCC 22214) with the pore range from 2 to 30 nm [40]. Amine-modified silica and the carboxyl group of SL interaction by electrostatics promote making porosity of silica. A different ratio of amino-functionalized silica nanoparticles shows different pores which were confirmed by the N_2 adsorption method. According to the method of making porosity and morphology of silica particles tuned by adjusting SL; at a low ratio of SL the morphology deteriorated and the authors confirmed that calcination of the materials makes a clear porosity with a narrow pore size distribution, at higher ratio porous micrometer-sized spherical particles are formed, the porosity is accessible by no calcination with the range of 30 nm. SL played a key factor in the formation of mesoporous silica nanoparticles

Another capping agent of SL is used in capped iron oxide nanoparticles (IONP) [41], SL prepared from *S. bombicola*. Due to the open acidic form of SL, used as a surface stabilizing agent to prevent the oxidation of metal/metal oxide nanoparticles particularly iron oxide nanoparticles. Their work explored that COOH from SL was modified with nitrodopamine (NDA) which is of high affinity with iron oxide nanoparticles to replace the ligand for strong interaction between iron oxide and SL. Further, the authors evaluated the colloidal stability of SL-IONP large variety of attractive interactions and led to nanoparticle aggregation. The high density of SL-NDA layer was observed from SL-IONP which has excellent colloidal stability in biological applications SL capped IONP are high protein and salt condition they have no cytotoxicity and negligible uptake by cells so that SL-IONP can be used for drug delivery. A similar observation has been reported for cobalt nanoparticle synthesis using SL. SL synthesized and extracted from the yeast of *S. bombicola* and acted as a capping agent. Synthesized nanoparticles were poly dispersive and more stable. The SL capped nanoparticles were biocompatible can be used for attachment of biomolecules and used as a diagnostic tool [42].

Mannosylerythritol lipids (MELs) are glycolipid biosurfactants produced by a variety of bacteria, fungi, and yeast that exhibit excellent biochemical and interfacial properties. MELs have several mannoses, 4-*O*-β-D-mannopyranosyl-erythritol, or 1-*O*-β-D-mannopyranosyl-erythritol as a hydrophilic head group fatty acid chain length and their saturation, number, and position of erythritol or acetyl group on mannose or both. MELs synthesized from Ustilago maydis to produce gold nanoparticles [43] Ustilago maydis CGMCC 5.203 used to synthesize MELs, using MELs, synthesized gold nanoparticles (AuNPs) with simple procedures. MELs act as reducing and capping agents in AuNPs synthesis. Owing to their electrostatic charges influence the shape and size of the particles and prevention of aggregation as well because of their excellent stabilizing capacity and hinder the aggregation. MELs AuNPs have good physical characters that help to bioactivities good physical characteristics which helps to improve bioactivities including antibacterial activity, antioxidant capacity, and toxicity toward HepG2 cells. The authors concluded that MELs stabilized AuNPs used for drug delivery and diagnostics tools.

33.3 Conclusion

Biological system has various opening for utilization of nanotechnology to develop the eco-friendly process for material synthesis. Biosurfactants, an amphiphilic compound from microbes have promising diversity in applications including textiles, varnish, mining, pharmaceuticals, oil recovery, and biomedical applications. The process of BS production is an emerging technique as an alternative source for the synthesis of nanoparticles and one of the most important to make more valuable in industrial applications due to their chemical and biological properties. Lower liquid surface tension has been a widely known key factor of BS in nanoparticle synthesis. The role of BS in NPs synthesis has been described used as capping agents with decreased toxicity, functional precursors with luminescence. The microemulsion method using an oil-water-surfactant mixture is a promising approach for the synthesis of nanoparticles. The BS-mediated nanoparticle synthesis is one of the superior methods of bacterial strains or fungal strains. Reduced aggregation of nanoparticles due to electrostatic attraction facilitates the formation of morphologically uniformed NPs. In this chapter, we highlight that BS-assisted NPs have been explored with different sizes, various shapes, strong stability, reduced agglomeration, controlled synthesis. Hence, BS-mediated NPs synthesis moves forward effectively to achieve an eco-friendly synthesis method in industrial development (Tables 33.1 and 33.2).

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CHAPTER

Application of biosurfactants in the food industry: supply chain and green economy perspectives

34

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34.1 Introduction

In the last decade, the demand for green and sustainable ingredients in food and cosmetic products has increased. The growing consumer awareness on utilization of natural and organic ingredients instead of chemically synthesized additives has driven the research of surfactants and emulsifiers to be derived from a natural origin. Surfactants are amphiphilic chemical compounds with hydrophilic (polar) and hydrophobic (nonpolar) fractions and hydrogen bonds between fluid phases like, air/water or oil/water that minimize the interfacial and surface tensions and makes emulsion [1,2]. Due to the global environmental awareness, recently biosurfactants, derived by microorganisms are getting more attention rather than chemical surfactants (e.g., sulphonates, carboxylates, sulfate acid esters) for its environmentally friendly nature, low toxicity, biocompatibility, greater selectivity, and efficacy under paramount environmental conditions (e.g., temperature, salinity, pH, etc.). The superficial-active properties of biosurfactant make them a more popular candidate. Most literature surveys on the application of biosurfactants are focused on bioremediation of pollutants as well as microbial enhanced oil recovery [3,4]. Nevertheless, these compounds reveal an array of valuable properties for the food industry particularly as emulsifiers, antiadhesive, wetting, foaming solubilizers [1,5], and antimicrobial agents [6]. Furthermore, to reduce the usage of synthetic compounds, there is an increasing demand for manufacturing greener and more sustainable food additives [7]. It is used for different purposes like modifying the texture of dough for cookies, cake, and bread, improving the viscosity, inhibiting pathogenic microorganism growth, stabilizing the salad dressings, etc. [2]. Regardless of the advantages shown by biosurfactants, there are few reports on hand concerning their use in the food industry.

34.1.1 Classification of biosurfactants

Biosurfactants are mostly fabricated from microorganisms growing aerobically from a carbon source feedstock in aqueous media. The source of the carbon feedstock could be carbohydrate, fat,

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oil, hydrocarbon, or any of their mixtures. Now the artificially synthesized surfactants are usually categorized depending upon the dissociation patterns in water, while the biosurfactants are classified by composition, molecular weight, mode of action, physicochemical properties, and microbial origin [8].

Biosurfactants are discharged into the culture to aid in the development of the microorganism. Their secretion assists the transportation and translocation of insoluble substrates across cell membranes. Depending upon the type of secretion from the cell, biosurfactants can be categorized into intracellular, extracellular, and adherent to microbacterial cells. All biosurfactants are of nonionic or anionic structures while cationic biosurfactants consist of amine groups. The hydrophilic moiety can be any type of amino acid, peptide, alcohol, phosphate carboxyl acid, or carbohydrate and the hydrophobic moiety usually consists of long-chain fatty acids. According to their microbial origin, they can also be categorized. Now classical literature suggests that according to molecular weights, biosurfactants are of two categories—(1) Low-Molecular-Mass biosurfactants (LMMBs) and (2) high-molecular mass biosurfactants (HMMBs) [9,10]. The LMMBs include glycolipid, phospholipids, and lipopeptides whereas the HMMBs involves polymeric surfactants as well as proteins, lipoproteins as they are mainly efficient as emulsion stabilizing agent. On the other hand, the LMMBs are effective in reducing surface and interfacial tensions [11].

34.1.2 Biosurfactant properties

There are some characteristic properties of biosurfactants that are important for the food processing industry. These include surface tension, pH tolerance, temperature tolerance, biodegradability, low toxicity, etc. These are illustrated in detail in the following subsections.

34.1.2.1 The surface and interfacial activity

Several biosurfactants are more proficient and effectual than conventional surfactants, decreasing the surface tension of liquids at very low concentrations. For example, Surfactin can lower the surface tension of water from 72 to 27 mN/m at 20 nM which is a relatively low concentration [12]. Now when a biosurfactant is developed or included in the medium, micelles are produced which consists of both hydrophilic and hydrophobic parts. Based on the nature of the system, the hydrophilic portion of the biosurfactant could be placed toward the external or internal part of the system so that the micelle becomes protected [13]. These micelles lead to lower surface tension and also are openly influenced by the boost in the concentration of the biosurfactant. Now the critical micelle concentration (CMC) is the minimum concentration of biosurfactant required for the maximum drop in surface and interfacial tension [14,15].

Different CMC values are observed for different chemical structures of different biosurfactants. The self-aggregation of micelles has a deep effect on the CMC values which in turn is managed by other properties such as temperature, pH, polarity, ionic strength, etc.

34.1.2.2 pH, temperature, and tolerance to ionic strength

The superficial activity and other properties of several biosurfactants are not affected by temperature and pH. Also, many biosurfactants can show their ability in extreme conditions. Nitschke and Pastore reported that a *B. subtilis* LB5a derived lipopeptide was steady after autoclaving (394K/20 minutes). Even after half year it was also stable at 255K. The superficial activity of the

lipopeptide was unaffected in the pH range 5−11 as well as in up to 20% NaCl solution [16]. It was reported *Bacillus licheniformis* JF-2 derived lichenysin was unaffected by up to 333K temperature, pH range of 4.5−9.0, and by Ca and NaCl concentrations up to 25 and 50 g/L, respectively [17]. de Freitas Ferreira et al. [18] obtained the consequence of environmental conditions on rhamnolipid activity. It operates as an antimicrobial agent in food, controlling the escalation of pathogens. It was recognized that rhamnolipids are pH-sensitive and therefore work best in more acidic circumstances.

34.1.2.3 Biodegradability

Biosurfactants are prone to bacterial degradation along with other microorganisms present in nature. This is responsible for making these molecules predominantly appropriate for environmental applications such as bioremediation processes as well as for waste treatment.

34.1.2.4 Low toxicity and availability

Biosurfactants are believed to be nontoxic and sustainable molecules. Some groundwork testing also displayed low toxicity, which makes them appropriate for use in food products, pharmaceuticals, and cosmetics. Sahnoun et al. [19] anticipated the critical concentration of biosurfactant in an animal model to be extremely higher than the expected concentrations of food additives permitted by the Food and Agricultural Organization and World Health Organization [19]. In their work, the nontoxic nature of lipopeptide biosurfactant was established via the study of the brine shrimp cytotoxicity and phytotoxicity assays. Biosurfactants can be developed from extensively accessible sustainable raw materials and they can be readily available as they can also be produced using industrial waste as substrate.

34.1.2.5 Emulsification and demulsification

Emulsions have an internal and an external phase and as a result, they are of typically two types: oil-in-water (o/w) and water-in-oil (w/o) emulsions. The emulsions usually possess titular stability which may be emphasized by surfactants. Biosurfactants may stabilize (emulsifiers) or destabilize (de-emulsifiers) the emulsion. Generally, the HMMBs are better emulsifiers than LMMBs. Sophorolipids produced from *Torulopsis bombicola* were found to reduce surface tension as well as interfacial tension but they are not good emulsifiers [20]. On the other hand, liposan does not lessen surface tension, but it has been utilized effectively to emulsify edible oils. Polymeric surfactants suggest further advantages as they coat droplets of oil thereby resulting in the formation of very stable emulsions which never coalesce. This is a particularly handy property for developing oil-water emulsions for use in the food industry [21]. Recently, Gaur et al. [22] reported that biosurfactants isolated from yeast strains *C. albicans* and *Candida glabrata* were observed to reduce surface tension as it acts as an emulsifier, which was sophorolipids. The biosurfactants demonstrated noteworthy antibacterial activity against *B. subtilis* and *E. coli* [22].

34.1.2.6 Antimicrobial activity

Various biosurfactants have revealed antimicrobial action against bacteria, algae, fungi, and viruses. The lipopeptide iturin from *B. subtilis* displayed effective antifungal activity [23]. Inactivation of enveloped viruses such as herpes and retrovirus was observed with 80 mM of surfactin [24]. Sophorolipids and rhamnolipids were found to be proficient antifungal agents against plant and

seed pathogenic fungi. Mycelial growth of Phytophthora sp. and Pythium sp. was 80% repressed by 200 mg/L of rhamnolipids and 500 mg/L of sophorolipids [25]. These could also be potentially utilized as food emulsifiers and as antibacterial agents.

Although surfactants are very useful in various applications, their chemical nature restricted their uses in food industries. In such a situation biosurfactants can replace the position due to their biodegradable and nontoxic nature. But, there is still some lack of research in a various applications using biosurfactants. This chapter mainly focuses on the variety of properties and the exploitation of biosurfactants applications in different sectors of food industries. Their advantages, classification, and their production from different food and agro-wastes also have been reviewed. Our objective is to deliver knowledge that can help biosurfactants more viable in various fields of application in the food industry.

34.2 Methodology

The chapter is primarily based on existing literature followed by further analysis. A thorough literature review was carried out to gather information on biosurfactants and their application in the food industry. Several standalone and combination of keywords were utilized for the literature review, such as—"biosurfactant"; "biosurfactant + food industry application"; "biosurfactant + waste"; "biosurfactant + food waste"; "biosurfactant + Supply chain"; "biosurfactant + circular economy," "Green economy approaches", etc. The collected papers were sorted and segregated. The cross-references were also evaluated wherever necessary. Further analysis was done to identify information for the composition of the chapter. Finally, a brainstorming session was carried out to identify possible routes for the development of a resource-efficient system following the principles of a Green Economy.

34.3 Biosurfactant production from food and agro-waste

Recently, the term "circular economy" has been coined by the European Commission. The concept is to aim for the prevention of waste generation or when that is inevitable, generated waste should be utilized for bioeconomical purposes [26]. Another evolving concept, which is very much in line with the Circular Economy is Green Economy, which is a great strategy to enhance biosurfactant production [27,28]. In the last few years, a broad range of products has been fabricated with the help of renewable sources as raw materials. Biosurfactant production using the core metabolic pathways via the microorganisms is not always a feasible process of biosurfactant production. Besides, the yield is affected by physiological state, cell density, nutrient availability, etc. To facilitate the production process, it is essential to include substrates like oils, fats, solid and liquid hydrocarbons in water as they are insoluble [29,30]. Biosurfactants can be developed in the course of distinctive metabolic routes in the stationary growth phase of the microorganism. Depending upon the prior kinetic survey to establish the variables, it is also probable to produce biosurfactants under the most advantageous growth conditions [31,32]. Although biosurfactants have several leads over synthetic surfactants, they are not however competent in challenging chemical surfactants cost-wise [33]. Recently the increasing environmental awareness has guided to the requirement for the advancement of biosurfactants as a substitute to the already accessible products.

The cost of biosurfactant production is higher than that of those synthetic surfactants [34]. Now to reduce this cost and propose environmentally advantageous preferences, the use of agro-industrial waste products has become a definite need as they can be treated as alternative substrates. The use of agro-industrial waste products as the raw materials is possible in biosurfactant production although there should be a nutritional balance and also the quality of the end product should not be compromised. Though there is no well-approved method yet by which one can achieve larger productivity and economic recovery on a biosurfactant [35,36] there has been constant strategic improvement recently to attain enhanced economic viability and competitiveness of the biosurfactants [37,38].

In most of the countries with agriculture as one of the chief aspect of the economy, the accessibility of agro-industrial by-products is reasonably noteworthy in every one of them. The waste generated by the soap stock, by the processing-refining soybean oil, palm oil, and other vegetable oils refining products consisting of sunflower, soybean, olive, groundnut, sesame, coconut, mustard oils are all familiar [39,40]. The literature also reports that the production of biosurfactants includes various types of substrates such as by-products, such as glycerol [41], sugarcane molasses [41], and corn steep liquor [42]. The utilization of further potential raw products has also lately been reported, for instance, sugarcane bagasse, green coconut, and straw from the carnauba palm [43]. In 2019, Chen et al. used corncob hydrolyzate as substrate for surfactin production which is loaded with xylose by *B. subtilis* BS-37, achieving 0.523 g/L [44]. Samad et al. (2014) reported the production of sophorolipid (3.6 g/L) by the microorganism *Candida* (*Starmerella*) *bombicola* which was grown on hydrolyzates from sweet sorghum bagasse. Among different dairy based products, cheese whey appears to be a much popular choice as a substrate [45]. In 2008, *L. pentosus* CECT-4023 was reported by Rodrigues et al. as a strong BS producing strain on whey cheese [46] while in 2015, Gudiña, et al. reported BS production from different *Lactobacillus* strains on conventional Mrs medium [47]. This medium is eminent for the growth and production of BS from lactic acid bacteria. Studies have further indicated that the waste products can also act as sources of carbon and nitrogen. Moreover, most of these products are procured from the food processing industry although there are a small amount of limitations concerning use for the development of microorganisms and the consequent manufacture of biosurfactants that can be used in the food, cosmetic, pharmaceutical, and agrochemical industries [48,49]. Actually, the greatest benefits from reducing waste disposal reside in making use of such wastes and reducing associated wastewaters generated from industries [50]. Therefore, the utilization of waste manufactured goods is not only beneficial in terms of reducing the cost of the substrates but also due to the accessibility and stability of each substrate.

34.4 Potential food applications of biosurfactants

34.4.1 Antioxidants and antiadhesives

In the food industry, the antioxidant property is the very prime factor that minimizes the various diseases (e.g., degenerative diseases, risk of heart disease) and consecutively intensifies the self-life of the product by delaying the oxidation reactions. Biosurfactants are highly desirable for their antioxidant property due to the existence of unsaturated fatty acids and better tricky reducing capacity with radical-segregation scope [2]. Different types of biosurfactants are used such as

Mannosylerythritol lipids (MELs), polysaccharide emulsifiers from Klebsiella, etc. *B. subtilis* RW-I also can be used for the development of biosurfactants as antioxidants [51]. Biosurfactants are also used to enhance the helpful products life due to the antiadhesive and antimicrobial properties of the surfactants by preventing microbial proliferation through controlling the attachment of microorganisms to the surfaces of equipment, pipeline, food, and food processing materials [2]. Such types of biosurfactants are used as a coating agent on equipment or food utensils for minimizing antifouling rate [51]. Giri et al. [52] studied antioxidant, antimicrobial, and antiadhesive properties from biosurfactants evaluated against *Bacillus licheniformis* VS16 and *Bacillus subtilis* VSG4. They also investigated the antiadhesive property developed by Bacillus cereus ATCC 11778, *Staphylococcus aureus* strain ATCC 29523, and *Salmonella typhimurium* strain ATCC 19430. The results showed potential application in food industries [52]. Thymol nanoemulsions developed from biosurfactant, for example, Quillaja Saponin and solvents prepared by tricaprylin, cinnamaldehyde, and a high level of oleic sunflower oil, which is an excellent antioxidant agent used in the food industry [53].

34.4.2 Salad dressings

Biosurfactants have good potential for application in the use of salad dressings. Researchers have achieved improved texture and high consistency of mayonnaise and salad dressings using biosurfactants as emulsifiers. Shepherd et al. [7] isolated a bioemulsifier from *C. utilis* which exhibited a high prospective for utilization in salad creams. Campos et al. [54] isolated and characterized a chemical compound which is essentially carbohydrate-protein-lipid complex derived from *C. utilis*. The compound increased the consistency of salad dressing when mixed at a concentration of 0.7% with carboxymethylcellulose and guar gum after 1 month storage [54]. According to Santos et al. [55], a mannoprotein is synthesized by *S. cerevisiae* that can stabilize oil-water emulsions in mayonnaise [55]. It has been reported that red algae such as *Porphiridium cruentum* and yeasts such as *Hansenula anomala, Rhodospiridium diobovatum* can produce extracellular bioemulsifiers [51,56]. These bioemulsifiers exhibit better stability when under extreme conditions such as temperature and pH compared to Arabic gum, guar gum, and carboxymethylcellulose [1,57,58].

34.4.3 Ice cream and bakery products

Biosurfactants can be used for various purposes including freshness extension, texture improvement, consistency control, creaminess development, solubilizing aromatic oils in the product in the bakery industry as well as ice-cream industry [58,59]. The idea that triggered the usage of biosurfactants in bakery products is finding eco-friendly replacement for available commercial alternatives. Another big reason for research inclination in this area is the growing demand for healthier composition and strict dietary portfolio [60]. Recent studies have confirmed the industrial potential of biosurfactant application for improvement of dough texture and reduction in calorific value of cookies and muffins [61]. The rhamnolipid biosurfactants have been found to improve the dough texture, stability, volume, and shelf life of bakery items. Glycerol monostearate is a commercial emulsifier used in bakery items. *B. subtilis derived bioemulsifier* was used to replace the commercial one which led to a better quality of bread and cookies in terms of texture and reduced sensitivity toward microbial attack. A softer texture in muffins was achieved through the addition of 0.75% concentration of a lipopeptide biosurfactant produced from *Nesterenkonia* sp. in muffin

formulation. The improvements were found in terms of less hardness and chewiness and improved cohesion and elasticity [62,63]. Replacement of egg yolk was achieved with a glycolipid biosurfactant derived from *S. cerevisiae* URM 6670 in cookies formulation suitable for vegan and health-conscious customers [64]. Research shows that it is also possible to replace vegetable oils in muffins with *C. bombicola* URM 3718 derived biosurfactants which can significantly increase the nutritional value as the amount of trans fatty acids is reduced significantly [65].

Utilization of biosurfactants in ice-cream is a possibility as explored by the researchers but there are no reports that specifically focus on the application of biosurfactants in ice-cream. Rosenberg and Ron [9] reported that the inclusion of emulsifiers in ice-cream can enhance the creaminess of low-fat dairy products. Some researchers claim that combining biosurfactants as emulsifiers in ice-cream can be beneficial in improving creaminess and product quality. The biosurfactants are useful for preserving consistency as well as physical properties. Additionally, they help in solubilizing aromatic oils which helps to retain as well as intensify the flavor of ice-creams [66,67]. Recently, low molecular weight biosurfactants have been reported to show promising results as a stabilizing agent in ice cream [68].

34.4.4 Emulsifying and stabilizing agents

In food products, emulsification plays a key role in consistency, texture, solubilization of atoms, phase dispersion, and self-life of products. The amphiphilic nature of biosurfactants makes them more effective in increasing them for dissolving the polarity of two immiscible liquids by minimizing the surface tension. Emulsification is mainly required in preparing cream, butter, mayonnaise, chocolates, and salad dressing food, etc. [59]. The purpose of emulsification is to stabilize of emulsion process by managing the spherical clustering and stabilize the aerated system which is done by minimizing the surface tension and diminishing the energy of the surface between the phases that prevent the amalgamation of particles by forming electrostatic and steric barriers. Biosurfactants are mostly produced from bacteria and yeasts under wide ranges of pH and temperatures. *Candida utilis, Rrhodotorula graminis, Candida valida, Hansenula anomala* types of yeast and bacteria like, *Acinetobacter calcoaceticus, Klebsiella sp* are used for the production of bioemulsifiers with greater stabilizing property [51]. Gaur et al. [22] isolated biosurfactants from *Candida glabrata* CBS138 and *Candida albicans* SC5314 that showed the excellent emulsifying ability of 53% and 51% against castor oil at 277−393K temperature between pH 2−10 and 2%−14% salt concentration [22].

34.4.5 Food additives and flavoring agents

Biosurfactant is also used as a food additive and flavoring agent which effectively enhances the consistency, texture, shelf life, and freshness of foods. Different types of food additives can be used such as hydrocolloids, enzymes (lipases, amylases, hemicellulases), and pentosanases for intensifying the property of bakery products such as bread, buns, pizza, cake, croissants, butter, and cream, etc. [59]. Glycolipids developed from various microorganisms are microbial surface-active compounds, constructed with carbohydrates linked with fatty acids which have great potential as food preservatives and food additives [62]. Rhamnolipid biosurfactant is found as an excellent food additive and also Rhamnolipid produced from *Pseudomonas aeruginosa* for obtaining L-rhamnose is known as a very good flavoring agent for the production of higher quality flavoring compounds [59].

34.5 Discussion and analysis

34.5.1 Techno-economic challenges

Synthesis of biosurfactants is primarily guided by the fact that scientists wish to substitute synthetic ones with them. However, high production economics is a major concern. According to Sidkey et al. [69], in 2013 early output of biosurfactants was 344 kilotons in the global market while it is expected to reach nearly 462 kilotons by 2020, with a CAGR of 4.3% [69]. Industrial production of biosurfactants is constrained by costly raw materials and low productivities [70]. Recovery, purification, and downstream processing of biosurfactants cost nearly 60%−80% of the overall manufacturing cost [71,72]. The downstream processing steps require extensive usage of chemicals and solvents. However, the scenario is expected to change in recent times as the focus has shifted to waste produced from different industries rather than using virgin raw materials as feedstock. Utilizing such waste products as raw materials is an essential step that can reduce the production economics to 30%−50% [73].

Advancement in research is required to increase the production yields along with the ideas of new types of biosurfactants. It is also important to explore the application roles of the biosurfactants other than the food industry such as—bioremediation processes, antimicrobial agents, environmental and industrial applications, etc. [74,75]. Moreover, the revival and purification cost of biosurfactants are still comparatively greater than the optimal [76]. It will be wiser to choose a less pricey substrate as it offers frequent accessibility and varies country-wise, offering in situ utilization as the alternative [77,78]. For instance, countries having overproduction of corn will have cheaper corn steep liquor compared to the other part of the world. Drop-in production cost of biosurfactants will project them as economically striking. This is primarily dependent on the selection of new microorganisms, usage of economical raw resources, upgraded inexpensive procedures, superior fabrication yield. Additionally, genetically modified microorganisms and their superlative mutants can be useful when amalgamated with cost-effective processes via the implementation of statistical models followed by decontamination of the yield product [79].

Although the biosurfactants help in the improvement of food formulations as well as in their nutritional value, their full application in the food industry is still partial. The reason behind this is that food matrices are relatively complex owing to their different origin of formulations [80]. Thus, the efficient use of biosurfactants in food formulations is still an obstacle. In addition, the biosurfactants are still highly costly to be considered in a proficient application in the food industry. Recent studies showed that to achieve the expected results, large concentrations of biosurfactants were used. Thus, it is crucial to build-up some economic policies for both reducing the amounts of biosurfactants used and for growing their effectiveness in low concentrations [1,58].

34.5.2 Supply chain framework

Biosurfactants can be synthesized from a wide array of substrates, such as—hydrocarbons, hydrophobic mixtures, vegetable oils, dairy products, waste products, etc. [47,71,75]. As mentioned in the introduction section, the waste streams from different industries serve as source or feedstock for biosurfactant synthesis. Hence, it is easily apprehensible that, a well-defined supply chain network (SCN) is essential for the biosurfactant industry to flourish following a cleaner production line.

From the perspective of supply chain sustainability, there are four pillars—environmental, economic, social, and demand [81]. It is imperative to prioritize and balance all four pillars of supply chain sustainability for business proliferation. As stated in Section 34.5.1, there are several issues and challenges toward developing a sustainable business model out of biosurfactant production. The perfect solution to this is to develop a sustainable supply chain network.

With the growing concern of resource circulation and cleaner production, we focus on the waste products that are used as a substrate for biosurfactant synthesis. A generic SCN structure for biosurfactants is presented in Fig. 34.1. As shown, the SCN has three sections—Supply Side, Internal operations, and Demand-side. The Supply-side starts with the industries whose waste will be feedstock for biosurfactant production, that is, agro-industrial waste, oil waste, and dairy waste. Through 3rd Party Logistics (3PL) they are connected to the internal operation section. Internal operations are the production line that performs all the processes. This section is responsible for the production of biosurfactants that will reach the customers on the demand side through 3PL. Since the application of biosurfactants is limited to industries, the tail end of the supply chain will terminate in the end-application industry, replicating a gate-to-gate supply chain scenario. It can be well apprehended that future expansion of the biosurfactant industry can lead toward a green industrial symbiosis.

34.5.3 Green economy perspectives

Waste materials, green materials, sustainably sourced materials can be used as the feedstock for biosurfactant production. Cleaner production strategies including low emission technologies, use of green catalysts, energy-efficient reaction pathways, etc. can be adopted for the production process of these biomolecules. Green chemistry can play an important role here. The above arrangement as a whole translates to a sustainable consumption and production (SCP) regime [82]. There is a very significant role that Govt should be playing toward converting the production of such efficient

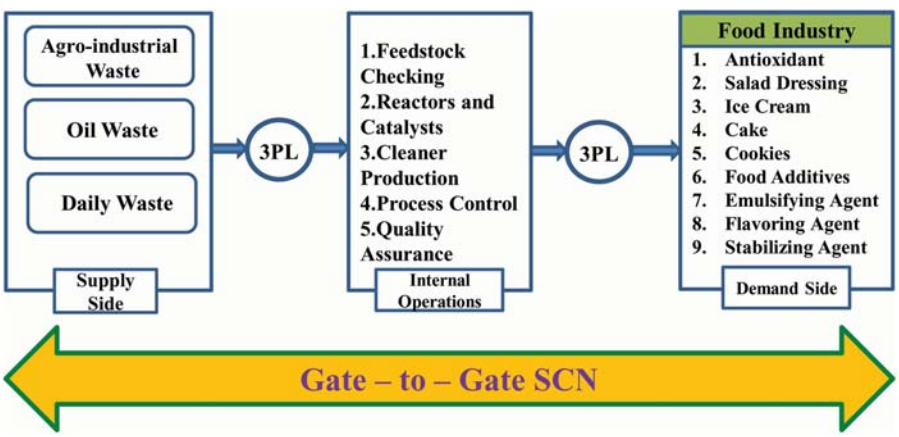

FIGURE 34.1

Generic supply chain network for biosurfactants.

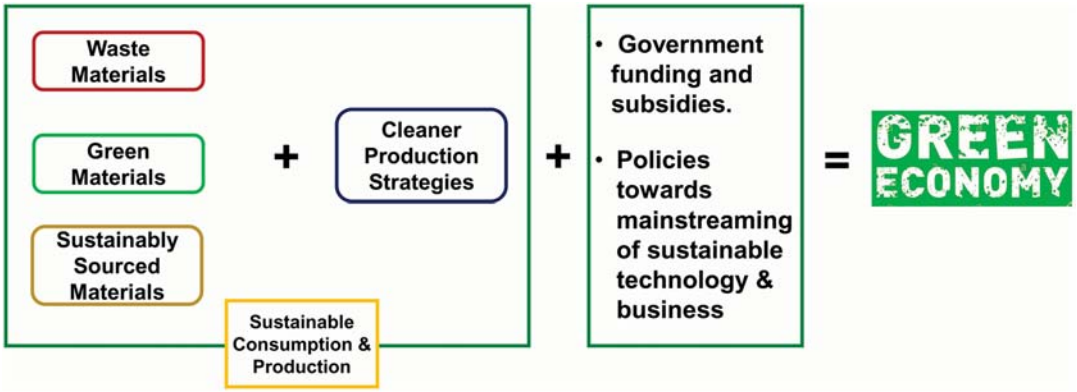

FIGURE 34.2

Conceptual framework toward Green Economy.

molecules toward a main-stream macroeconomy. Government aids in terms of funding R&D and subsidies on the final product is imperative. Additionally developing policies toward mainstreaming of sustainable technologies and green business should be prioritized by the government. As shown in Fig. 34.2, the combination of the above two strategies, that is, SCP & sustainable govt aided policies will lead toward a green economy environment [28].

34.6 Conclusion

Biosurfactants are beautiful biomolecules with robust application areas. One of the most important areas of its application in the food industry. In this chapter, we have discussed the several applications of biosurfactants in the food industry. Antioxidants, Salad Dressing, Ice Cream, Cake, and Cookies are a few potential areas of application for biosurfactants. Additionally, applications of biosurfactants were found as food additives, emulsifying agents, flavoring agents, stabilizing agents, etc. The techno-economic aspect shows that biosurfactants are in a nascent stage and it requires more research in the areas of efficient reactor design, catalyst development, yield, production economics, and feedstock purification. The utilization of waste materials is the potential future feedstock for expansion in this area. The supply chain mapping revealed that the overall development in this area can lead toward a green industrial symbiotic environment. Some strategies were also suggested toward green economy. More research in this direction will be beneficial and help advancing this area.

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CHAPTER

Understanding mechanisms underlying genes regulating the production of biosurfactant

35

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35.1 Introduction

Biosurfactants are multifunctional molecules of the 21st century, with diverse structures, mostly produced by microorganisms as surface-active compounds for their various requirements [1]. They are now most interestingly followed for their usefulness in various industries like petroleum, food, pharmaceutical, agriculture and are also having biomedical and therapeutic applications. Though laboratory production of biosurfactants is a success, plant scale production is still a challenge as final product composition depends on environmental, nutrient, and micronutrient factors [2]. Moreover genetics of the biosurfactant-producing strains are responsible for the low-level production which makes the use of recombinant varieties an important strategy to increase production and for this, a deep insight into its genetic makeup is very important. Operons like *lic* operon, *srf* operon, iturin operon, lichenysin operon, Quorum sensing are some of the regulatory mechanisms involved in biosurfactant production [3].

35.2 Mechanism of working of biosurfactants

Biosurfactants cause the emulsification of oils into hydrocarbon–water mixture to enhance its degradation and dispersal from its polluted environment. Chemical surfactants are usually not preferred owing to their toxicity and recalcitrant existence in the environment [4]. Biosurfactants have good solubilization and emulsification ability, are biodegradable, possess less toxicity, and hence are better choices compared to chemical surfactants [5]. They interact with compounds having lower solubility and enhance their relocation into the aqueous phase allowing the mobilization of persistent pollutants rooted in the soil leading to their elimination [6]. On the other hand, they induce changes in cell membrane properties resulting in enhanced adherence of microbes. This helps in the uptake of the substrate when two immiscible phases are involved [7,8]. Besides they have innumerable properties such as lubrification, detergency, food foaming characteristics, and so on [9]. They may be classified as cationic, anionic, nonionic, and zwitterionic surfactants based on their polarity. The effectiveness of a biosurfactant can be measured from its

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capacity to reduce surface tension. A good surfactant possesses the ability to lower the surface tension of the aqueous phase from 72 to 35 mN/m. Similarly, they also lower the interfacial tension which is the tension between polar and nonpolar lipids in water [9].

An efficient biosurfactant should have low critical micellar concentration (CMC), that is, a lesser amount of surfactant should be available to decrease the surface tension. CMC is the least concentration of surfactant required to instigate micelle formation. It is affected by various parameters such as pH, temperature, the concentration of ions, etc. and varies according to the interfacial tension, the surface tension of the medium, and solubility of the surfactant. Another parameter that measures the effectiveness of a surfactant is the hydrophilic−lipophilic balance (HLB) which determines which type of emulsion is favored by the emulsifier (an emulsion of oil in water or water in oil). Biosurfactants mediate bioremediation through two mechanisms: increase in surface area of hydrophobic substrates that are insoluble in water and elevating the bioavailability of lipophilic substances [10].

35.3 Enhancing the surface area of water-insoluble hydrophobic substances

The microbes involved in bioremediation are restricted by the surface area at the interface between soil and water [10]. Emulsification aids the microbe in increasing cell concentration by elevating the bioavailability of oil in an oil-polluted environment [9].

35.4 Increasing biological availability of water-insoluble substances

Biosurfactants increase the solubilization or desorption of substances that are water-insoluble. Poor solubility (e.g., polyaromatic hydrocarbons) is a hindrance to the bioavailability of the substrate to microbes in polluted sites. Surfactants that are nonbiological in origin have both positive and negative effects on their biodegradation. Agro-industrial wastes have reduced the cost of production of biosurfactants for enhancing the microbes that produce them and also increase the mobilization of hydrophobic substances in soil. Some glycolipids and lipopeptide biosurfactants from bacterial isolates such as *Achromobacter, Bacillus*, and *Ochrobactrum* act by decreasing the surface tension of crude oil in oil spills (aliphatic and aromatic fractions)—they depict elevated emulsifying action showing lower surface tension [11]. Emulsification Index is an example used to illustrate the emulsification ability in a biosurfactant. Rhamnolipids synthesized by *Pseudomonas aeruginosa* decreased surface tension of water and also the interfacial tension in the water−hexadecane system to below 1 mN/m along with lowering of CMC. Sophorolipids, also known for reducing the surface tension at the water−oil interface were used as humectants in cosmetics, soil, bioremediation of hydrocarbons, and also for their anticancer activity when the chain length was increased [12].

35.5 Molecular genetic mechanisms of microbial synthesis of biosurfactants

A wide range of microorganisms synthesizes biosurfactants as surface-active molecules. They are viable alternatives because of their unique characteristics such as high biodegradability, low

toxicity, eco-friendly, high foaming, increased surface activity, and selectivity, and also specificity at extreme conditions such as temperature, pH, salinity, and low CMC [11].

Many current studies show that the production of biosurfactants is usually carried out commonly in *Acinetobacter, Pseudomonas, Bacillus, Serratia, Candida* spp. The glycolipid rhamnolipid gene sequence found in the gram-positive *Pseudomonas aeruginosa* and the lipopeptide-based surfactin synthesized by *Bacillus subtilis* were most commonly known [13]. The molecular genetics and biochemical analysis of the biosurfactant synthesis have unraveled the underlying operons, enzymes, and metabolic pathways necessary in their extracellular development and expression [3].

Among all the biosurfactants identified to date, glycolipid biosurfactants from *P. aeruginosa* also known as rhamnolipids are formed utilizing carbohydrates as the singular source of carbon during growth [14]. Multiple lipopeptides are produced from *Bacillus* spp., but only a few are effective in reducing surface tension on par with surfactin and lichenysin obtained from *Bacillus subtilis* and *B. licheniformis*, respectively [13]. *Acinetobacter calcoaceticus* RAG-1 was commonly considered in terms of the biosynthesis and discharge of a high molecular weight bioemulsifier [15]. Lichensin, a lipopeptide obtained from *B. licheniformis* found in oil reserves. Its synthesis is uninhibited by the high oil levels in the medium and is active over a broad temperature range. It is a preliminary biosurfactant for industrial applications such as improving oil recovery using extremophilic microbes [16].

Quorum sensing system plays a pivotal role in the production of Biosurfactants. Autoinducers which are the signal molecules of regulatory pathways are actively involved in biosurfactant synthesis depending on the cell density [17]. Quorum sensing engrosses *N*-acylhomoserine, a membrane-permeable lactone signal molecule in Gram-negative bacteria that acts by binding to a transcriptional activator thereby controlling the expression of multiple genes [18]. Whereas, the signal molecule involved in Gram-positive bacteria is usually an extracellular peptide, that activates a two-component regulatory mechanism involving a sensor or signal transducer and a response regulator [19].

We begin to discuss the genetic aspects of biosurfactant production by various microbes based on their polarity; which is classified into phospholipids, lipoproteins, and glycolipids.

35.5.1 Phospholipids and fatty acids (mycolic acids) biosurfactants

The phospholipids form the major microbial membrane composition. When the hydrocarbon-degrading bacteria or yeast grow on alkanes as substrates, the extent of phosphorylation increases significantly. This was observed in the *Acinetobacter* sp, grown in hexadecane. HO1-N, rich vesicles were developed with phospholipids (primarily phosphatidylethanolamine). Phospholipids were synthesized using *Thiobacillus thiooxidans*, which are responsible for segregating the elemental sulfur required for metabolism. *Rhodococcus erythropolis* produces Phosphatidylethanolamine wherein growth on n-alkanes leads to reduction of less than 1and 30 mN/m surface tension at water−hexadecane interface [20].

Fatty acids formed from alkanes due to microbial oxidation have been known to be surfactants. Examples of abovementioned fatty acids include mycolic acids [20]. Mycolic acids possess a long hydrocarbon-chain and are β-hydroxy fatty acids with a relatively long aliphatic chain at the α-carbon atom. The overall number of carbon atoms ranges from 30 to 86. They are synthesized by bacterial of the genus *Mycobacterium*, *Rhodococcus, Nocardia*, and *Corynebacterium*, and also

other species of the minor genera (e.g., *Gordonia, Bacterionema, Micropolyspora*, and *Brevibacterium*) [21]. Corynomycolic acids (R_1-CH (OH)-CH (R_2)-COOH) are a class of surface-active molecules with differences in a number of carbon atoms. The substrate in the growth medium has a major impact on the synthesis of biosurfactants of different chain lengths. *Corynebacterium lepus* has been isolated from a combination of corynomycolic acids with outstanding surfactant properties. This induced a major decrease in surface tension in aqueous media and in the interface of water- hexadecane system at all pH values ranging from 2 to 10 [3].

35.5.2 Lipoproteins or lipopeptides biosurfactants

Lipopeptides are a cluster of low-molecular-weight biosurfactants synthesized from amino acids. These lipopeptides are cyclic depsipeptides synthesized by various microorganism strains, including the bacteria of genera *Bacillus, Lactobacillus, Streptomyces, Pseudomonas*, and *Serratia*. Due to the nonribosomal nature, they are also comprised of unnatural amino acids such as the d-enantiomers [22]. A similar type of regulation is found in biosurfactants synthesized by various species of *Pseudomonas* such as Viscosin, Amphisin, and Arthrofactin. Lichensin is a similar lipopeptide produced from oil reservoirs by *B. licheniformis*. It is structurally similar to surfactin in protein moiety except for the substitution of leucine to isoleucine and dissimilar in the lipophilic moiety. The lichensin synthetase encoding gene *lch*A shows structural similarity to *srf*A operon. The promoter is homologous to *srf*A promoter. The competence-related gene *com*A from both these *Bacillus* species shows high homology indicating the similarity with which these biosurfactants are regulated and produced [15].

35.5.2.1 Surfactin

It is a heptapeptide bound to the β-hydroxy fatty acyl chain that forms a cyclic lactone ring. Surfactin biosynthesis is brought about by a nonribosomal peptide (NRP) synthetase system, incorporated in a srfA operon, composing three genes (srfA, srfB, and srfC) guarded by a Quorum sensing mechanism [23]. The operon contains a gene required for cell competence and quorum sensing and regulates the expression of *srf*A through a signal peptide ComX which accumulates in the adjacent medium in which cell density is elevated [24]. Surfactin obtained from various species of *Bacillus* is deemed to be one of the most functional biosurfactants of all reported to diminish air/water surface tension from 72.5 to 27 mN/m [25]. Nonribosomal peptide synthetases are involved in lipoprotein biosurfactants elicit high structural similarity among various microbial species. In *B. subtilis*; the biosurfactant production is inhibited by the occurrence of hydrocarbons in the surrounding medium unlike in other bacterial biosurfactants [24]. It was found that the involvement of yerP caused resistance to surfactin production in some strains of *B. subtilis* [25].

35.5.2.2 Lichenysin

It is a biodegradable surfactant with tremendous ability to extract crude oil from the oil reserve. Lichenysin is unidentical compared to surfactin in the replacement of leucine with isoleucine in the amino acid of the very last peptide and also in a lipophilic moiety, which comprises a combination of straight and branched 3-hydroxy fatty acids [13]. The operon responsible for the biosynthesis of lichensin from *B. licheniformis* ATCC 10716 has been studied by cloning and sequencing it. Lichenysin operon comprises three peptide synthetase genes known as *lic*A, *lic*B, and *lic*C which

are transcribed in the same direction [26]. The surfactants obtained from *Bacillus licheniformis* possess the ability to reduce the surface tension of the water to 27mN/m and the interfacial tension between the water and the n-hexadecane to 0.36 mN/m [3].

35.5.2.3 Iturin

Iturin A is a biosurfactant that is a lipopeptide in nature produced by certain strains of *Bacillus subtilis*, such as *B. subtilis* RB14. It has the antifungal ability and consists of four peptides, *itu*D, *itu*A, *itu*B, and *itu*C [27]. The open reading frame of the *itu*D gene codes for the putative malonyl coenzyme A transacylase. It was found that the disruption of *itu*D gene results in truncated and inactive iturin A. The subsequent gene, *itu*A, codes for a 449-kDa protein that comprises three functional sequences homologous to the peptides fatty acid synthetase, amino acid transferase, and peptide synthetase. The next and third gene, *itu*B, and the fourth gene, *itu*C, translate to 609- and 297-kDa peptide synthetases, respectively [27].

35.5.2.4 Arthrofactin

A bacterium of *Pseudomonas* sp. MIS38 synthesizes arthrofactin, an efficient biosurfactant which is a cyclic compound and a lipopeptide produced by a macro complex comprising three peptide synthetases that are not associated with ribosomes [28]. Three genes form the arthrofactin synthetase gene cluster named *arf*A, *arf*B, and *arf*C code for ArfA, ArfB, and ArfC proteins, which are combined to form a composite structure. *Arf*A, *Arf*B and *Arf*C possess two, four, and five functional moieties, correspondingly [29].

35.5.2.5 Viscosin

Another efficient peptide cum lipid biosurfactant is Viscosin which is also a mediator for plant pathogens synthesized in the pectolytic variant of *Pseudomonas fluorescens*. One of the reports showed that *P. fluorescens* strain *SBW25*, site-directed gene mutation encoding one among the two *Lux*R-type regulators identified, *Visc*AR and *Visc*BCR, dramatically decreased the rates of the expression of *visc*ABC genes leading to the deterioration of viscosin production. Viscosin produced by *P. fluorescens*PfA7B is encoded by 25 kbp long chromosomal DNA. Its biosynthesis requires a peptide synthetase complex [30].

35.5.2.6 Amphisin

Amphisine is obtained from *Pseudomonas* sp. DSS73. The two-component regulation by GacA/GacS (GacA is a regulatory gene and GacS, a kinase) regulates the expression of amphisin synthetase (*ams*Y) (28). Its biosynthesis is controlled by *gac*S as its mutant reobtains the surface motility property when a plasmid carrying the heterologous *Pseudomonas syringae* wild-type *gac*S was introduced [3].

35.5.2.7 Putisolvin

Two cyclic lipopeptide surfactants called putisolvins I and II are obtained from *Pseudomonas putida* PCL1445. The ORF (open reading frame) of the gene responsible for the biosynthesis of putisolvine depicts amino acid homology when compared to several lipopeptide-based synthetases [31]. These biosurfactants are formed by a putisolvin synthetase called *pso*A. Three genes, *dna*K, *dna*J,

and *grp*E which are heat-shock proteins are involved in the positive regulation of putisolvin synthesis [32].

35.5.2.8 Serrawettin

Serrawettins are nonionic biosurfactants formed by *Serratia marcescens*. Analysis of the *S. marcescens* mutants showed the presence of three novel genes in the synthesis of W1 serrawettin. The *psw*P gene encodes the phosphopantetheinyl transferase cluster enzyme, the *swr*W gene codes for a unimodular synthetase that belongs to nonribosomal peptide synthetase (NRPS) family, and *hex*S codes for *Lys*R-type transcriptional regulator that downregulates the production of *Serratia* exolipids and certain extracellular enzymes [33].

35.5.3 Glycolypid biosurfactants

Most of the glycolipid and lipoprotein biosurfactants are regulated by operons. Most of the bacterial biosurfactants' production is controlled by quorum sensing. These organisms are found to produce a diffusible signal that accumulates to more amount that is sufficient enough for gene activation. Such type of regulation is seen in Gram-positive and also in Gram-negative bacterial species that produce biosurfactants. The synthesis of glycolipids is brought about by glycosyl transfer reactions catalyzed by glycosyl transferases. One such enzyme is the rhamnosyl transferase-1 that is encoded by four genes *rhl* A, B, R, I on a plasmid that can be expressed in a heterologous host. These genes are clustered together as an operon and are expressed collectively in the form of bicistronic RNA. This system includes the *rhl*R gene that is involved in quorum sensing and the regulatory gene *rhl*A that regulates the structural genes *rhl* A, B. Similar quorum sensing system was seen in the chromosomal genes encoding rhamnolipid synthesis in *P. aeruginosa*. These genes in both the above-mentioned microbes can induce biosynthesis of rhamnolipids and various genes responsible for the virulence of the bacterium [34].

Rhamnolipids, are a type of glycolipids, amongst the most comprehensively experimented and characterized of the different classes of biosurfactants. They are the most potent biosurfactants due to various features such as reduced surface tension (30−32 mN/m), high emulsifying potency, and greater affinity for organic lipophilic molecules. A unique advantage of producing biosurfactants from *P. aeruginosa* is the increased rhamnolipid production and its strict regulation in response to environmental factors. [33]. The biosynthesis of *P. aeruginosa* rhamnolipids was initially recorded by Jarvis et al., in 1949, when Glycolipids containing various moieties of sugar were found (e.g., rhamnose, sophorose, and trehalose) bound to long-chain fatty acids [35].

Quorum sensing is more prevalent among various bacteria and regulates various metabolic events such as the expression of surfactants and the involvement of secondary metabolites in various activities such as luminescence, genetic material relocation, and swarming [13]. The *rhl* quorum sensing mechanism of *P. aeruginosa* controls the development of biosurfactants. Production of the rhamnolipids depends on the type of strain, culture condition, and medium composition. With *Pseudomonas* sp. *AK6U* strain can simultaneously generate biosurfactants from rhamnolipids and use organosulfur compounds as sole sources of sulfur [36]. In one of the case studies, it is evident that the source of sulfur regulates the expression of the genes *rhl*A, *rhl*B, *rhl*C associated with the biosurfactants. In bacterial cultures with DBT and DBT-sulfone, the increased expression of *Rhl*C expression illustrates the increased production of dirhamnolipids compared to those containing

Magnesium sulfate as the source of sulfur [37]. Genes implicated in the biosynthesis of rhamnolipid occur in the plasmid in the form of *Rhl*A, B, R and I which could be employed for expression in another host [3]. *Rhl*ABC genes that encode the biosynthesis of major enzymes required in the synthesis of rhamnolipid-based surfactants. *Rhl*A protein is involved in the production of dimers of hydroxy-alkanoates that reflect the hydrophobic movement of rhamnolipid biosurfactant that several *Pseudomonas* spp. produce [3].

*Rhl*B and *Rhl*C were found to be rhamnosyltransferases that catalyze the transport of dTDP-L-rhamnose to the hydroxyalcanoates (producing monomeric rhamnolipids) or even to existing rhamnolipid molecules for the production of dirhamnolipid, respectively [37]. *P. aeruginosa* possesses two individual quorum sensing arrangements known as *las* and *rhl*. The las system comprises *Las*R: a transcriptional activator and *Las*I, which synthesizes an auto-inducer N-(3oxododecanoyl) homoserine lactone (PAI-1). Various virulence genes including the *las*B (elastase encoding) are induced by the *Las*R and PAI-1. The *rhl* system includes RhlR, and RhlI, presumed transcriptional activators involved in the regulation of *N*-butyrylhomoserine lactone (PAI-2) synthesis. It was stated that rhamnolipid production in *P. aeruginosa* requires both *rhl* system and *rhl*AB (rhamnosyltransferase encoding). Pearson et al. in 1997 worked on a bioassay in *E. coli* which resulted in the findings that PAI-2 and *Rhl*R were necessary for ample expression of *rhl*A gene product [38]. To illustrate the supposed interface between PAI-2 and *Rhl*R, it was found that [3 H] PAI-2 molecule binds only to those *E. coli* cells producing *Rhl*R protein rather than to those of *Las*R. Ultimately, the specificity of proteins bound to the las and *rhl* systems was studied in these bioassays. The *las* system has the potential to mildly activate *rhl*A, and correspondingly, the *rhl* system activates *las*B to a certain extent. Nevertheless, these effects were insignificant compared to that of activation of *rhl*A by the *rhl* consortium along with *las*B by the *las* consortium [38]. The various genes involved in biosurfactant regulation are summarized in Fig. 35.1.

The production of emulsan, a high molecular weight glycolipid emulsifier produced by *Acinetobacter calcoaceticus* RAG-1 has a peculiar composition. It is made up of repeating sugar units in a polysaccharide backbone to which fatty acids are joined by amide and ester linkages along with noncovalently bound protein moiety. An esterase was found to affect the release of emulsan from the cell surface which is produced from the structural gene: *est*. Emulsification is due to its lipase activity produced by an operon which is regulated by a lipase chaperone gene *lip*B downstream to structural gene *lip*A. Alasan is another biosurfactant produced by *A. radioresistens* KA53 which is composed of anionic polysaccharides and three proteins encoded by three different genes (*Aln*A, *Aln*B, *Aln*C). The bacterium was found to release these three proteins under stress conditions [3].

35.6 Gene regulation in fungal biosurfactants

Trichoderma reesei produces low-molecular-weight protein biosurfactants known as hydrophobins possessing high levels of cysteine, higher surface, and amphiphilic characteristics. The expression of this protein is regulated by two regulatory genes hfb1 and hfb2. Several *Candida* species produce sophorolipids, in which biosynthesis of the biosurfactants is mediated by monooxygenase enzyme, NADPH-dependent cytochrome P450. These proteins are implicated in fatty acid conversions as well as their genes are induced by unknown mechanisms. Also, the addition of glucose sugars to the fatty acid is mediated by the enzymes glycosyl transferases I, II [3].

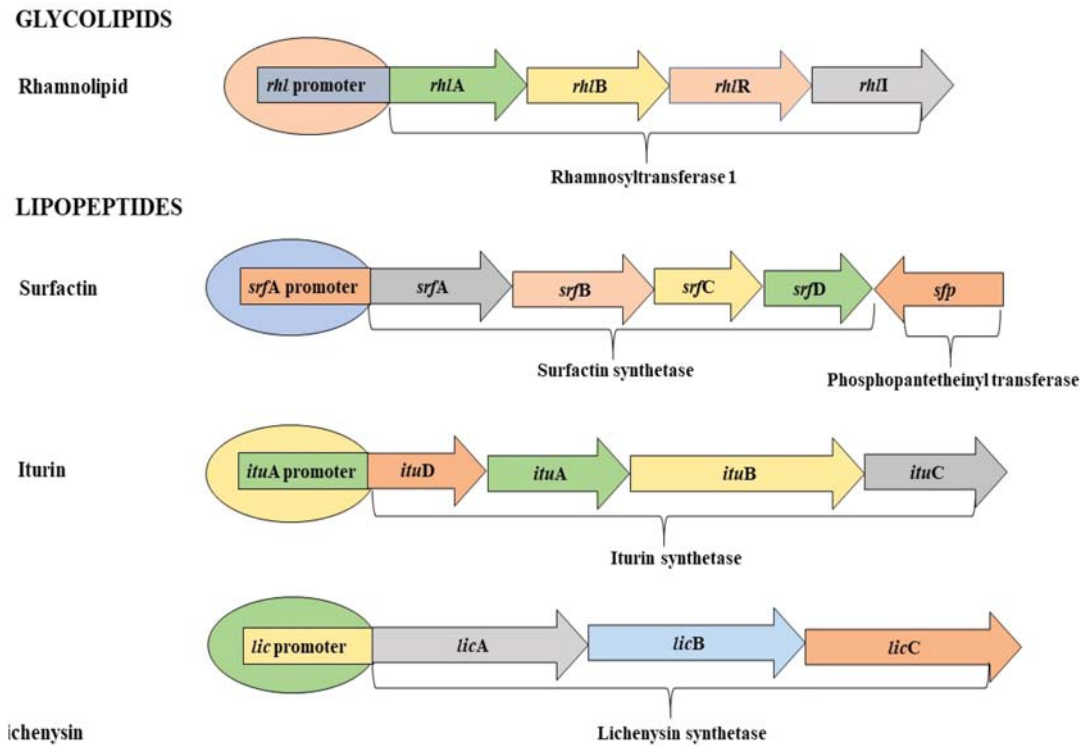

FIGURE 35.1

Genes implicated in the expression of biosurfactants.

Pseudozyma antarctica produces glycolipid biosurfactant in which the genes were studied using mutants for extracellular glycolipid production in *emt*1 (glycosyltransferase); *cyp*1, *cyt*P450 monooxygenase (lipid synthesis). The gene *emt*1 has five open reading frames for three peptides Mac1, Mac2, and Mat1 essential for the production of Mannosylerythritol lipids (MELs) [3]. *Ustilago maydis* is unusual among glycolipid fungal producers because it secretes two groups of glycolipids which are structurally unrelated during nitrogen depletion. Those are the MELs and the cellobiose lipid ustilagic acid (UA) [39]. UAs are disaccharides containing cellobiose lipids. The disaccharide moiety is bound to 15,16-dihydroxyhexadecanoic acid through an O-glycosidic bond. The term Ustilipids is exclusively used for MELs that are derived from acylation of β-D-mannopyranosyl-D-erythritol [40].

35.7 Molecular engineering facets for novel and customized biosurfactants

Mutants of *P. aeruginosa* PTCC1637 were found to produce 10 times more biosurfactants when mutations were induced in the promoter region. Similar effect was observed in *B. subtilis* SD901,

B. subtilis MI113, *B. licheniformis*KGL11 for biosurfactant production. Other strategies employed for enhanced biosurfactant production were to identify the quorum sensing producers in bacteria or to modify the biosynthetic pathway. Naphthalene degradation was found to increase when pUTK21plasmid was genetically engineered with the catabolic genes along with the bioluminescent gene from *P. fluorescens*HK44. An increased biofluorescence was observed in response to the bioavailability of soil hydrocarbons, when *lux* gene was fused with the promoter for naphthalene catabolic genes [17]. Correspondingly, when the iturin promoter of the operon was replaced with *rep*U promoter of the plasmid pUB110, a threefold amplification in biosurfactant production was observed [27]. Another promising upcoming strategy is to identify autochthonic microbes and introduce genes for biosurfactant production through genetic engineering and release of recombinant microbes for in situ bioremediation for the reason that certain microbes pollute the environment by depleting the carbon sources [3].

35.8 Commercial applications of biosurfactants

An alternate reason for heightened industrial interest in microbial surfactants is the frequency of reports on antibiotics and a wide range of bioactive properties of certain biosurfactants. For instance, the lipopeptide antibiotics polymyxin B, daptomycin, and rhamnolipids used in medicine, also commonly employed in agriculture. Several advantages of these aforementioned biosurfactants point toward the greater benefits alongside the decline in surface tension. This united effect of interfacial activity with bioactive properties has to lead to foresee potential in food, pharmaceutical, agricultural, environmental, and other unexplored applications for many biosurfactants. Lipopeptide-based biosurfactants are cyclic peptides that undergo acylation with fatty acids of a varied number of carbon atoms and compositions. They are secreted by several gram-positive bacteria as extracellular compounds such as *Bacillus* or *Streptomyces* and also by gram-negative bacteria such as *Pseudomonas* [20].

35.8.1 Biosurfactants in food industry

They are known to diminish the interfacial and surface tension mediate the formation and stabilization of emulsions. They are specifically used due to their properties of controlling aggregation of fat globules, enhancing texture, shelf-life of starch, rheological features of wheat dough, enhancing consistency and texture of lipid based commodities, slowing staling of flavor oils, solubilzation of flavor oils, enhancing volume, texture, and shelf-life of bakery products by using rhamnolipid biosurfactants [41]. Rhamnolipids also act as growth inhibitors against *E. coli, S. marcescens, Aspergillus niger, Penicillium chrysogenum, Alcaligenes faecalis*, and many other organisms that contaminate food-based ingredients. Rhamnose is difficult to isolate from plants, hence it is hydrolyzed from rhamnolipids. It is used for the production of flavor compounds [42].

Monoacylglycerols or MAG and their derivatives (lactate and lactate esters of MAG) are decomposable and relatively biocompatible surfactants commonly in dairy and confectionery food; in pharmaceuticals as emulsifying agents and drug delivery medium, and also in cosmetics as emollients, emulsifying agents, and viscosifying agents. The most common biosurfactant used in laundry

detergents is Glycerol monostearate [43–45]. Glycolipid biosurfactants such as the sophorolipids, mannosylerythritol liposurfactants, rhamnolipids, and trehalose lipids find applications as alternatives to alkylated sugar derivatives. Sugar esters are important biosurfactants used mainly as emulsifiers in foodstuff, cosmetics, and pharmaceuticals owing to their high antimicrobial activity as well as biodegradability. Many fatty acid esters derivatives of polyglycerol and ethylene and propylene glycol are highly placed in foodstuff, cosmetics, and pharmaceuticals, principally as emulsifiers [46]. The compounds fatty amine ethoxylates are derived from fatty amides such as kernel of palm fruit or coconut oil or tallow. The two ethoxylate chains are bound to the fatty amine at the amine Nitrogen. These compounds have tremendous potential as acid thickeners, adjuvants of various crop seeds in agriculture, antistatic agents, textile processing agents, detergents, and also as lubricants [46].

35.8.2 Biomedical and therapeutic applications of biosurfactants

Betaines are amphoteric surfactants that offer detergency along with high foaming ability and mild contact on the skin. For water solubility formulation, alkyl and alkyl amidobetaines are cationic surfactants that work even under extreme temperature and pH. They can be formulated for creating thick liquids that can shear thin and have efficient skin compatibility and can be dispersed through squeeze bottles or pumps. Amphoteric surfactants are usually mild and used for personal care products. They are primed with anionic primary surfactants to form complexes that reduce irritation potential. Lauryl betaine mediates the dissemination of model compounds in the skin of murine models and human skin itself. It was suggested for development as an adjuvant to augment the dermal preparations of pharmaceutical preparations [47].

Nonbacillus lipopeptides such as polymixins are well-known group of antibiotics. Polymixins are cationic branched cyclic decapeptides. They are identified as Polymixin A, B, C, D, E which are known to hinder the growth of gram-negative bacteria by integrating into the cell membrane. Viscosin is a cyclic tripeptide isolated from *P. fluorescens* and *P. viscosa*. The compound has antiviral properties and growth promotion of rhizobacteria in plants. Serrawettin is a lipopeptide responsible for the virulence of *Serratiamarcescnes*. Hence many lipopeptides are studied for their pathological importance as targets of many drugs or as antimicrobial agents. The ester-based biosurfactants derived from phenylalanine, tyrosine and fatty alcohols, form an ester-derived product that was found to be bioactive against Gram-positive bacteria [47]. The applications of biosurfactants are summarized in Fig. 35.2.

35.9 Toxicological and ecological aspects of biosurfactants

Biodegradation and toxicity to the aquatic ecosystem are critical safety concerns for betaines, as they tend to pave their path into the environment in many applications. Experimenting on their biodegradation in aerobic conditions showed that the immediate biodegradability of alkyl betaines was uninfluenced by alkyl chain length. According to the information found in product safety data sheets, the crucial point in the vulnerability to lauryl betaine lies in its toxicity in the oral cavity. The acute toxicity experiments conducted in rats reveal that the oral concentrations of LD50 value

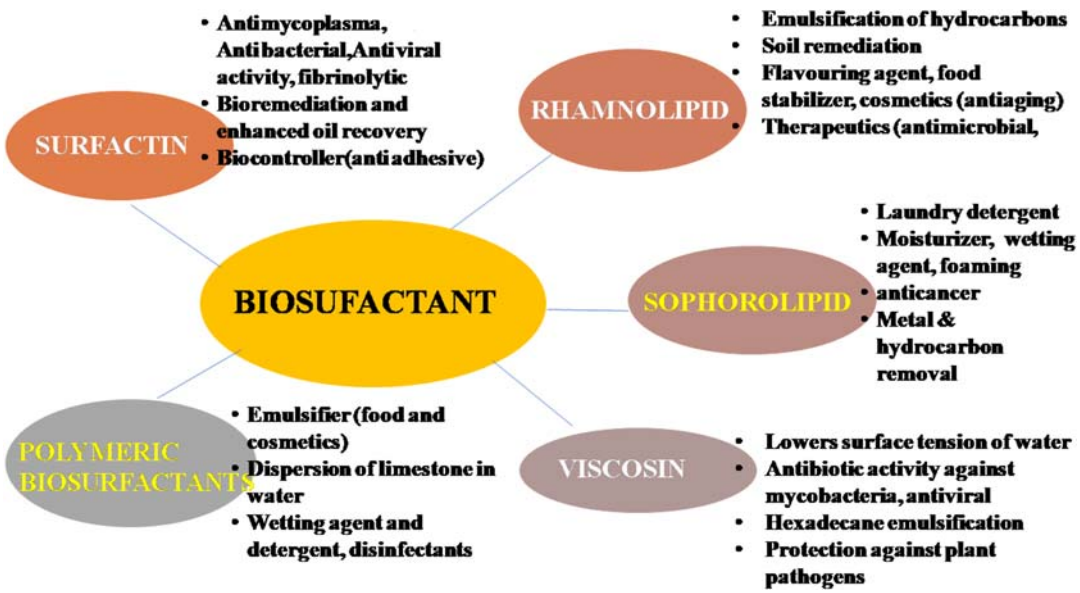

FIGURE 35.2

Applications of biosurfactants.

of lauryl betaine and alkyl betaines belonging to C12−C14 were found to be 0.071 g/kg and 3 mg/kg. Toxicity studies were conducted for applicability as inert constituents of pesticides in which C12 and C16 alkyl betaines were assayed for oral toxicity in acute exposures and found to elicit low or moderate toxicity. The alkyl betaine of C12 length was an excessive irritation to the skin and eyes in acute exposure studies. Also, impurities found in amphoteric surfactants are monochloro and dichloroacetic acids known to be carcinogens in trace concentrations, even in ppm. Rhamnolipids are also used as additives in cosmetics due to their compatibility with skin, low irritancy, and low toxicity [47].

35.10 Bioremediation using biosurfactants

Biosurfactants are used in various modes for removing lipophilic and other pollutants from soil and water. Both positive and negative and also nil effects have been observed in the case of supplementation with biosurfactants for bioremediation. Rhamnolipids have caused a positive effect on the elimination of phenanthrene [48], anthracene [49], and also various Polyaromatic hydrocarbons [45] by solubilizing the compound whereas a negative effect was seen in the case of phennathrene remediation when the rhamnolipids were produced from *Sphingomonas* and *Paenibacillus* spp. [50]. Rhamnolipids had a negative effect on the bioremediation of Cadmium [51] whereas a positive effect on the bioremediation of Copper [52].

Mixed consortia were also used for biodegradation compared to single cultures where one or few microbes enhanced the growth by altering the cell surface properties or by solubilization of hydrocarbons for uptake and biodegradation as found with rhamnolipids [53]. Biosurfactants also have higher biodegradability compared to chemical surfactants which makes them a greener alternative for the environment. The addition of biosurfactants produced by *B.s megaterium* to contaminated samples enhanced biodegradation of Fluorine [54]. Biosurfactants were found to enhance the desorption of polyaromatic hydrocarbons from agricultural soil at concentrations less than CMC of a soil−water system; elevate contact angle between mud and the containment and mediate the separation of contamination from soil [55].

35.11 Conclusion

Biosurfactants are highly useful substances produced by microorganisms including fungi and have wide applications. For their production on an industrial-scale requires the proper understanding of their genetics which unfortunately is not available for many. As genetic engineering is the way forward for the large-scale synthesis of biosurfactants, the underlying mechanisms as studied in genes regulating their products in detail is a requirement.

35.12 Acknowledgments

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35.13 Conflict of interest

Authors have no conflict of interest to declare.

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Further reading

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GREEN SUSTAINABLE PROCESS FOR CHEMICAL AND ENVIRONMENTAL ENGINEERING AND SCIENCE

Biomedical Application of Biosurfactant In Medical Sector

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Biomedical Application of Biosurfactant in Medical Sector highlights the numerous applications of biosurfactant in the field of medicine, most especially as a replacement to synthetic drugs which has been reported to develop several levels of resistance over the years. Special emphasis is laid on the application of biosurfactant as a nonpyrogenic and nontoxic immunological adjuvant and inhibitory activity against H+, K+-ATPase, and defense against gastric ulcers as well as their practical application as an antiadhesive coating agent for medical insertional materials.

Several scientific advancements have been recorded in the application of biosurfactant in the field of medical sciences during the past decade. Biosurfactants refers to surfactants from microbial origin and can be synthesized by several identified microorganisms including bacteria, yeast, and fungi. Biosurfactants have been identified as a natural molecule that has special and unique biological properties such as the capability to decrease surface tensions that exist between various phases. Biosurfactants offer many attractive properties, but with research into their production and its use across the literature, it can be hard for researchers to identify available options and consider how they can be applied to improve the sustainability of different medical applications. Biomedical Application of Biosurfactant in Medical Sector addresses that issue by combining knowledge of their production with information on their application in a wide range of medical applications.

Drawing on the knowledge of its expert team of global contributors: this book provides useful insight for all those currently or potentially interested in developing or applying biosurfactants in their own work.

Key features

- Reflects on differing strains of fungi, bacteria, actinomycetes, and yeast, and reviews genetic modification of such strains for enhanced biosurfactant production
- Explores the use of biosurfactants across a broad range of medical applications
- Provides mathematical modeling, metabolomics, bioinformatics, metabolic engineering, systems biology, and computer technology for solving real-life challenges using biosurfactants
- Presents biosurfactants as an innovative green, biotechnological solution to improve human health
- Highlights the numerous applications of biosurfactants in the field of medicine, most especially as a replacement to synthetic drugs which have been reported to develop several levels of resistance over the years

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