BIODIVERSITAS
Volume 21, Number 9, September 2020
Pages: 4034-4040

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d210914

Distribution and molecular characterization of biosurfactant-producing bacteria

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Manuscript received: 4 July 2020. Revision accepted: 10 August 2020.

Abstract. *Alyousif NA, Luaibi YYYA, Hussein W. 2020. Distribution and molecular characterization of biosurfactant-producing bacteria. Biodiversitas 21: 4034-4040.* Biosurfactants (BSs) are biological surface-active compounds produced by several microorganisms with many areas of application, as such become an important product in biotechnology and consequence to be used in industries. In recent years, many researchers pay attention to BSs producers' microorganisms. The present study was aimed to isolate, identify, and screening BS producing bacteria from six various sites in two different cities in Iraq. Four samples were collected from four sites in Basrah governorate and the rest two samples from Al-Garraf oilfield in Thi-Qar governorate. A total of 33 different bacterial isolates were obtained, 20 out of the 33 were found to be biosurfactants producing isolates that detected through the emulsification index (E24%), oil spreading test, and emulsification activity. The isolated bacterial strains were more identified by 16S rRNA gene sequencing. The results showed that the biosurfactants producing isolates belonged to genera *Bacillus*, *Pseudomonas*, *Enterobacter*, *Staphylococcus*, *Acinetobacter*, and *Aerococcus*. *Bacillus jeotgali* and *Aerococcus viridans* are reporting as biosurfactant producing bacteria for the first time and *Bacillus jeotgali* is isolated for first time from crude oil of oilfield reservoir in this study in world. Moreover, six bacterial isolates were identified as new strains and deposited at NCBI Genbank under accession numbers MT261834 (*Bacillus subtilis* strain IRQNWYA3), MT261835 (*Bacillus licheniformis* strain IRQNWYB4), MT261836 (*Pseudomonas stutzeri* strain IRQNWYF2), MT261837 (*Pseudomonas zhaodongensis* strain IRQNWYF3), MT261838 (*Pseudomonas* sp. IRQNWYF4) and MT261839 (*Bacillus licheniformis* strain IRQNWYF5). A2 isolate that was identified as *Pseudomonas aeruginosa* has shown the highest values of emulsification activity and emulsification index (1.678±0.050 absorbance at 540 nm and 56.6% respectively) that show efficient potential of biosurfactant production. Phylogenetic tree was also constructed in this study based on 16S rRNA gene sequences of biosurfactant-producing bacteria to evaluate their close relationship and evolution between them.

Keywords: 16S rRNA gene sequencing, biosurfactant-producing bacteria, emulsification activity

INTRODUCTION

Biosurfactants (BSs) are biological surface-active compounds produced by several microorganisms which either secreted into the surrounding environment or adhere to the plasma membrane of the producer cell (Antoniou et al. 2015; Ndlovu et al. 2016). BSs have unique properties such as high surface activity, non-toxic nature, environmentally friendly, biodegradable, and tolerance of extreme temperatures, pH, and salinity. These properties allow biosurfactants to be a preferable alternative to chemical surfactants (Thavasi et al. 2011; Liu et al. 2013).

Biosurfactants are amphipathic molecules containing two moieties, hydrophobic moiety having long-chain of fatty acids, which is less soluble in water and a hydrophilic part having carbohydrates or carboxylic acids, which is more soluble in water. They are divided into diverse types based on chemical structure, namely: glycolipids, phospholipids, lipopeptides, neutral lipids, fatty acids, and polymeric biosurfactants (Banat et al. 2014; Soltanighias et al. 2019).

Biosurfactants producing bacteria are ubiquitous and inhabiting several environments in a wide range of temperatures, pH values, and salinity. Several bacterial belong to different genera such as *Bacillus*, *Pseudomonas*, *Burkholderia* and *Flavobacterium* are reported to produce biosurfactants, however, the bacteria are considered generously biosurfactant producers. The soil and water contaminated with hydrocarbons and oilfields produced water is abundant in biosurfactant producing bacteria, where they produce biosurfactants to exploit hydrocarbons as a carbon source (Femi-Ola et al. 2015; Ewida and Mohamed 2019; Sohail and Jamil 2020).

BSs have gained much interest in recent years which considered as one of the high values of microbial products, that have many areas of application and become an important product of biotechnology that can be used in industries, environmental and medical application as antimicrobial, wetting, coagulating, anti-adhesive, thickening, dispersion, moisturizing, foaming, emulsifiers and agents, where BSs have exploited in various industries such as enhanced oil recovery, medicine, food processing, agriculture, pharmaceutical, cosmetics and bioremediation of organic pollutants in the environment (Vedaraman and Venkatesh 2011; Elazzazy et al. 2015).

The aim of this study is to isolate and screen biosurfactants producing bacteria from different sources, characterize bacteria by molecular technique, determine their potential to produce biosurfactants, and construct phylogenetic tree.

MATERIALS AND METHODS

Samples collection

Soil, water and crude oil samples were collected from six sites (soil sample from the area around the generator (A), soil sample from the area around the petrol station (B), produced water from Al-Garraf oilfield (C), crude oil from Al-Garraf oilfield (D), contaminated water from Shatt Al-Arab (E) and soil sample from area around Nehran Omer oilfield (F). A, B, E and F sites in Basrah governorate (30°22′N 47°22′E), while C and D in Thi-Qar governorate (31°14′N 46°19′E). Ten grams Soil samples were collected from five points under depth five cm using a sterile shovel and placed in sterile containers, while 500 ml of water samples and 500 ml of crude oil samples were collected. Then, all samples were transferred in a cool box to the laboratory for the investigation.

Isolation of biosurfactant-producing bacteria

Isolation of biosurfactant-producing bacterial strain was conducted by enrichment method using modified mineral salt medium (MSM) adopted from Deng et al. (2014). The MSM has consisted of g/l of 5 NaCl, 3 of Na_2HPO_4, 2 of KH_2PO_4, 1of NH_4NO_3, 0.7 of $MgSO_4.7H_2O$, and 1 ml /l trace salt solution with 1% (v/v) olive oil as the sole carbon source and pH 7. The trace salt solution was defined in mg of 20 $CaCl_2$, 30 of $FeCl_3$, 0.5 of $CuSO_4$, 0.5 of $MnSO_4.H_2O$, and 10 of $ZnSO_4.7H_2O$ per liter. The MSM was sterilized by autoclaving at 121 °C for 20 min. Two grams of the soil samples and 2 ml of contaminated water samples were enriched separately with 50 ml of MSM in 250 ml conical flasks supplied with 1% olive oil. The Erlenmeyer flask was incubated at 30 °C and 150 rpm for 7 days. The enrichment culture products were sequentially diluted, and spread on the nutrient agar (without olive oil) for incubation at 30 °C. After 24 hrs, the colonies with different morphologies were picked out and purified based on their Gram staining characteristic, cell shape, and colony morphology. The bacteria were maintained on nutrient agar slants and stored at 4 °C.

Preparation of the bacterial inoculum

The isolated bacteria were activated in fifty ml of autoclaved nutrient broth in 250-ml flasks and incubated for 24 hrs at 30 °C. The uninoculated nutrient broth was used as a negative control. The MSM containing Erlenmeyer's flasks were inoculated with 5 % cfu/ml of the prepared bacterial inoculum (OD600, 1).

Screening for biosurfactant production

For screening biosurfactant production by the isolated bacteria, 50 ml MSM with 1% of olive oil as carbon source in 250 ml Erlenmeyer flasks was inoculated with 5% inoculum and incubated in a rotary shaker at 30 °C and 150 rpm for 7 days. After 7 days of fermentation, bacterial cells were removed by centrifugation 5000 rpm at 4C° for 20 min (Xiangsheng et al. 2010). The cell-free supernatant was subjected to different screening methods to obtain biosurfactant-producing strains.

Oil spreading test

The oil spreading test was carried out by adding 40 ml of distilled water to a Petri dish with a diameter of 15 cm. Subsequently 20 µl of crude oil was added onto the surface of the water, which was formed a thin layer. Then, 10 µl of culture supernatant was added onto the center of the crude oil layer. The area of the clear zone on the oil surface was measured and compared with 10 µl of distilled water as a negative control (Satpute et al. 2010).

Determination of the emulsification index

A mixture of two ml supernatant and two ml kerosene was vertically stirred for two min and the height of the emulsion layer was measured after 24 hrs to determine the emulsification index (Ozdal et al. 2017). The equation used to determine the emulsification index (E24%) is as follows:

$$E24\ (\%) = \frac{\text{The height of emulsion layer}}{\text{The height of total solution}} \times 100\%$$

Determination of emulsification activity

Cell-free supernatant (0.5 ml) of the sample was added to a screw-capped tube containing 7.5 ml of Tris-Mg [20mM Tris HCl (pH 7.0) and 10 mM $MgSO_4$] and 0.1 ml of kerosene. The tubes were vortexed for 2 min and allowed to sit for 1 hour. Absorbance was measured at 540 nm. Emulsification activity (EA) was defined as the measured optical density (Sifour et al. 2005).

Biomass determination

Biomass was determined by centrifuging 10 ml samples at 5000 rpm for 15 min at 5 °C and the cell pellet was dried in an oven at 105°C for 24 hrs (Santos et al. 2018).

Bacterial identification by 16S rRNA

The bacterial isolates were identified up to species level by targeting 16S rRNA gene. Chromosomal DNA was extracted according to the procedure of Presto™ Mini g DNA bacteria kit from the (Geneaid) company. DNA was amplified by the polymerase chain reaction (PCR) using universal primers 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5-GGTTACCTTGTTACGACTT-3). PCR Reactions were made in a total volume of 50 µl. An initial denaturation step of 96 °C for 3 min followed by 27 cycles of 96 °C for 30s, annealing temperature of 56°C for 25s and extension at 72°C for 15s and final extension at 72°C for 10 min (Miyoshi et al. 2005). PCR products were separated based on molecular weight using a 1% (w/v) agarose gel made with TBE buffer (Thermo Fisher Scientific). DNA was visualized under UV light using ethidium bromide DNA stain (Thermo Fisher Scientific). Amplified DNA was purified and sequenced by Macrogen company (South Korea). The obtained 16S rRNA gene sequences were corrected and compared with nucleotide sequences of NCBI using BLAST tools "http://www.ncbi.nlm.nih.gov" to estimate the sequence homology and identification of isolates. Multiple sequence alignment was performed using CLUSTAL Omega "https://www.ebi.ac.uk/Tools/msa/clustalo/". The phylogenetic tree was constructed using MAFFT (Multiple alignment program for nucleotides sequences) "http://mafft.cbrc.jp/alignment/server/" (Katoh et al. 2002).

Table 1. Screening of bacterial isolates for biosurfactant production

Site	Isolates	Gram staining	Emulsification activity/ 540 nm	Emulsification index (E24%)	Oil spreading/ cm	Biomass gm/L
A	A1	-	0.741±0.050*	53.3	12	2.787±0.002*
	A2	-	1.678±0.050	56.6	14	2.787 ± 0.100
	A3	+	0.353±0.055	-	-	1.142±0.344
	A4	+	0.048±0.006	10	4	3.268±0.583
	A5	-	0.038±0.038	-	-	0.942±0.363
	A6	+	0.050±0.038	-	0.4	1.413±0.163
B	B1	+	0.051±0.009	13.3	3	2.704±0.111
	B2	-	0.041±0.007	-	0.2	1.664±0.433
	B3	+	0.041±0.003	-	-	0.612±0.248
	B4	+	0.271±0.041	10	3	2.752±0.526
	B5	+	0.294±0.017	13.3	5.5	2.864±0.123
	B6	-	0.048±0.003	16.6	5	2.681±0.342
C	C1	-	0.011±0.008	-	-	1.468±0.319
	C2	-	0.062±0.009	-	-	0.750±0.227
	C3	+	0.065±0.008	40	3	3.369±0.557
	C4	-	0.054±0.010	16.6	5	3.559±0.469
	C5	+	1.197±0.195	40	10	2.355±0.144
D	D1	+	0.868±0.103	30	5.5	3.307±0.289
	D2	+	0.896±0.082	53.3	14	2.237±0.080
	D3	+	0.064±0.009	13.3	2	2.297±0.111
	D4	-	1.461±0.094	53.3	12	3.435±0.375
E	E1	-	0.480±0.007	10	4	3.268±0.583
	E2	-	0.048±0.011	13.3	2	3.269±0.145
	E3	-	0.031±0.002	-	0.3	1.062±0.212
	E4	-	0.011±0.005	-	-	0.767±0.061
	E5	-	0.038±0.006	-	-	0.724±0.303
	E6	-	0.049±0.003	-	0.2	1.621±0.375
	E7	-	0.045±0.003	16.6	4	2.947±0.246
F	F1	+	0.198±0.069	10	3	1.760±0.153
	F2	-	0.020±0.012	30	5.9	1.819±0.246
	F3	-	0.051±0.009	-	-	0.849±0.102
	F4	-	0.087±0.005	10	2.5	3.268±0.144
	F5	+	0.028±0.003	-	-	0.435±0.257

Note: Mean ± SD, n=3

Statistical analysis

The average values presented in emulsification activity and biomass were estimated by triplicate and expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Isolation of bacteria

The six samples of different sources in the present study had used to isolate the bacteria. Thirty-three bacterial isolates were isolated by enrichment culture techniques from collected samples. The Gram staining and microscopic measurements of bacterial cells were recorded 19 (57.57%) Gram-negative isolates and 14 (42.43%) Gram-positive isolates. Number of bacterial isolates in each site were distributed as follows: 6 isolated from soil sample of A-site (3 –ve and 3+ve), 6 from soil sample of B site (2 –ve and 4 +ve), 5 from soil sample of C site (3 -ve and 2 +ve), 4 from water sample of D site (3 +ve and 1 -ve), 7 from water sample of E site (7 –ve) and 5 from crude oil of F site (3 –ve and 2 +ve) (Table 1).

Screening of biosurfactant producing bacteria

Qualitative screening tests (emulsification index and Oil spreading test) and quantitative test (emulsification activity) were performed to assess biosurfactant production by bacterial isolates. The Emulsification index (E24%) referred to that 20 (60.6%) isolates (out of 33) could emulsify the kerosene with values ranging from 56.6% to 10% and considered positive for biosurfactant production (Table 1). Out of the 33 bacterial isolates screened for the oil spreading test, 24 isolates (72.7%) showed a clear zone of oil displacement with diameters were ranging from 14 to 0.2 cm (Table 1), but the four isolates (A6, B2, E3, and E6) are negative for the emulsification index and showed weak positive results (0.4, 0.2, 0.3 and 0.2 cm) for the oil spreading test, therefore, they considered negative for biosurfactant production. Quantitative tests (emulsification activity) were analyzed for each isolate to determine which isolates are active producers for biosurfactant. The results showed that A2, D4, C5, D2, D1 and A1 isolates have the highest values of emulsification activity. A2 isolate shows efficient potential of biosurfactant production and can be used for future applications. Higher biomass concentration was obtained from biosurfactant producing isolates than non-producing bacteria.

Twenty isolates (60.6%) were determined as biosurfactant producing bacteria according to screening tests distributed among the sampling sites including 10 Gram-negative isolates A1, A2, B2, B6, C4, D4, E1, E2, E7 and F2, and 10 Gram-positive isolates A4, B1, B4, B5, C4, D1, D2, D3, F1, and F4. All bacterial isolates of Al-Garraf oilfield crude oil showed the ability of biosurfactant production.

Bacterial identification by 16S rRNA

All bacterial species were identified by amplification and sequencing of the 16S rRNA gene. The sequences were analyzed by using Basic Local Alignment Search Tool (BLAST) followed by the National Center for Biotechnology Information (NCBI) "http://www.ncbi.nlm.nih.gov ".

The 16S rRNA gene sequence analysis of all the bacterial isolates demonstrates that these isolates at genus level (Figure 1) belongs to: *Bacillus* (11 isolates), *Pseudomonas (8 isolates)*, *Enterobacter* (6 isolates), *Aerococcus* (2 isolates), *Acinetobacter* (1 isolate), *Staphylococcus* (1 isolate), *Achromobacter* (1 isolate), *Klebsiella* (1 isolate), *Cedecea* (1 isolate) and *Stenotrophomonas* (1 isolate). Six bacterial isolates (A3 from A site, B4 from B site and F2, F3, F4 and F5 from F site) were identified as new strains and their sequences were deposited at the National Center for Biotechnical Information (NCBI) under the Genbank accession number MT261834 (*Bacillus subtilis* strain IRQNWYA3), MT261835 (*Bacillus licheniformis* strain IRQNWYB4), MT261836 (*Pseudomonas stutzeri* strain IRQNWYF2), MT261837 (*Pseudomonas zhaodongensis* strain

IRQNWYF3), MT261838 (*Pseudomonas* sp. IRQNWYF4) and MT261839 (*Bacillus licheniformis* strain IRQNWYF5). Figure (2) demonstrated the frequency distribution of biosurfactant-producing genera within each site. *Pseudomonas* and *Bacillus* genera are the most distribution genera in four sites. Phylogenetic tree was constructed based on 16S rRNA gene sequences of biosurfactant-producing bacteria to evaluate their close relationship and evolution between them. The analysis of phylogenetic tree placed eight *Bacillus* isolates into five subgroups. The first subgroup included *Bacillus pumilus* and *Bacillus safensis* along with closely related species. The second sub-group included *Bacillus subtilis* and *Bacillus velezensis*. The third sub-group comprised *B. licheniformis*. The fourth and fifth sub-group consisted of *B. cereus* and *B. jeotgali* respectively. The *Pseudomonas* species and their closely related species placed into three sub-groups, including *P. aeruginosa*, *P. stutzeri*, and *P. mendocina* each sub-group, respectively. The other biosurfactant producing bacteria were placed in different sub-groups with their closely related species (Figure 3).

Discussion

The present study is aimed to isolate biosurfactant producing bacteria from different sources contaminated with petroleum products including samples of crude oil and contaminated water of Al-Garraf oilfield. Biosurfactant producing bacteria can be isolated from various ecosystems, but the habitats that are polluted with petroleum products are more yielding than unpolluted habitats (Soltanighias 2019). The biosurfactant may be involved in protection of microorganisms against unfavorable environmental conditions. In addition, biosurfactant production is important for survival of the microorganisms to facilitate the attachment and adhesion of microbial cells to natural substrates (Fenibo 2019). The bacterial isolates that gave positive results for primary screening tests were only selected as biosurfactant producers. Where, the primary screening tests that selected to present study (emulsification index, Oil spreading test, and emulsification activity) constitutes a quick and easy method to screen and predict biosurfactant production. About 60% (20 isolates) of total isolates (33 isolates) are recorded as biosurfactant producing bacteria. Satpute et al. (2008) who found that identification of potential biosurfactant producers should be selected by more than one screening method.

Table 2. Identification of biosurfactant-producing bacteria by 16S rRNA gene sequencing

Site	Isolates code	Accession no. of closet species	Closet species	Sequence identity (%)	Accession no. of new strain
A	A1	GU204966.1	*Pseudomonas mendocina*	100	
	A2	MK607451.1	*Pseudomonas aeruginosa*	100	
	A3	JF932296.1	*Bacillus subtilis*	99	MT261834.1
	A4	MN932266.1	*Aerococcus viridans*	100	
	A5	KX657687.1	*Klebsiella pneumoniae*	100	
	A6	MK859966.1	*Bacillus cereus*	100	
B	B1	GU551935.1	*Bacillus cereus*	100	
	B2	KY492312.1	*Enterobacter cloacae*	100	
	B3	MN513224.1	*Aerococcus viridans*	100	
	B4	KY328838.1	*Bacillus licheniformis*	99	MT261835.1
	B5	MT040837.1	*Bacillus subtilis*	100	
	B6	KF783212.1	*Pseudomonas stutzeri*	100	
C	C1	MF996504.1	*Stenotrophomonas sp. strain YFC1.2*	100	
	C2	MN022536.1	*Achromobacter* sp. strain RABA7	100	
	C3	LN999937.1	*Bacillus safensis*	100	
	C4	GU339277.1	*Pseudomonas stutzeri*	100	
	C5	MN365041.1	*Bacillus velezensis*	100	
D	D1	MK521069.1	*Bacillus pumilus*	100	
	D2	MK883070.1	*Staphylococcus epidermidis*	100	
	D3	KR347228.1	*Bacillus jeotgali*	100	
	D4	KX585258.1	*Pseudomonas stutzeri*	100	
E	E1	EU258608.1	*Acinetobacter venetianus*	100	
	E2	MK459527.1	*Enterobacter* sp. strain TC159	100	
	E3	MG516114.1	*Cedecea neteri*	100	
	E4	KJ184910.1	*Enterobacter* sp. CZGRN4	100	
	E5	MK522131.1	*Enterobacter asburiae*	100	
	E6	MK641315.1	*Enterobacter tabaci*	100	
	E7	GQ406570.1	*Enterobacter cloacae*	100	
F	F1	KJ437489.1	*Bacillus cereus*	100	
	F2	MH384990.1	*Pseudomonas stutzeri*	99	MT261836.1
	F3	MH725483.1	*Pseudomonas zhaodongensis*	99	MT261837.1
	F4	LT601028.1	*Pseudomonas* sp. A31/70	99	MT261838.1
	F5	HM753621.1	*Bacillus licheniformis*	99	MT261839.1

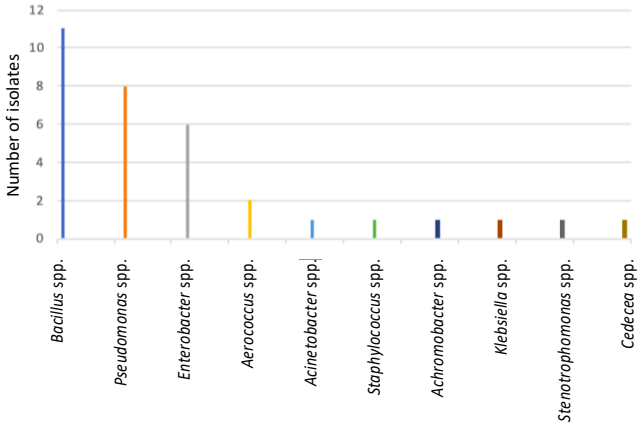

Figure 1. The number of total isolates of the respective genera (n=33)

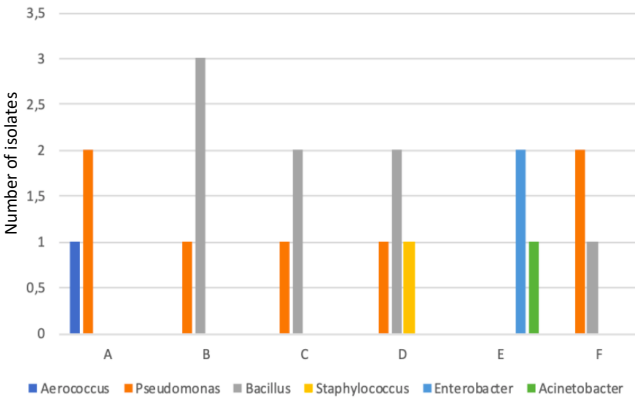

Figure 2. Distribution of biosurfactant producing bacteria at various sites

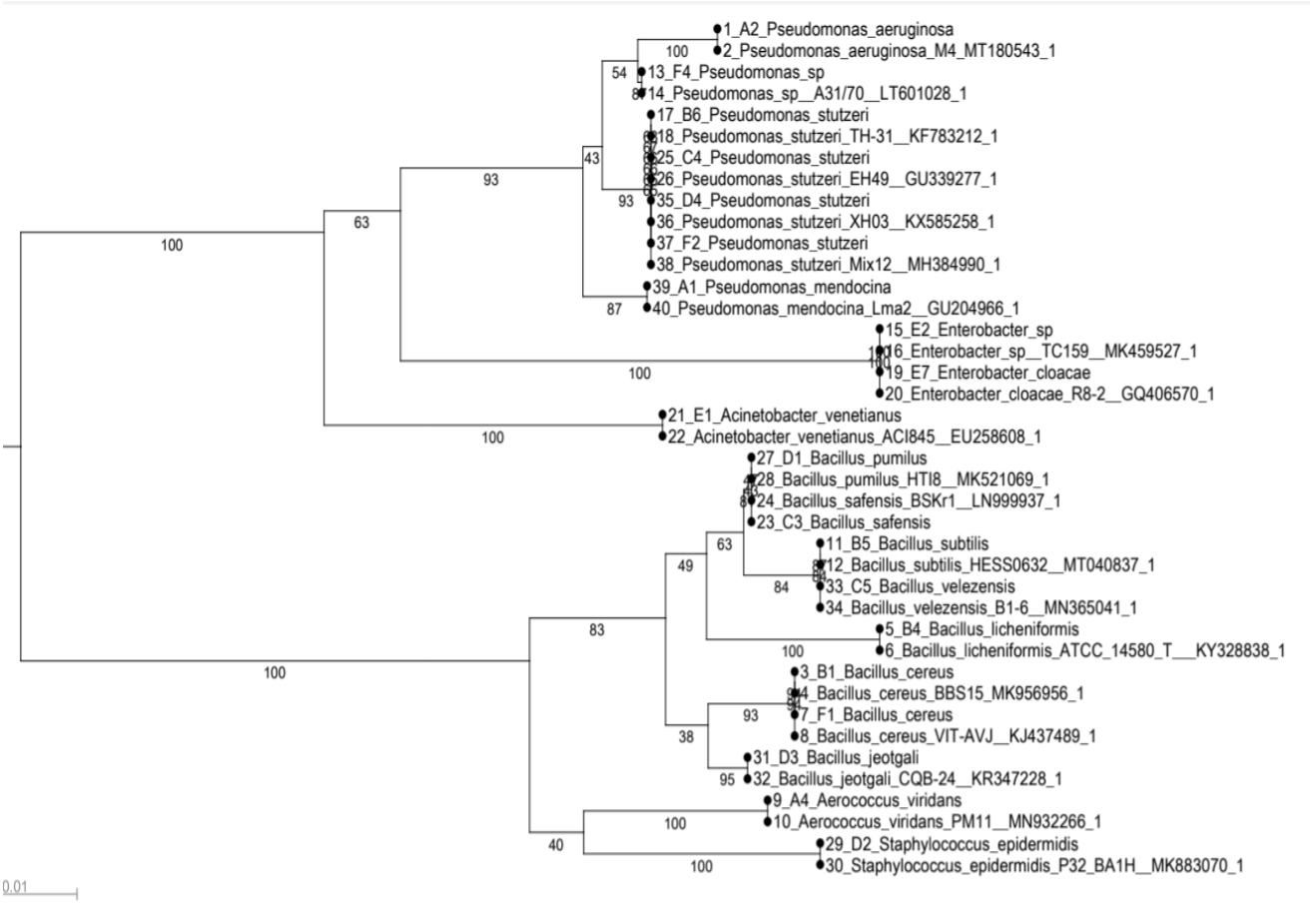

Figure 3. Neighbor-joining phylogenetic reconstruction tree showing evolutionary relationships of biosurfactant producing bacteria with close relatives available in NCBI GenBank database

Emulsification index (E24%) is one of the important methods to support the selection of potential biosurfactant producers. The low values of emulsification index indicate that the isolates produce a low amount of biosurfactant. Mounira and Abdelhadi (2015) indicated that the oil spreading test is reliable in detecting biosurfactant production and determining the presence of biosurfactant in the supernatant of culture broth. Soltanighias et al. (2019) reported 29 % of bacteria were isolated from three oil-contaminated considering as biosurfactant producing bacteria. While, Dang et al. (2015) were isolated 176 marine bacteria from hydrocarbon-contaminated sites along the Norwegian coastline. Eighteen isolates among them showed the ability to produce biosurfactants. The

identification of bacteria based on 16S rRNA gene sequences showed nine isolates of biosurfactant producing bacteria belong to *Bacillus* genus, six isolates to *Pseudomonas*, two isolates to *Enterobacter*, one strain of *Staphylococcus* and *Aerococcus*.

The isolates of *Bacillus* and *Pseudomonas* genera are dominated in the study sites (figure 3). The species of the genus *Pseudomonas* distributed in five sites out six sites, while the species of the genus *Bacillus* distributed in four sites out six sites. The *Pseudomonas* is prevalent genera due to the diversity of mechanisms by which *Pseudomonas* promotes its survival and persistence in various environments (Moradali et al. 2017). The *Bacillus* is predominant and prevalent genera in the hydrocarbon-contaminated environments due to their ability of *Bacillus* to produce biosurfactants and presence of their resistant endospores, therefore they have been termed the more tolerant bacteria to high levels of hydrocarbon contaminants (Viramontes-Ramos et al. 2010).

The isolates of *Bacillus* (*Bacillus subtilis, Bacillus licheniformis, Bacillus cereus, Bacillus pumilus, Bacillus safensis,* and *Bacillus velezensis*) were obtained in the current study have been reported for biosurfactant production in several previous studies (Liu et al. 2010; Joshi et al. 2013; Yadav et al. 2016; Das and Kumar 2019). A large variety of the *Bacillus* genus has been reported for biosurfactants production mainly a variety of lipopeptides biosurfactants, and the majority of *Bacillus* spp. are non-pathogenic, which allow their direct applications in food and pharmaceutical industries (Felix et al. 2019). *Bacillus jeotgali* was not reported for biosurfactant production in previous studies and was isolated for the first time from a Korean traditional fermented seafood, jeotgal in 2001 (Yoon et al. 2001), but in the current study, *B. jeotgali* isolated from crude oil in oilfield reservoir and reported its ability to produce biosurfactant for the first time in the world. *Aerococcus viridans* was not reported for biosurfactant production in previous studies, However, it is a saprophytic bacterium found in air, vegetation, soil, seafood and could be also found in the upper respiratory tract of healthy individuals as part of the microflora (Bradley 2002).

The member of *Pseudomonas* genus (*P. aeruginosa, P. stutzeri*, and *P. mendocina*) was obtained in the current study have been widely studied for their production rhamnolipids and lipoproteins biosurfactant, where rhamnolipid biosurfactants have excellent surfactant properties (Cheng et al. 2017; Shekhar et al. 2018; Twigg et al. 2019). *Acinetobacter venetianus*, *Enterobacter cloacae*, and *Staphylococcus epidermidis* have been reported for the production of biosurfactant in previous reports (Bach et al. 2003; Hamed et al. 2012; Ekprasert et al. 2019). The microbial biosurfactants are very important compounds that can be used in various areas of application such as the pharmaceutical application, agriculture, food industries, enhanced oil recovery and environmental restoration (Liu et al. 2013; Lovaglio et al. 2015). The production of biosurfactants in large amounts for industrial applications depends on the optimization of the media composition and condition, primarily carbon, pH, temperature, and nitrogen sources.

ACKNOWLEDGMENTS

The authors kindly acknowledge the deanship of College of Science, University of Basrah, Iraq for providing laboratory facilities.

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