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Screening potential of biosurfactant production from local bacterial isolates

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Abstract

A wide range of microorganisms can produce biosurfactants with attractive properties. Fifteen bacterial isolates have been collected (9 isolates from soil and 6 from water). The isolates characterized morphological 10 were gram-negative, and 5 were positive. All isolates were screened to perform their ability to produce biosurfactants (hemolysis and drop collapsing, emulsification, oil spreading, and foam production tests). The screening results for isolates' ability to produce biosurfactants showed that the highest value of the drop-collapsing test was recorded for the isolates F4, F5, F9, and F10. In contrast, isolate F13 recorded the highest value for the emulsification test. F11 had the highest value for the oil-spreading test, and the isolate F7 recorded the highest value for the foam test, while none of the isolates recorded hemolysis activity. The biosurfactant production of F9 and F6 show the highest yield 0.955 g/L and 0.68 g/L for the two isolates, respectively, is the highest compared with other isolates. The present study included five samples from each soil and water contaminated with petroleum hydrocarbons.

Keywords: Biosurfactant production, bacteria, drop collapse, oil spreading, emulsification index.

Introduction

Surfactant is an active compound that is used in industry. It consists of a hydrophobic and hydrophilic group, which is found active in soaps and detergents, can aggregate on air and water surfaces, and is commonly used to separate oily substances from a particular medium. These molecules can increase the water solubility of non-aqueous phase liquids by reducing the surface/interface tension between the surfaces of air, water, oil and water (Yin *et al*., 2009). These materials are widely used in industry, agriculture, food, cosmetic, and pharmaceutical applications. However, most of these compounds are chemically synthesized and may cause environmental and toxic problems due to the difficulty of decomposing these substances (Makkar and Rockne, 2003). To reduce the pollution in the environment, it can user-friendly and safe methods such as biosurfactants produced from microorganisms, which have complex structures that differ from one bacteria to another by their composition and effectiveness (Lang, 2002). It is produce by aerobic microorganisms in the aqueous phases in the presence of carbon sources as raw materials such as carbohydrates, hydrocarbons, fats and oils, and Biosurfactants excreted in the culture medium to support the growth of microorganisms by facilitating the translocation of insoluble substrates into cell membranes (Campos *et al*., 2013). These compounds classify mainly based on the origin of the microbes and their

chemical composition (Sharma and Saharan, 2016). Biosurfactants are divided into two types based on molecular weight, polymers of high molecular weight that are the most efficient stabilizing agents. Glycolipids, lipopeptides and phospholipids constitute the majority of low mass biosurfactants, low molecular weight compounds, which lower the interfacial surface tension. At the same time, particulate and polymeric surfactants come under the large mass biosurfactants (Saenz-Marta *et al*.,2015). Microorganisms produce these substances to enhance the bioavailability of hydrophobic unreachable and immiscible substrates, thus increasing the availability of unreachable and unmixed surfaces and thus providing a better chance of survival under severe conditions as low humidity. The production of these active compounds requires the presence of miscible hydrophobic and an oil/carbon source in the culture medium. This process may be cost-effective and alternate when waste products are used as feedstock (Dziegielewska and Adamczak, 2013). This study aims to screen the ability of local bacterial isolates to produce biosurfactants.

Material and methods

Sample collection

Five soil samples were collected from Al-Shuaiba near the oil refinery site. The samples were placed in sterile bags, transferred to the laboratory within 30 minutes, and stored at 4°C. Five water samples were collected from wastewater discharge of al Al-Najibiya thermal station in a sterile 50ml tube. The sample is immediately taken to the laboratory and storage at 4°C till usage to preserve the microbial consortium of water sample (Almansoory *et al*., 2019)

Isolation of bacteria

The water samples were enriched by inoculating with sterile mineral salt medium (MSM) containing (g/L): 15g $NaNO_3$, 1.1g KCl, 1.1g NaCl, 0.00028g FeSO4.7H2O, 3.4g KH_2PO_4, 4.4g K_2HPO4, 0.5g $MgSO_4.7H_2O$ and 0.5g yeast extract and pH was adjusted to 7.3 incubated at 33°C. The medium was autoclaved at 121°C for 15 min. one ml of water sample was inoculated with 100 ml of MSM in a shaker incubator (150 rpm) also 1 gram of soil sample was incubated with adding 1ml of crude oil to release adhering microorganisms. After 24 hours of incubation, the samples were selected based on the colony morphology on nutrient agar plates and the gram staining method (Nayarisseri *et al*., 2018; Huang *et al*., 2020).

Screening of biosurfactant

Bacterial cultures incubated in 500ml Erlenmeyer flask with 100 ml of mineral salt medium containing ($g.l^{-1}$) 10g NaCl, 0.5 $MgSO_4$, 0.5g NH_4Cl, 0.2g $CaCl_2$, 1.0g K_2HPO_3, 0.5g KH2PO4, 0.1g KCl, 0.03g $FeCl_3.6H_2O$ and crude oil (1.0%, w/v) with pH adjusted to 7. Supplemented with 1% (v/v) trace salt medium contained ($g.l^{-1}$) 3.0g $MnSO_4.4H_2O$, 1.0g EDTA, 0.1g $FeSO_4.7H_2O$, 0.1g $CaCl_2.2H_2O$, 0.1g $CoCl_2.6H_2O$, 0.1g $ZnSO_4.7H_2O$, 0.01g $CuSO_4.5H_2O$, 0.01g $AlK(SO_4)_2.12H_2O$, 0.01g H_3BO_3, 0.01g $Na_2MnO_4.2H_2O$. Flasks inoculated with a loopful of bacterial colony culture grown on nutrient agar plates and maintained in a shaker for 5 days at 150 rpm and 33°C. After 5 days of incubation, the supernatant was taken from each flask after centrifugation at 6000 rpm at 4°C for 15 minutes for biosurfactant screening (Gudiña *et al*., 2012; Yan *et al*., 2013).

Hemolytic activity

The selective isolates were tested for their hemolytic activity using human blood agar plates and incubated for 48 h at 37°C to assay hemolytic activity. The plates were visually examined for clearing zones around the colonies, indicative of biosurfactant production (Bicca *et al*., 1999).

Drop collapse

In this method, 25 µl of supernatant biosurfactant pipetted onto parafilm used methylene blue for photographic purposes.

The droplet was allowed drying for minutes and the diameter of the dried droplet was recorded by a ruler with 10 µl of a water droplet as a control sample. (Kuiper *et al*., 2003; Tugrul and Cansunar, 2005).

Oil spreading test

In this test, 20 µl of crude oil was added to 20 µl of distilled water in a petri dish, followed by adding 10 µl of the supernatant on the oil surface. The oil-free clear zone displaced by cell free broth indicates biosurfactant presence (Morikawa *et al*., 2000).

Emulsification index (E24)

The emulsification index (E24) was measured using the method described by Barakat *et al*. (2017). A mixture of 2 ml of supernatant of all isolates was taken separately with 2 ml of kerosene. The mixture vortexed for two minutes and left in the room for 24 hours. The percentage is calculated according to the equation.

Emulsification Index (E24) = Height of Emulsion formed /Total height of Solution.

Foaming activity

The foam height was measured after shaking 10 ml of supernatant by hand for two minutes. The foam activity was measured by equation (El-Sheshtawy, 2015).

Foaming = foam height / total height *100

Extraction of crude biosurfactant

The supernatant of selective isolates (100 ml) was acidified using 1 N HCL solution and left overnight at 4°C for complete precipitation of the biosurfactants. After centrifugation at 8000 rpm for 20 minutes, the precipitates dissolved in distilled water at pH 7.0, followed by biosurfactant extraction with a solvent of 2:1 chloroform-methanol ratio at room temperature (Nitschke and Pastore, 2006).

Results and discussion

Isolation of bacteria

Nine isolates were isolated from Al-Shuaiba contaminated soil and six isolates from Al-Najibiya thermal station water discharge. Gram staining showed a 66.3% ratio of negative bacteria and 33.3% for positive bacteria. The results also showed that most bacteria are bacilli to rod in shape, and some are spore forming, as shown in table (1). Saisa-Ard *et al*. (2013) , mention that the species belonging to *Bacillus* sp. , *Acinetobacter* sp. , *Pseudomonas* sp. , *Corynebacterium* sp. , *Rhodococcus* sp. and *Serratia* sp. Are the most common bacterial species that produce biosurfactants isolated from aquatic environments or soil contaminated with hydrocarbons. The presence of aerobic bacteria that form spores in different environments, helps the bacteria to survive for long periods in extreme conditions (Singh *et al*., 2007).

Screening of biosurfactant

All isolates show negative results for blood agar hemolysis on human agar plates table 1 and figure1. Thavasi *et al*. (2011) show that the evaluation of hemolytic activity is not a very reliable method for testing the production of biosurfactants caused by compounds produced by other microorganisms fig (1). For this reason, more tests are needed to verify the production of biosurfactants. Bernheimer and Avigad (1970) first discovered the hemolytic activity of biosurfactants produced by *Bacillus subtilis* when they produced surfactin, a red blood cell analyzer. Two isolates F4 and F5 showed the highest drop collapsing ability followed by F9 and F10, which show a positive result as it is shown in table 1. The investigation of droplet collapse indicated the ability of isolates to produce biosurfactants (Chandran and Das 2010). Supernatant droplets, which contain biosurfactants, spread or collapse between liquids and hydrophobic surfaces due to decreased strength or tension (Walter *et al*., 2010).

Table 1. Gram staining of isolates and screening of biosurfactant activity

isolate	Cell shape	Gram stain	Hemolytic activity	Drop collapse	Oil spreading	Emulsification index%	Foaming activity%
F1	rod bacilli	-	Gamma	+	0	2.86	1.79
F2	rod	+	Gamma	+	0	2.86	1
F3	rod	_	Gamma	+	5	25.00	0.89
F4	rod	+	Gamma	+++	0	44.12	11.67
F5	rod bacilli	-	Gamma	+++	0	0	9.25
F6	rod	+	Gamma	++	1.5	45.45	40.90
F7	rod bacilli	-	Gamma	+	2	35.71	47.05
F8	rod	+	Gamma	++	0	39.44	30.76
F9	Cocci	+	Gamma	+++	0	48.65	10.71
F10	bacilli	-	Gamma	+++	2.5	44.12	23.07
F11	rod	-	Gamma	+	7	54.38	0.74
F12	rod	-	Gamma	+	0	55.56	0.21
F13	rod	-	Gamma	+	5	72.73	0.02
F14	rod	-	Gamma	++	3	55.40	37.14
F15	rod	-	Gamma	+	1	40.91	0.18

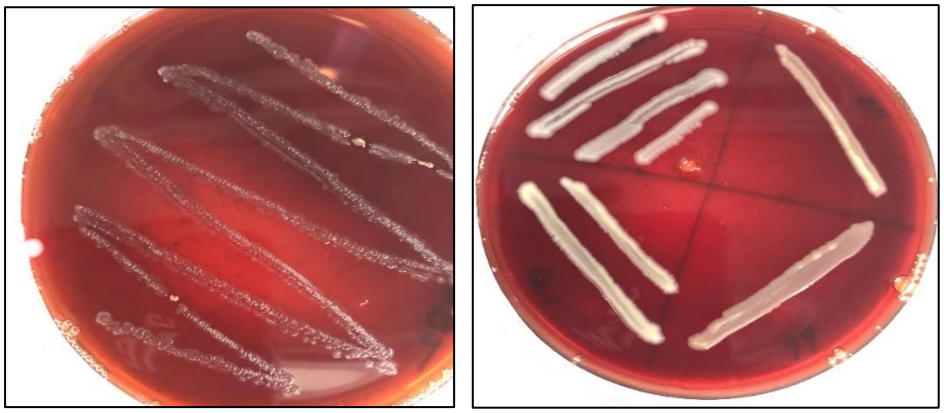

Figure1: gamma hemolytic activity of some isolates.

The emulsification index shows a high percentage of 72.73% to F13 isolate, while F1 shows 2.86% in table 1, fig 2. Jadhav *et al*. (2011) recorded emulsification index (E24) using biosurfactant production by *Enterobacter* sp. MS16 70.5 %. Secato *et*

al. (2016) recorded emulsification index (E24) using biosurfactant production by *Bacillus subtilis* 75% and 83% for soy oil and motor oil, respectively. Karmakar *et al*.(2019) pointed emulsification index (E24) using biosurfactant production by *Enterobacter cloacae* KY231211 47.5K% using Soybean oil. Mandal *et al*. (2013) indicated that some bacterial strains belonging to *Enterobacteriaceae* could produce lipopeptide biosurfactants with high emulsifying properties potential. Oil spreading test shows high spreading activity 7cm for F11 isolate, followed by F3 and F13 with 5 cm diameter, table 1. Fig 3. A larger diameter of clear zone represents the higher activity of the supernatant (Rodrigues *et al*. 2006)

The foaming activity shows the highest percentage of 47.06% and 40.90% for F7 and F6, table 1, fig. 4. Obayori *et al*. (2009) mention that the production of biosurfactants is a strategy used by microorganisms to influence the absorption of hydrocarbons. Willumsen and Karlson (1996) indicated that a good emulsifier could mix with liquid hydrocarbon molecules at an emulsification rate of more than 50% and stabilize for 24 hours or more. The production of foaming is a good test to examine biosurfactant production (Meenal and Madhura, 2016).

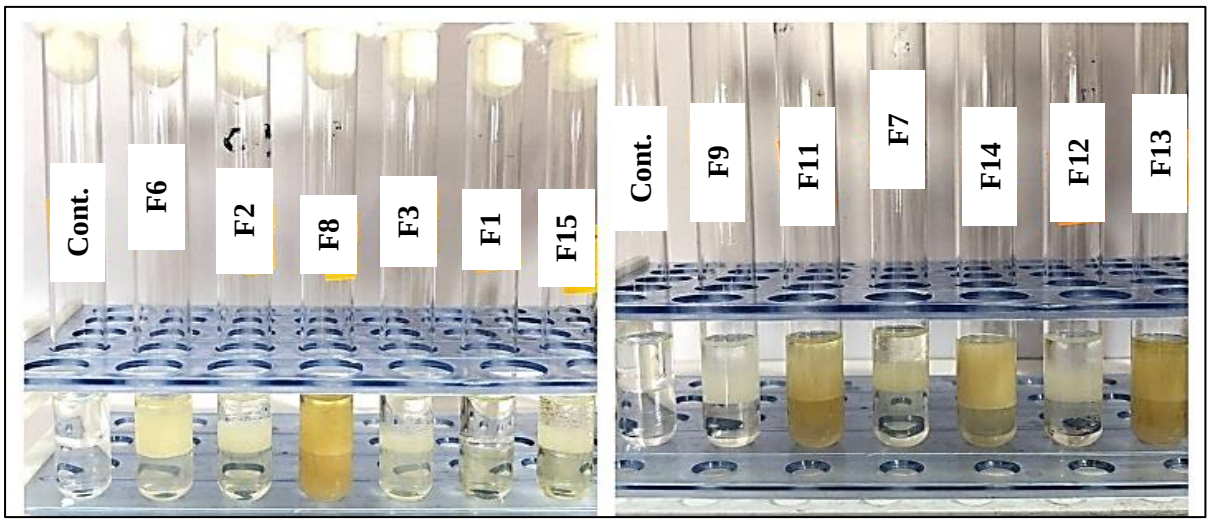

Fig. 2 Emulsification index

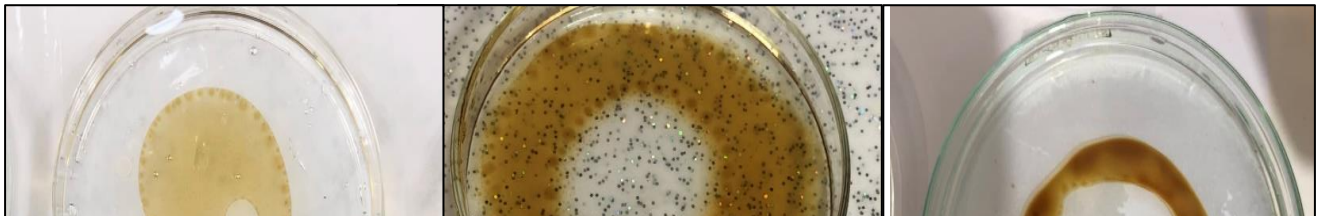

Figure 3: Oil spreading test

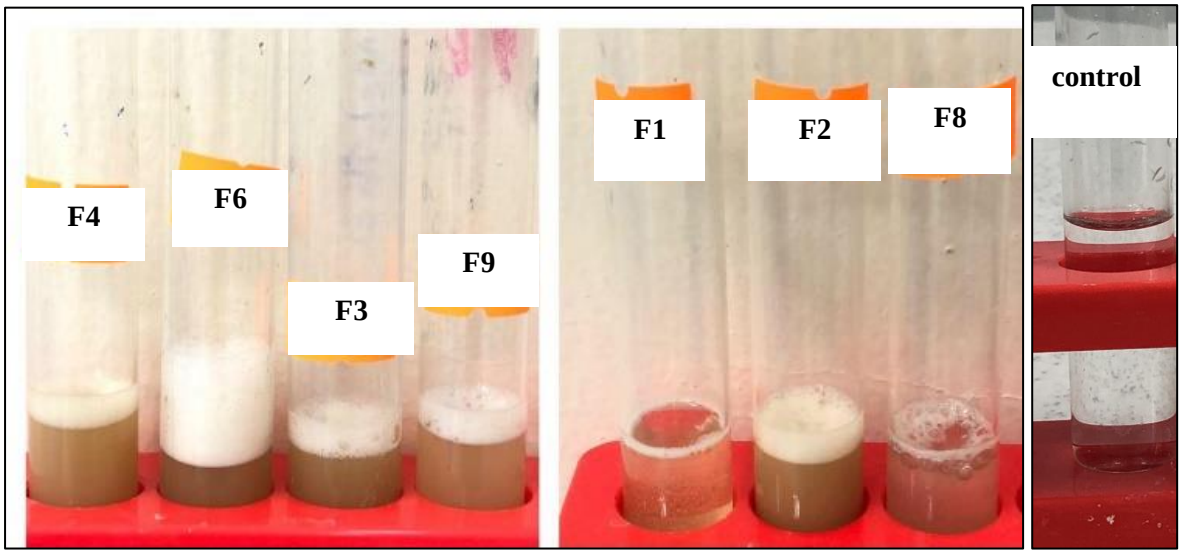

Figure 4: Foaming activity

Biosurfactant production

Six isolates chose for the second screening of biosurfactant production from preliminary screening methods. F9 and F6 show the highest yield of dried biosurfactant (0.95) and (0.68) g/l, respectively. While (0.61, 0.34, 0.3 and 0.27)
for F11, F10, F4, and F5, respectively. The extraction of the biosurfactants from the supernatant of medium containing cultured bacterial cells depends on its solubility in water, its ionic charge, and its location (Al-Wahaibi *et al.*, 2016). Several extraction methods, such as solvent extraction, acetone precipitation, crystallization, and acid deposition, can be included (Gautam and Tyagi, 2006). Cameotra (1997) Indicated that the acid deposition method is a relatively inexpensive technique for extracting a biomaterial such as surfactin, in which fatty surfactants are insoluble under conditions of high acidity (2.0-4.0pH). Kuiper *et al.* (2004) mention, Solvent extraction using ethyl acetate is the most common method for a group of peptide biosurfactants.

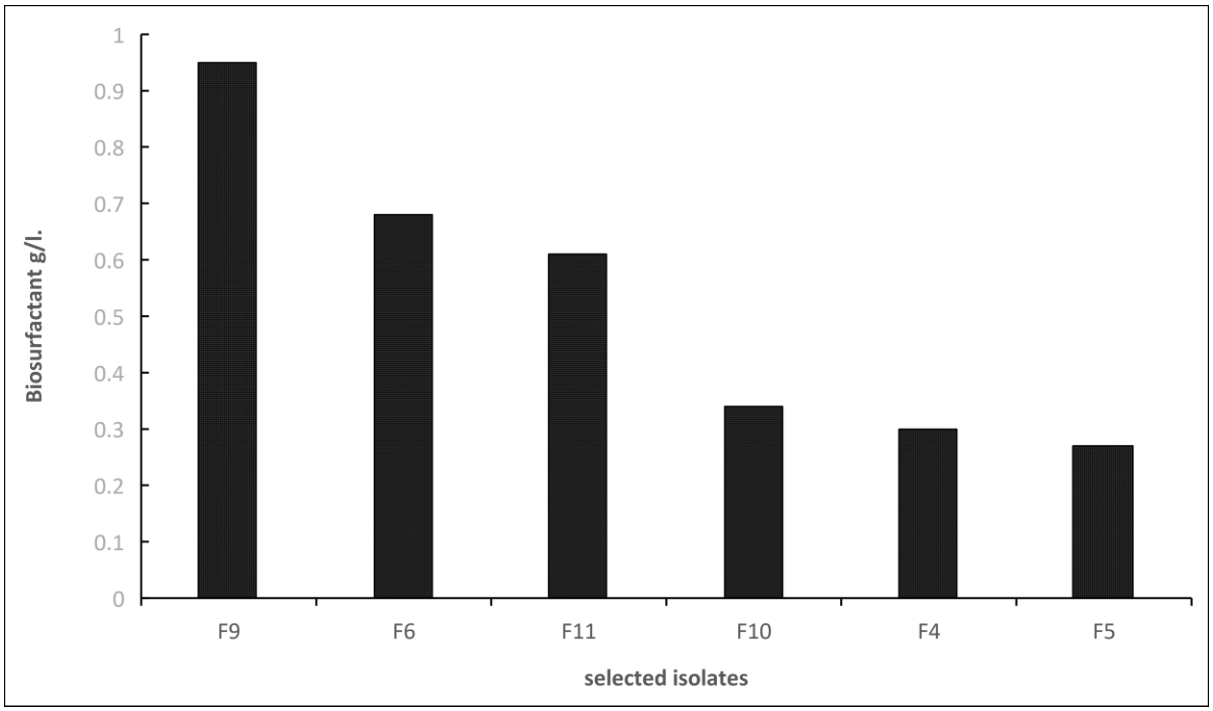

Fig. 5 the production of biosurfactants by selected six isolates

Conclusions

isolated from contaminated soil and water were able to produce biosurfactants by different methods. This study showed that two species of bacteria ability to produce biosurfactants showed the biosurfactant production by F9 and F6 show the highest yield 0.955 g/L and 0.68 g/L for the these isolates respectively. While the highest value to the drop-collapsing test recorded by the isolates F4, F5, F9, and F10 but isolate F13 showed the highest value for the emulsification test. F11 registered for the oil-spreading test, and the isolate F7 recorded the for the foam test.

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غربلة العزلات البكتيرية المحلية الكفؤة في انتاج المستحلبات

فرح ضياء بدر[1] , اسيا فاضل عبدالله المنصوري[1] و وجدان حسين التميمي[2]
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المستخلص

تمتلك العديد من الاحياء المجهرية المنتجة للمركبات الحيوية الخافضة للشد السطحي خصائص مميزة. في الدراسة الحالية تم عزل 15 نوع من البكتيريا المنتجة للمركبات الخافضة للشد السطحي. تسعة من العزلات تم عزلت من التربة وخمسة عزلات عزلت من المياه. عشرة عزلات كانت سالبة لصبغة كرام بينما خمسة منها كانت موجبة لصبغة كرام. تم اختبار قدرة هذه العزلات على انتاج المركبات الحيوية الخافضة للشد السطحي ومن ضمنها (اختبار تحلل الدم، واختبار انهيار القطرة، واختبار الاستحلاب، واختبار انتشار الزيت، واختبار انتاج الرغوة). أظهرت نتائج غربلة قدرة العزلات البكتيرية على انتاج المركبات الحيوية الخافضة للشد السطحي اعلى قيمة للعزلات F4 و F5 و F9 و F10 في اختبار انهيار القطرة، بينما أظهرت العزلة F13 اعلى قيمة لاختبار الاستحلاب، كما أظهرت العزلة F11 اعلى قيمة لاختبار انتشار الزيت والعزلة F7 اعلى قيمة لاختبار انتشار الرغوة. بينما لم تظهر أي من العزلات قدرة على تحلل الدم.

وفي اختبار انتاج المركبات الخافضة للشد السطحي أظهرت العزلتان F6 و F9 اعلى إنتاجية قدرت 0.68 غم/لتر و 0.955 غم/لتر على التوالي بالمقارنة مع العزلات الأخرى.

الكلمات المفتاحية: انتاج المركبات الخافضة للشد السطحي، البكتيريا، الاستحلاب، انهيار القطرة، انتشار الزيت.