Journal of Agricultural Chemistry and Biotechnology

Journal homepage & Available online at: www.jacb.journals.ekb.eg

Qualitative Assessment of Indigenous Bacteria Isolated from Oil-Polluted Sources for Their Capability to Produce Biosurfactants

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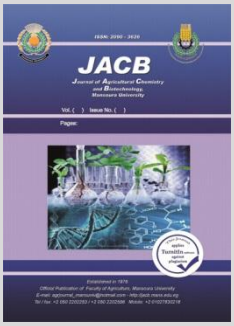

Article Information
Received 4 / 10 /2025
Accepted 14/ 10 /2025

ABSTRACT

Biosurfactants are surface-active compounds produced by microorganisms that serve as eco-friendly alternatives to synthetic surfactants due to their biodegradability, low toxicity, and production from renewable substrates. Their amphiphilic nature enables them to reduce surface and interfacial tension, emulsify hydrophobic compounds, and enhance the bioavailability of hydrocarbons. These properties make biosurfactants valuable for industrial and environmental applications, including bioremediation of oil-contaminated sites, enhanced oil recovery, pharmaceuticals, cosmetics, and food processing. This study aimed to isolate and characterize biosurfactant-producing bacteria from petroleum oil-polluted soils. Nine samples were collected from Port Said, Gharbia, Dakahlia governorates, and the western desert, as well as artificially polluted soils with used cooking oil and waste engine oil. Isolation was carried out using Bushnell-Haas (BH) and Mineral Salt Medium (MSM), resulting in 63 distinct bacterial isolates. Four techniques were performed for screening biosurfactant producers (drop collapse, oil spreading, emulsification index (E_{24}%), and the CTAB-methylene blue agar plate method). Several isolates exhibited significant biosurfactant activity, with emulsification index values reaching 64% and oil displacement zones up to 2.8 mm, indicating strong surface activity. The drop collapse test confirmed the production of surface-active compounds, whereas no dark blue halos were observed in the CTAB-methylene blue assay, suggesting the absence of detectable anionic biosurfactants under the tested conditions. These findings demonstrate the diversity of indigenous bacterial communities in oil-polluted environments and their potential for sustainable biosurfactant production. The most potent isolates identified in this study represent promising candidates for future optimization and application in bioremediation and various industrial processes.

Keywords: Bacteria, Biosurfactant, Hydrocarbons, Petroleum oil

INTRODUCTION

Biosurfactants are surface-active microbial molecules that have gained popularity due to their distinct physicochemical features and wide variety of industrial and environmental uses. (Pardhi *et al*., 2022; Nagtode *et al*., 2023). Unlike chemically created surfactants, biosurfactants are biodegradable, less toxic, and may be made from renewable substrates, making them environmentally beneficial alternatives. Their amphiphilic structure allows them to lower surface and interfacial tension, stabilize emulsions, and increase hydrophobic chemical solubility (Faccioli *et al*., 2024).

In recent years, biosurfactants have been extensively researched for their involvement in a variety of industries, including increased oil recovery, bioremediation of hydrocarbon-contaminated environments, food processing, cosmetics, medicines, and agriculture (Abdoli *et al*., 2025). Glycolipids (Thakur *et al*., 2021), lipopeptides (Lin *et al*., 1994), and phospholipids are the most investigated biosurfactants due to their high emulsifying activity and stability in harsh environments (Pardhi *et al*., 2022 and Faccioli *et al*., 2024).

Biosurfactants are widely produced by microorganisms such as *Pseudomonas* sp. (Siegmund & Wagner 1991 and Agarry *et al*., 2015), *Bacillus* sp. (Lin *et al*., 1994; Marchut-Mikołajczyk *et al*., 2021 and Sultana *et al*., 2024), and *Rhodococcus* sp. (Andreolli *et al*., 2023). These bacteria are frequently isolated from hydrocarbon-rich environments such as oil-polluted soils, wastewater, and petroleum reservoirs, where biosurfactant synthesis confers a selection advantage by increasing the availability of hydrophobic substrates. As a result, screening for biosurfactant-producing microbes in contaminated locations is a viable technique for identifying novel strains with high production potential (Uyar and Sağlam 2021).

Thus, the hypothesis for this research is based on the possibility of isolating bacteria producing biosurfactants, examining the isolated bacteria using preliminary assays to evaluate the potential of biosurfactants production and seeking to identify the most potent strain that can be utilized in further study.

MATERIALS AND METHODS

Samples Collection

For this study, nine soil samples were collected from various sites in Egypt. One sample of crude petroleum oil (PO) was kindly got from petroleum refining station, western desert. Six petroleum-polluted soil samples were obtained from various locations. Four samples were collected from Port Said Governorate, where the pollution sources included gasoline 80 (SG80), gasoline 92 (SG92), turbine (ST), and kerosene (SK). In addition, a soil sample-polluted with waste engine oil (SOE) was collected from Gharbia Governorate, while another one was collected from Dakahlia Governorate that polluted with residues of irrigation machine oil (SOI). Moreover, two soil samples were artificially polluted, one was amended with used cooking oil (SOPWK), and the other with waste car oil (SOPWC). Both artificially polluted soils were maintained for one month with manual mixing every

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DOI: 10.21608/jacb.2025.429454.1125

two days to allow aeration, growth and microbial adaptation. All collected samples were preserved in the refrigerator at 4°C until further use.

Isolation and purification of Biosurfactant-producing bacteria

Bacterial isolation was carried out using two enrichment media: Bushnell-Haas (BH) medium and Mineral salt medium (MSM), each following a slightly different procedure.

Ten grams of a soil sample or 2% of crude petroleum oil was aseptically inoculated into Erlenmeyer flasks containing 90 ml of sterilized BH broth. One liter of BH medium contains (g/L): $MgSO_4$ (0.20), $CaCl_2$ (0.02), KH_2PO_4 (1.0), K_2HPO_4 (1.0), NH_4NO_3 (1.0) and $FeCl_3$ (0.05), supplemented with 2% (v/v) crude oil as the sole carbon source. The pH was adjusted to 7.0 prior to sterilization (Sahoo *et al*., 2011). The culture medium was incubated at 30 °C for 96 h with shaking (JSR JSSI-100C) at 150 rpm. Subsequently, 10 ml of the enriched broth was transferred again into fresh Erlenmeyer flasks containing 90 ml of sterile BH medium supplemented with crude oil and re-incubated under the same conditions to enhance the growth of hydrocarbon-degrading bacteria, followed by incubation at 30°C for 96h. After the second subculture, 0.1 ml of the broth culture was spread onto nutrient agar plates prepared by adding 18 g/L of agar to nutrient broth (HiMedia product). The inoculated plates were incubated at 30°C for 24 to 48 hours. (Sahoo *et al*., 2011 and Chinnasamy *et.al.,* 2021). As well as approximately 10 g of homogenized soil sample was suspended in 100 ml of physiological saline (1% NaCl), shaken vigorously for 1-2 h, and left to stand for one hour. From this suspension, 5 ml of supernatant was inoculated into Erlenmeyer flasks containing 45 ml of sterilized basal mineral salt medium (MSM) supplemented with 2% (v/v) crude oil as the sole carbon and energy source. One liter of MSM medium contains (g/L): NH_4NO_3 (1.0), $MgSO_4\,7H_2O$ (0.7), Na_2HPO_4 (3.0), KH_2PO_4 (2.0) and 1 ml/L of a trace element solution. The trace element solution contained (mg/L): $CuSO_4\,5H_2O$ (0.5), $FeCl_3$ (30), $ZnSO_4{\cdot}7H_2O$ (10), $MnSO_4{\cdot}H_2O$ (0.5) and $CaCl_2$ (20), and the solution was adjusted to pH 7.2 ± 0.2 (Yalaoui-Guellal *et al*., 2018 and Abdel-Mawgoud *et al*., 2008). Cultures were incubated at 30°C on an orbital shaker (JSR JSSI-100C) at 150 rpm for 7 days. All enrichment experiments were conducted in triplicate. Following incubation, single bacterial colonies were obtained by spreading aliquots of BH and SMS enriched cultures onto nutrient agar plates and incubated at 37°C for 3–4 days. Morphologically distinct colonies were picked, purified by re-streaking nutrient Agar plates. All selected pure bacterial isolates were then transferred to nutrient agar slants for preservation and stored at 4°C until further use.

Morphological characterizations of bacterial isolates

Bacterial isolates were studied based on some cultural and morphological characteristics given in Bergey's Manual of Determinative Bacteriology (1994). Separate colonies of bacterial isolates from 24-day-old nutrient agar plate cultures were examined to be characterized visually and through light microscopy. Also, the direct microscopic examination of stained smear of the isolates' culture was stained and carried out for studying shape, aggregation of the bacterial cells, also Gram and spore staining were done. These specific patterns (colony and cells) aid in the preliminary identification of the isolates for further study.

Biosurfactant production conditions

Inocula of bacterial isolates were prepared by culturing them in nutrient broth at 30°C for 24 h with shaking at 150 rpm to obtain actively growing cultures. These 24-hour-old cultures were then used as inocula for biosurfactant production experiments (Saimmai *et al*., 2013; Greenwell *et al*., 2016). Crude petroleum oil was used as a sole carbon and energy source, which was added at a 2% (v/v) concentration. The media (HB or MSM) were sterilized by autoclaving at 121°C for 15 min. Each 250 ml Erlenmeyer flask contained 50 ml of sterile medium was inoculated with 2% (v/v) of the actively growing bacterial inoculum. Cultures were incubated at 30°C in a rotary shaker at 150 rpm for 7 days (Saimmai *et al*., 2013; Youssef *et al*., 2004 and Sahoo *et al*., 2011). The broth cultures were then centrifuged at 6000 rpm for 30 minutes at 4°C in order to obtain the cell-free supernatant which, was carefully collected and used for detection on biosurfactant through subsequent assays (Nayarisseri *et al*., 2018).

Screening techniques for detecting biosurfactant producers

Production of biosurfactant by bacterial isolates was evaluated using four standard qualitative and semi-quantitative techniques performed on the obtained culture supernatants.

Drop collapse test: A thin layer of crude oil (5–10 µl) was placed on a clean glass slide or parafilm. Then, 10 µl of cell-free supernatant was carefully added to the surface of the oil. After 1 minute, the droplet was observed for spreading or flattening, which indicated the presence of biosurfactants (Jain *et al.*1991).

Oil spreading assay: A Petri dish containing 50 ml of distilled water was layered with 20 µl of cooking oil. Subsequently, 10 µl of culture supernatant was gently added to the center of the oil layer. The displacement of oil created a clear zone, and its diameter (cm) was measured. Larger diameters corresponded to higher biosurfactant activity. Each isolate was tested in three replicates (Chigede *et al*., 2024 and Jui *et al*., 2024).

Emulsification index (E_{24}): Equal volume of cell-free supernatant and hydrophobic substrate (cooking oil) in glass test tube were well mixed by vortex for 2 min and the mixture was allowed to settle at room temperature for 24h. (Iqbal *et al*., 1995 and Jui *et al*., 2024). The emulsification index was calculated as the following equation:

$$E_{24}\% = \frac{Height\ of\ emulsion\ layer}{Total\ height\ of\ liquid\ column} \times 100$$

All tests were conducted in triplicate, and the average were calculated accordingly.

CTAB-MB agar plate method: To prepare CTAB-MB agar plates, MSM agar was prepared with a carbon source (crude oil 2%, w/v), and supplemented with CTAB (0.5 mg/ml), and methylene blue (0.2 mg/ml). Using heated point of a 10 ml glass pipette, shallow wells were made into the surface of the plates and 10 µL of the broth culture was placed into each well. At 30°C, agar plates were incubated and checked over 24 to 48 h incubation time for formation of a dark blue halo around the colonies that confirm anionic biosurfactant production (Siegmund and Wagner 1991).

RESULTS AND DISCUSSION

Isolation of bacterial isolates

A total of sixty-three bacterial isolates were successfully obtained from nine different samples which were a petroleum oil sample and eight soil samples either from petroleum-polluted areas or artificially polluted with waste engine oil as well as used frying oil. It is interesting of the data represented in Table (1) that out of 63 isolates, 32 isolates were obtained from petroleum oil sample both grown on BH

(B) and MSM (M) media, 22 and 10 isolates respectively. This may be attributed to the higher concentration of hydrocarbons, which act as a selective factor for microbes capable of utilizing these compounds as a carbon source and biosurfactant-producing microorganisms.

Table 1. Cultural and microscopic characterizations of bacterial isolates

No.	Isolate Code	Colony Characterizations							Microscopic Morphology			
		Size	Color	Margin	Form	Elevation	Opacity	Texture	Shape	Arrangement	Gram Stain	Spore Stain
1.	PO-B1	Med.	Creamy white	Entire	Circular	Convex	Opaque	Smooth	Cocci	Single	-	-
2.	PO-B2	Med.	Yellow	Entire	Circular	Low Circular	Opaque	Smooth	Short Rod	Single	-	-
3.	PO-B3	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
4.	PO-B4	Med.	Creamy white	Undulate	Circular	Low Circular	Opaque	Smooth	Short Rod	Chain	-	-
5.	PO-B5	Med.	Off-white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
6.	PO-B6	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Short Rod	Chain	-	-
7.	PO-B7	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
8.	PO-B8	S	milky	Entire	Circular	Raised	Opaque	Smooth	Cocci	Single	-	-
9.	PO-B9	Med.	White	Entire	Circular	Low Circular	Semi*	Smooth	Cocci	Single	-	-
10.	PO-B10	Med.	White	Entire	Circular	Low Circular	Opaque	Smooth	Short Rod	Single	-	-
11.	PO-B11	Med.	White	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	+	+
12.	PO-B12	Med.	White	Entire	Circular	Low Circular	Opaque	Dry	Cocci	Single	-	-
13.	PO-B13	Med.	White	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
14.	PO-B14	Med.	White	Undulate	Circular	Flat	Opaque	Smooth	Cocci	Irregular	+	-
15.	PO-B15	Med.	Off-white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Chain	+	+
16.	PO-B16	Med.	Off-white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
17.	PO-B17	Med.	Creamy white	Entire	Filamentous	Low Circular	Opaque	Smooth	Cocci	Single	-	-
18.	PO-B18	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Short Rod	Single	-	-
19.	PO-B19	Med.	Creamy white	Entire	Circular	Low Circular	opaque	Smooth	Cocci	Single	-	-
20.	PO-B20	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
21.	PO-B21	Med.	Pinkish white	Entire	Circular	Raised	Opaque	Smooth	Cocci	Single	-	-
22.	PO-B22	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
23.	PO-M1	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
24.	PO-M2	Med.	Creamy whit	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
25.	PO-M3	Med.	Off-white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Chain	-	-
26.	PO-M4	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Chain	+	-
27.	PO-M5	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
28.	PO-M6	Med.	Creamy white	Undulate	Circular	Raised	Opaque	Smooth	Cocci	Single	-	-
29.	PO-M7	Med.	Yellow	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
30.	PO-M8	Med.	Creamy white	Entire	Circular	Raised	Opaque	Smooth	Short Rod	Diplo	-	-
31.	PO-M9	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
32.	PO-M10	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Short Rod	Single	-	-
33.	SG80-B1	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
34.	SG80-B2	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Dry	Cocci	Single	-	-
35.	SG80-B3	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Short Rod	Single	-	-
36.	SG80-B4	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
37.	SG80-B5	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
38.	SG90-B1	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
39.	SG90-B2	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
40.	SG90-B3	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Short Rod	Single	-	-
41.	SG90-B4	Med.	Yellow	Entire	Circular	Low Circular	Opaque	Smooth	Short Rod	Single	-	-
42.	SG90-B5	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
43.	SG90-B6	Med.	Creamy white	Entire	Circular	Flat	Opaque	Smooth	Cocci	Single	-	-
44.	ST-B1	Med.	White	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
45.	ST-B2	Med.	White	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
46.	SK-B1	Med.	Yellow	Entire	Circular	Low Circular	Opaque	Wrinkled	Cocci	Chain	-	-
47.	SK-B2	Med.	Pinkish white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
48.	SK-B3	Med.	Creamy whit	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
49.	SK-B4	Med.	Yellow	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
50.	SOE-M1	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
51.	SOI-B1	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Short Rod	Single	+	-
52.	SOI-B2	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Irregular	-	-
53.	SOPWK-M1	Med.	Creamy white	Entire	filamentous	Low Circular	Opaque	Smooth	Short Rod	Single	-	-
54.	SOPWK-M2	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Short Rod	Single	-	-
55.	SOPWK-M3	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Single	-	-
56.	SOPWK-M4	Med.	White	Entire	Circular	Low Circular	Opaque	Dry	Cocci	Single	-	-
57.	SOPWK-M5	Med.	Pinkish white	Entire	Circular	Low Circular	Opaque	Dry	Cocci	Single	-	-
58.	SOPWK-M6	Med.	Off-white	Undulate	Circular	Low Circular	Opaque	Smooth	Rod	Single	-	-
59.	SOPWC-M1	Med.	Creamy white	Undulate	Circular	Low Circular	Opaque	Smooth	Cocci	Irregular	-	-
60.	SOPWC-M2	Med.	Creamy white	Entire	Circular	Low Circular	Semi*	Smooth	Cocci	Single	-	-
61.	SOPWC-M3	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Irregular	-	-
62.	SOPWC-M4	Med.	Creamy white	Entire	Circular	Flat	Opaque	Smooth	Cocci	Chain	-	-
63.	SOPWC-M5	Med.	Creamy white	Entire	Circular	Low Circular	Opaque	Smooth	Cocci	Chain	-	-

(B) isolated on BH, (M) isolated on MSM, (+) positive, (-) negative, (Med.) medium, (S) Small, (Semi*) Semitransparent

Moreover, microscopic characterization showed that 4٩ isolates exhibited coccoid morphology, while 13 isolates appeared as short rods. Gram staining also indicated that 58 isolates were Gram-negative, whereas only 3 isolates were identified as spore-forming bacteria. Such diversity in morphology and Gram reaction suggests the presence of different bacterial groups with potential metabolic versatility, which may contribute to effective hydrocarbon degradation and biosurfactant production.

These findings indicate that petroleum-polluted soils are rich habitats for hydrocarbon-degrading bacteria with potential biosurfactant production abilities. Similar observations were reported by Gogoi *et al.,* (2016), who highlighted that oil-contaminated environments favor the growth of microorganisms possessing catabolic pathways for hydrocarbon degradation and biosurfactant synthesis.

Characterizations of biosurfactant-producing bacterial isolates

The obtained bacterial isolates (sixty-three) were further examined for their colony features, Gram reaction, and microscopic features. The detailed characteristics of the biosurfactant-producing isolates, along with their screening results, are presented in Table (1).

Microscopic characterizations of 63 bacterial isolates showed that 49 isolates exhibited coccoid morphology, while 13 isolates appeared as short rods. Gram staining indicated that 58 isolates were Gram-negative, whereas 3 isolates were identified as spore-forming bacteria. Such diversity in morphology and Gram reaction suggests the presence of different bacterial groups with potential metabolic versatility, which may contribute to effective hydrocarbon degradation and biosurfactant production.

Screening bacterial isolates for Biosurfactant Production

To identify the most promising biosurfactant-producing strains, all 63 isolates were assessed using four qualitative and semi-quantitative assays: drop collapse test, oil spreading test, emulsification index (E_{24}%) and CTAB-MB agar plate method. The results of these screening tests are summarized in Table (2).

Drop Collapse Test

Among the 63 isolates tested, 55 isolates gave a positive reaction in the drop collapse assay. A positive result was indicated by the flattening or spreading of the droplet on the oil surface, whereas the persistence of a spherical droplet represented a negative result (Table 2 and Fig. 1).

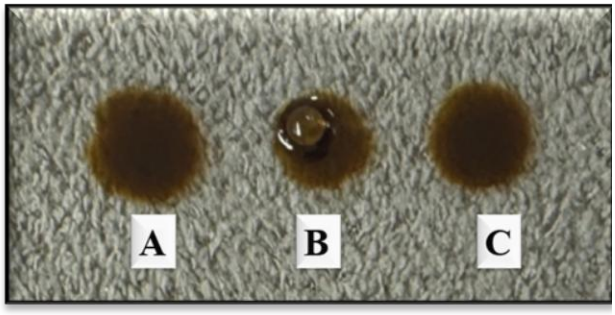

Fig. 1. Photograph of drop collapse assay shows a comparison of different samples: Samples A and C exhibited spreading and flattening of the drop, indicating a positive result and presence of surface-active compounds (biosurfactants). In contrast, sample B retained a spherical droplet shape, representing a negative result and suggesting the absence of biosurfactant production.

The very high percentage of positive isolates demonstrates that petroleum-polluted soils are a rich source of microorganisms capable of producing surface-active compounds. This suggests that the majority of isolates have the potential to secrete biosurfactants. The drop collapse assay is widely recognized as a rapid and reliable qualitative screening method for the preliminary detection of biosurfactant production. Similar findings were reported by Jain *et al*., (1991), who observed that the drop collapse test is highly sensitive for identifying biosurfactant-producing strains, especially those associated with hydrocarbon-rich environments.

Oil Spreading Test

The bacterial isolates exhibited variable activity in this assay, where clear zone diameters ranging from 0.4 to 2.8 cm. In contrast, several isolates showed very small or negligible displacement zones (<0.5 cm), indicating weak or no biosurfactant production. The oil spreading assay is a sensitive and quantitative method for detecting biosurfactant production, as the size of the clear zone is directly related to the ability of the culture supernatant to reduce surface tension and displace the oil (Table 2).

The wide variation in zone diameters among the isolates reflects differences in their biosurfactant production potential. Walter *et al*., (2010) reported similar findings, emphasizing that larger diameters correspond to strains that produce greater amounts of biosurfactants. These findings are consistent with the drop collapse test, as isolates that exhibited higher oil spreading diameters also showed strong positive activity in the drop collapse assay, confirming their potential for efficient biosurfactant production.

Emulsification Index (E_{24}%)

Emulsification activity was clarified in Figs. (2, 3) and the data represented in Table (2) indicate that the bacterial isolates exhibited variable emulsification activity, with E_{24}% values ranging between 20 and 64%.

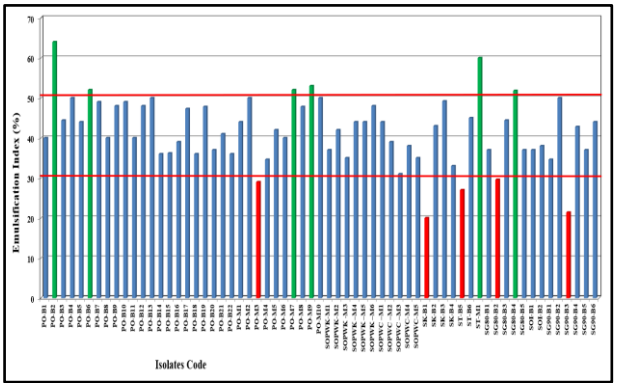

Fig. 2. Emulsification Index (E_{24}%) of all the bacterial isolates indicating potential for biosurfactant production. Red lines for the values range between low (red columns, <30%) and high (green columns, up to 50%).

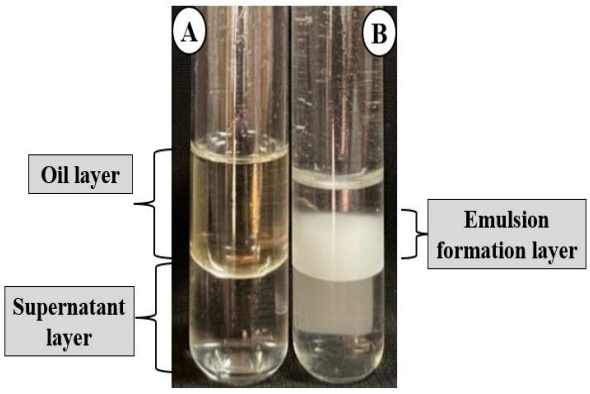

Fig. 3. Photograph of emulsification activity of bacterial culture supernatant. (A) showing no emulsion formation. (B) display a stable emulsion layer

Table 2. Screening results of the bacterial isolates for detecting their ability to produce biosurfactants

Isolate Code	Drop Collapse	Oil spreading (cm)*	E_{24} (%)*	CTAB-MB test
PO-B1	+	0.9	40.0	-
PO-B2	+	2.8	64.0	-
PO-B3	+	1.4	44.4	-
PO-B4	+	1.2	50.0	-
PO-B5	+	1.1	44.0	-
PO-B6	+	2.0	52.0	-
PO-B7	+	1.72	49.0	-
PO-B8	+	0.8	40.0	-
PO-B9	+	1.9	48.0	-
PO-B10	+	1.7	49.0	-
PO-B11	+	1.0	40.0	-
PO-B12	+	1.6	48.0	-
PO-B13	+	1.9	50.0	-
PO-B14	+	0.5	36.0	-
PO-B15	-	0.7	36.2	-
PO-B16	+	1.2	39.0	-
PO-B17	+	2.0	47.3	-
PO-B18	+	0.8	36.0	-
PO-B19	+	1.0	47.8	-
PO-B20	+	0.8	37.0	-
PO-B21	+	0.8	41.0	-
PO-B22	+	0.9	36.0	-
PO-M1	+	1.3	44.0	-
PO-M2	+	1.6	50.0	-
PO-M3	+	0.4	29.0	-
PO-M4	-	1.1	34.6	-
PO-M5	+	1.5	42.0	-
PO-M6	+	0.9	40.0	-
PO-M7	+	1.7	52.0	-
PO-M8	+	1.0	47.8	-
PO-M9	+	1.4	53.0	-
PO-M10	+	1.2	50.0	-
SG80-B1	-	0.5	29.6	-
SG80-B2	+	1.8	44.4	-
SG80-B3	+	2.1	51.8	-
SG80-B4	+	0.4	37.0	-
SG80-B5	+	0.6	37.0	-
SG90-B1	+	1.8	50.0	-
SG90-B2	-	0.6	21.4	-
SG90-B3	+	1.5	42.8	-
SG90-B4	+	0.7	37.0	-
SG90-B5	+	1.2	44.0	-
SG90-B6	+	2.4	60.0	-
ST-B1	-	0.6	27.0	-
ST-B2	+	1.3	45.0	-
SK-B1	-	0.5	20.0	-
SK-B2	+	1.0	43.0	-
SK-B3	+	1.6	49.2	-
SK-B4	+	0.8	33.0	-
SOE-M1	+	1.1	37.0	-
SOI-B1	+	0.9	38.0	-
SOI-B2	-	0.8	34.6	-
SOPWK-M1	+	0.6	37.0	-
SOPWK-M2	+	0.9	42.0	-
SOPWK-M3	+	0.7	35.0	-
SOPWK-M4	+	1.3	44.0	-
SOPWK-M5	+	1.1	44.0	-
SOPWK-M6	+	1.8	48.0	-
SOPWC-M1	+	1.3	44.0	-
SOPWC-M2	+	0.9	39.0	-
SOPWC-M3	-	0.7	31.0	-
SOPWC-M4	+	0.9	38.0	-
SOPWC-M5	+	0.9	35.0	-

(+) positive , (-) negative, * mean value of three independent experiments

Whereas the highest emulsification activity was recorded with bacterial isolate PO-B2 (which was isolated from petroleum-oil on BH medium). Based on the E_{24}% values, the emulsification activity can be categorized into

three levels: high activity (above 50%, indicating strong and stable emulsifying capability); moderate activity (Between 30% and 50%, reflecting moderate biosurfactant production) and low activity (below 30%, indicating weak or negligible emulsifying potential) as shown in Fig. (2). The wide variation in E_{24}% among the tested isolates reflects diversity in their biosurfactant production abilities. The emulsification index (E_{24}%) is a key parameter for evaluating the potency and stability of biosurfactants in forming stable emulsions between oil and water (Fig. 3). A high emulsion-formed layer indicates the biosurfactant's concentration and effectiveness.

According to Meena *et al*., (2021), biosurfactants with E_{24}% values above 50% are considered highly efficient and suitable for industrial applications.

These findings are consistent with the results obtained from the drop collapse and oil spreading assays, where isolates with higher E_{24}% values also demonstrated strong positive activity, confirming their superior biosurfactant-producing potential.

CTAB-MB agar plate method

The CTAB-MB test is a rapid screening method that applies to detect anionic biosurfactants, through the formation of dark blue halos around the colonies. However, our data (Table 2) shows that no color change or precipitation zones were observed for any of the tested isolates. The lack of blue halo formation (negative) for all tested bacterial isolates confirmed that they may not be capable of producing anionic biosurfactants such as rhamnolipids, which specifically react with CTAB and methylene blue to form visible complexes. These findings indicate that the biosurfactants produced might belong to different classes, such as lipopeptides or polymeric biosurfactants, which cannot be detected by this method. Similar observations were reported by Sabnis and Juvale (2016), who explained that the CTAB-MB assay is highly specific for detecting anionic biosurfactants and may not show positive results if the produced compounds are non-ionic or cationic.

It could be concluded that the isolation and screening of bacterial strains from crude oil itself, oil-contaminated and artificially enriched soil revealed a wide diversity of bacterial communities with variable biosurfactant-producing potential. Several isolates exhibited promising emulsification activity and oil displacement capacity, highlighting their significance for future applications. Although none of the tested strains produced detectable anionic biosurfactants under the given conditions, the outcomes provide a strong basis for further optimization and advanced characterization. In general, these results confirm the potential of local and indigenous bacteria as valuable candidates for sustainable biosurfactant production and their application in bioremediation and related industrial processes.

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التقييم النوعي للبكتيريا المحلية المعزولة من مصادر ملوثة بالنفط لقدرتها على إنتاج المواد الخافضة للتوتر السطحي

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الملخص

تُعد المواد الخافضة للتوتر السطحي من المركبات النشطة سطحياً والتي تنتجها الكائنات الحية الدقيقة، وتستخدم كبدائل صديقة للبيئة بدلاً من المخلقة كيميائياً، حيث إنها قابلة للتحلل الحيوي، ومنخفضة السمية، ويمكن انتاجها من مصادر بكتيرية متجددة. وتتميز تلك المواد بطبيعتها المحبة للماء التي تمكنها من خفض التوتر السطحي واستحلاب المركبات الكاره للماء مما يجعلها ذات قيمة في التطبيقات الصناعية والبيئية مثل العلاج الحيوي للمواقع الملوثة بالبترول والمستحضرات الصيدلانية والتجميلية وكذلك الصناعات الغذائية. استهدفت هذه الدراسة عزل وتوصيف البكتيريا المنتجة للمواد الخافضة للتوتر السطحي من عينات ملوثة بزيت البترول. وأمكن الحصول على ٦٣ عزلة بكتيرية باستخدام بيئات MSM & BH . اختبرت تلك العزلات لقدرتها على إنتاج تلك المواد الحيوية الخافضة للتوتر السطحي وذلك باستخدام أربع اختبارات وهي: drop collapse - oil spreading - emulsification index (E_{24}%) - CTAB-methylene blue agar plate . أظهرت العديد من العزلات نشاطًا حيويًا كبيرًا للمواد الخافضة للتوتر السطحي، حيث وصلت قيم مؤشر الاستحلاب emulsification index (E_{24}%) إلى ٦٤% ، كما بلغت مناطق oil spreading ايضاً الى ٢،٨مم، مما يشير إلى نشاط سطحي قوي. أكد اختبار drop collapse إنتاج مركبات نشطة سطحياً، في حين لم تتم ملاحظة أي هالات زرقاء داكنة في اختبار CTAB-methylene blue مما يشير إلى عدم وجود مواد أنيونية خافضة للتوتر السطحي في ظل الظروف المختبرة. ونستنتج من هذه النتائج مدى تنوع المجتمعات البكتيرية المتوطنة في تلك البيئات الملوثة بالنفط وقدرتها على إنتاج المواد الخافضة للتوتر السطحي. وتمثل أفضل العزلات التي تم تحديدها خلال هذه الدراسة اختيار واعد لدراسات مستقبلية لتحسين الإنتاج وإمكانية تطبيقها في المعالجة الحيوية والعمليات الصناعية المختلفة.