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Extraction of biosurfactant from *pseudomonas aeruginosa* inhabiting oil-spilled soils

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Abstract

Background Screening for biosurfactant-producing microbes involves the process of sampling and isolating bacteria from various environmental sources. Hydrocarbon-contaminated locations are considered ideal sources for isolating bacteria that produce biosurfactants. This is because these microbes have adapted to living in oil-contaminated environments and have developed mechanisms to produce biosurfactants as a means of survival. Therefore, screening microbes from such locations increases the likelihood of finding bacteria capable of producing biosurfactants.

Results This investigation aimed to isolate bisurfactant-producing bacteria from oil-contaminated soil samples, with a particular focus on *Pseudomonas aeruginosa*. Out of the 10 samples collected, four were confirmed to be *P. aeruginosa*, and all strains showed positive results for antagonistic, hemolytic, and emulsification tests. The strains were effective against *B. subtilis*, *S. aureus*, and *E. coli* but less effective against *A. hydrophila*. The PS10 strain exhibited the highest emulsification index, while PS08 showed the lowest. Based on microscopic and biochemical examination, *P. aeruginosa* was identified as the most effective isolate.

Conclusion These findings highlight the potential of *P. aeruginosa* as a biosurfactant producer for industrial and environmental applications. However, further studies are needed to fully understand the biosurfactant production potential of these strains and to optimize the conditions for biosurfactant production.

Keywords *Pseudomonas aeruginosa*, Biosurfactant, Antagonistic test, Hemolytic assay, Emulsification index

Background

Oil pollution is a significant and long-standing environmental problem that has had adverse effects on the planet for many years. Accidental oil releases into the environment can have severe consequences on marine and coastal ecosystems, as well as on human health and the economy. Oil spills can result in wildlife damage, habitat destruction, and water contamination, leading to long-term environmental impacts [1, 2]. Various sources contribute to oil pollution, including offshore drilling, fossil fuel combustion, tanker accidents, pipeline leaks, and improper disposal of used oil, causing numerous oil spills, land contamination, and air pollution [3]. Over the past six decades, notable oil spills, such as the Exxon Valdez spill in 1989, the Deepwater Horizon spill in 2010, and the Wakashio spill in 2020, have drawn attention to the devastating consequences of oil pollution and the necessity for enhanced prevention, response, and cleanup efforts [4].

The use of biosurfactants has been proposed as an effective approach to enhance the biodegradation of oil. Biosurfactants, which are natural or microbially produced surface-active agents, can reduce the interfacial tension between water and oil, thereby improving the solubilization and bioavailability of hydrophobic compounds,

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such as oil, to microorganisms. By enhancing the bioavailability of oil, biosurfactants can promote the biodegradation of oil by microorganisms [5, 6]. Biosurfactants are naturally occurring or microbially produced surface-active molecules with the ability to decrease the surface and interfacial tension between immiscible phases, such as water and oil. Various microorganisms, including bacteria, fungi, and yeast, are capable of producing biosurfactants. These compounds possess unique properties, such as low toxicity, biodegradability, and substrate specificity, making them suitable for diverse applications in industries like bioremediation, food, pharmaceuticals, and cosmetics [7, 8].

Among the microorganisms, *Pseudomonas aeruginosa* is well known for its ability to produce a wide range of biosurfactants, which have potential applications in various industries, including oil recovery and soil remediation [9–12]. *P. aeruginosa* is a Gram-negative, rod-shaped bacterium belonging to the family Pseudomonadaceae [13]. In recent years, there has been growing interest in extracting biosurfactants from *P. aeruginosa* inhabiting oil-spilled soils due to their potential use in bioremediating oil-contaminated sites [14, 15]. Several studies have focused on the extraction of biosurfactants from *P. aeruginosa* inhabiting oil-spilled soils, involving steps such as soil microorganism isolation, cultivation, and biosurfactant extraction [16]. Various extraction methods, including solvent extraction, precipitation, and chromatography, have been investigated to isolate and purify biosurfactants. The extraction yield and purity of the biosurfactants can vary significantly depending on the extraction method and the specific strain of *P. aeruginosa* used [17–21]. Rhamnolipids, a class of glycolipid biosurfactants, have been extensively studied, and *P. aeruginosa* is one of the most well-known and widely studied producers of rhamnolipids. These biosurfactants consist of one or two rhamnose (a sugar) molecules linked to one or two β-hydroxy fatty acid molecules through a glycosidic bond. They find numerous applications in industries such as pharmaceuticals, cosmetics, and bioremediation [22–24].

The objective of this study was to investigate the biosurfactant-producing potential of *P. aeruginosa* isolated from oil-spilled soils. The study involved screening soil samples, isolating *P. aeruginosa* strains, and characterizing the biosurfactants produced by the isolated strains.

Methods

Collection of soil samples

Oil-contaminated soil samples, including petrol, diesel, kerosene, etc., were collected from 10 different locations in Chennai, India. The soil was collected from the contaminated area at a depth of 2–3 inches from the ground level using a clean spatula. Subsequently, the soil was transferred to a clean plastic cover and immediately processed without delay.

Processing of samples

The processing of soil samples to isolate and identify microorganisms present in the sample involved the following steps: Five grams of soil were weighed and transferred to a 250 ml conical flask containing 100 ml of distilled water. The flask was then placed on a shaker and agitated at 200 rpm for 48 h to ensure thorough mixing of the soil. From the resulting mixture, 1 ml was taken and serially diluted in distilled water. The pour plate technique was employed using nutrient agar medium, which serves as a solid basal medium supplying the basic carbon, nitrogen, and mineral requirements for organism growth. The plates were subsequently incubated at 37 °C for 24 h and observed for the presence of pigmented colonies. The pigmented colonies were then sub-cultured by transferring a small portion of the colony onto a new agar plate, followed by incubation to obtain a pure culture of the microorganism [25–27]. The samples were further identified through various biochemical tests and cultural characteristics, which aid in determining the type of microorganism present in the sample.

Characterization of biosurfactant-producing bacterial isolates

Gram staining and biochemical tests were conducted on the sub-cultured samples to identify the organisms [28, 29].

Grams staining

To prepare the bacterial culture for examination, a thin layer of the culture was spread on a glass slide. Crystal violet reagent was applied to the slide and allowed to soak in for one minute before being rinsed off with tap water. Next, a few drops of Gram's iodine were added to the slide and left for 30 s before being rinsed off. The slide was then decolorized using 95% ethanol and gently washed again with tap water. Finally, the slide was treated with safranin, washed with water, air-dried, and viewed under an oil immersion objective. Biosurfactant-producing bacterial isolates can be classified as either gram-positive (purple) or gram-negative (pink) depending on the colour of the stain retained.

Motility test

To determine the motility of a bacterial culture, the hanging drop method was employed. First, a uniform layer of Vaseline was applied to the edges of a cover slip. Next, a small drop of the bacterial culture was placed in the centre of the cover slip. An inverted cavity was then placed

over the cover slip with care. The slide was flipped, and the hanging drop was observed for motility under a high-power objective. This test is used to determine if the bacterial isolates are motile or non-motile. Motile bacteria will move away from the drop, while non-motile bacteria will remain in the drop.

Indole test

The Indole test is used to determine if certain bacteria can produce Indole, which is a byproduct of tryptophan metabolism. Tryptophan is found in the peptones of the culture media and is converted by the enzyme tryptophanase into Indole, skatol, and indole acetic acid. Indole reacts with aldehydes to produce a red color, indicating a positive result. To perform the test, bacteria are grown in Tryptophan medium and then tested for the presence of Indole using Kovac's reagent. A loop full of a 24-h culture is inoculated into tryptone broth and incubated for 24–48 h at 37 °C. Then, 0.20 ml of Kovac's reagent is added, and the color change is observed to determine the test result. Biosurfactant-producing bacterial isolates that produce indole will turn the reagent red.

Citrate test

The citrate test is a way to check if certain organisms can use citrate as their sole carbon source for growth. As they grow, they produce acetate and other alkaline carbonates, which cause a change in the colour of the indicator. To perform the test, a loop full of a 24-h culture from nutrient agar is inoculated onto a citrate agar slant and incubated for 24 h at 37 °C. The composition of the agar includes sodium chloride, magnesium sulphate, ammonium dihydrogen phosphate, dipotassium phosphate, sodium citrate, bromothymol blue, agar, and a pH of 6.9. The result of the test is then observed. Biosurfactant-producing bacterial isolates that can utilize citrate will turn the medium from green to blue.

Urease test

The Urease Test is a diagnostic test used to identify the presence of the enzyme urease in a bacterium. To perform the test, a medium is prepared with ingredients such as peptone, dextrose, sodium chloride, monopotassium phosphate, disodium phosphate, phenol red, and agar. The medium is sterilized, and a filter-sterilized 40% urea solution is added once the medium has cooled to 50 °C. A loopful of a 24-h culture from nutrient agar is inoculated in the urea agar slant and incubated for 24 h at 37 °C. The results are then observed. Biosurfactant-producing bacterial isolates that produce urease will turn the medium from yellow to pink.

Triple sugar iron (TSI) test

The Triple Sugar Iron (TSI) agar test is used to determine if an organism can utilize glucose, lactose, and sucrose sugars. The test medium contains beef extract, yeast extract, peptone, sodium chloride, glucose, lactose, sucrose, ferrous ammonium sulphate, sodium thiosulfate, phenol red, and agar. Phenol red acts as an acid–base indicator, changing the colour of the medium from orange-red to yellow in the presence of acids produced during carbohydrate fermentation. Sodium thiosulfate is used as a substrate for hydrogen sulphide production, and ferrous sulphate is used to detect this colourless end product. Only cultures of organisms producing hydrogen sulphide will cause extensive blackening in the butt due to the precipitation of the insoluble ferrous sulphide. To perform the TSI test, a loopful of a 24-h culture from nutrient agar is taken and stabbed into the centre of the TSI agar butt, then streaked on the surface of the slant. The medium is then incubated for 24 h at 37 °C, and the results are observed. Biosurfactant-producing bacterial isolates that ferment glucose, lactose, and/or sucrose will produce an acid that turns the medium yellow. If the bacteria produce hydrogen sulphide, it will react with the thiosulfate to produce a black precipitate in the medium.

Pigment production test

To analyse the characteristics of a bacterial culture, a streaking method was used to transfer the culture onto nutrient agar plates. The plates were then observed to identify the colony morphology, which refers to the shape, size, colour, and texture of the colonies. Additionally, the plates were examined for pigment production, which can be an indicator of certain bacterial species or metabolic pathways.

Storage of cultures

The culture was sub-cultured twice a week and stored in the refrigerator.

Screening for biosurfactants

Screening for biosurfactants was carried out for the isolates that produced a bluish-green pigment in the subculture and were identified as *P. aeruginosa*. The screening involved various methodologies, which are as follows: [30–32].

A Antagonistic test
B Hemolytic test
C Emulsifying test

Production of biosurfactant

To produce biosurfactant, fermentation was used with a mineral salt medium (MSM). The culture sample was inoculated in the medium and placed in a shaker at 200 rpm for 48 h to ensure complete aeration. After fermentation, the broth medium was subjected to tests to determine biosurfactant production. The MSM was composed of dipotassium hydrogen phosphate, potassium dihydrogen phosphate, magnesium sulphate, ammonium sulphate, and glycerol. The amounts of these components were 4.8 g/L, 1.5 g/L, 0.5 g/L, 1.0 g/L, and 2 mL/L, respectively, with a pH of 7.2.

Antagonistic test

To test the antimicrobial activity of the biosurfactant produced in the medium, the antagonistic test was performed using *Bacillus subtilis*, *Staphylococcus aureus*, *Aeromonas hydrophila*, and Escherichia coli. The purpose of this test is to determine the potential toxicity of the biosurfactant-spent medium. The method involves taking 10–20 mL of the medium and centrifuging it at 10,000 rpm for 10 min. The resulting cell-free supernatant is inoculated as 20 µl onto the wells made in the freshly grown cultures of the above-mentioned bacteria on Muller Hinton agar plates. The plates are then incubated at 37 °C for 24 h, and the zones of inhibition are measured.

Hemolytic assay

The hemolytic test is a method used to determine if bacteria can produce biosurfactants that can break down red blood cells. This is done by streaking the bacterial isolates on a fresh agar plate containing human blood and incubating the plate at 37 °C for 24 h. The presence of a beta-hemolytic band around the bacterial colonies indicates that the bacteria are capable of producing biosurfactants that can lyse the erythrocytes. After 24 h, the results are observed and interpreted.

Emulsification test

The emulsification test is a method used to determine if biosurfactants have the ability to mix with and disperse hydrocarbons like petrol, kerosene, and diesel. To carry out this test, a series of clean test tubes are taken and filled with a mixture of kerosene and water in a 1:4 volume ratio. To each tube, 1 mL of a fermentation medium is added along with a control (Tween 80). The setup is left undisturbed for 24 h, and the height of the emulsified layer is measured and used to calculate the emulsification index (E24). The E24 is determined by dividing the height of the emulsified layer by the total height of the hydrocarbon in the test tube and multiplying the result by 10.

Table 1 Shows the samples collected from different areas

S.no	Sample name	Area of collection
1	PS01	Mechanic shed (Petrol) West Mambalam
2	PS02	Ration shop (kerosene) Pushpa Nagar
3	PS03	Petrol filling station (Petrol) Ellango Nagar
4	PS04	Mechanic shed (Petrol) Kumaran Colony
5	PS05	Diesel filling station (Diesel) Nungampakam
6	PS06	Ration shop (kerosene) Trust Puram
7	PS07	Vadapalani Bus Depot (Diesel)
8	PS08	Automobile workshop (Diesel) Guindy
9	PS09	Petrol filling station (Petrol) Chetpet
10	PS10	Mechanic shed (Petrol) Anna Nagar

Table 2 Number of total viable bacteria from each of the soil sample *Pseudomonas aeruginosa* strains

S.no	Sample number	Average number of bacteria present
1	PSO3	2.6×10^6
2	PSO4	3.7×10^6
3	PS08	4.4×10^6
4	PS010	4.1×10^5
Non *pseudomonas aeruginosa* strains		
5	PS01	4.0×10^6
6	PS02	2.9×10^6
7	PS05	3.9×10^6
8	PS06	4.2×10^6
9	PS07	4.1×10^5
10	PS09	2.8×10^6

Results

For the present study on the "screening and extraction of *P. aeruginosa* from oil-contaminated soils," 10 oil-contaminated soil samples were collected from different areas, including petrol, diesel, and kerosene (Table 1).

Total viable populations were counted and recorded from the 10 oil-contaminated soil samples (Table 2). The viable cell counts ranged from 4.1×105 to 4.4×106 CFU/gm of soil. These results are consistent with the findings of Bodour, Navarro-Noya, and Li [33–35].

Among the 10 samples, all the soil samples produced bluish green-colored colonies. Out of these, only 4 samples were found to be positive for *P. aeruginosa* based on the following cultural characteristics. *P. aeruginosa* is a type of bacteria that produces different coloured pigments on nutrient agar, including the well-known pyocyanin and fluorescin. Pyocyanin is unique to this specific type of bacteria and has the ability to inhibit the growth of other bacteria, making *P. aeruginosa* dominant

Fig. 1 Colonies of nutrient agar

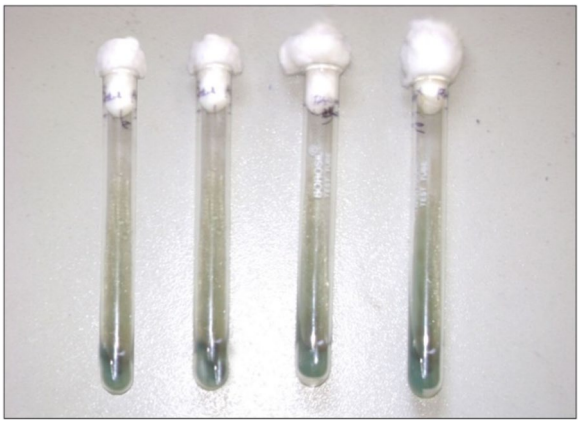
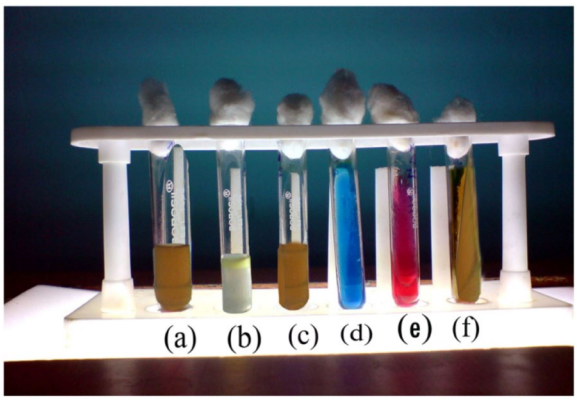


Fig. 2 Sub cultures



(a) Methyl Red (b) Indole

(c) Voges Proskauer (d) Citrate

(e) Triple Sugar Iron (f) Urease

Fig. 3 Biochemical tests

in mixed populations. On nutrient agar, colonies created by this bacteria are large, opaque, and irregular in shape, with a distinct musty, earthy odour. They also display an iridescent metallic sheen, as shown in Fig. 1. These colonies were sub-cultured for various experiments (Fig. 2). The isolated cultures were confirmed using gram staining, motility tests, and biochemical characteristic features (Fig. 3). The isolates were compared with the standard strain of *P. aeruginosa* (ATCC 9027), as shown in Table 3.

After the fermentation period the medium (Fig. 4) was used for the following purposes.

Antagonist test

An experiment was conducted to assess the toxicity of a fermented medium containing rhamnolipid produced by *P. aeruginosa*. The fermented medium was tested for its ability to inhibit the growth of various microorganisms,

Table 3 Showing the gram staining, motility and biochemical test results of 4 isolates compared with the standard strain

S.no	Test	Standard strain (atcc 9027)	Isolated sample
1	Gram staining	GNR	GNR
2	Motility	MR	MR
3	Indole	(–)	(–)
4	Citrate utilization	(+)	(+)
5	Urease	(–)	(–)
6	TSI	K/K	K/K

GNR: Gram negative rods, MR : Motile rods, (+) : Positive, (-) : Negative, K/K: Alkaline slant/Alkaline butt

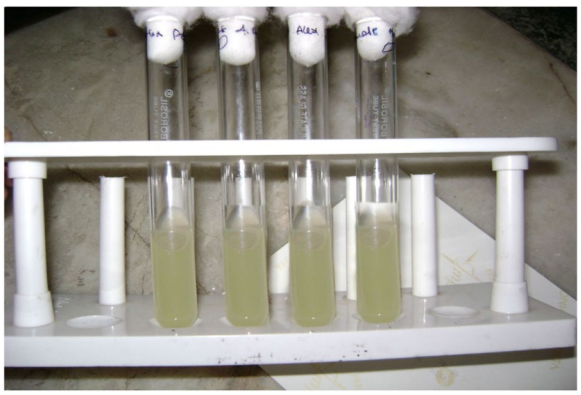


Fig. 4 Fermentation medium

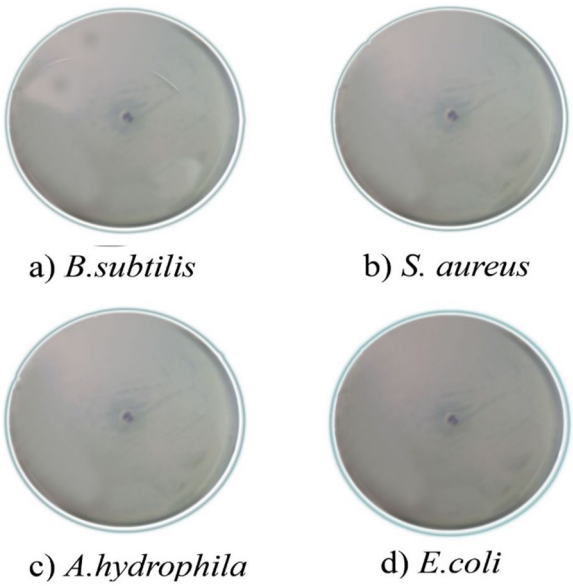


Fig. 5 Antagonist test

such as *B. subtilis*, *S. aureus*, *E. coli*, and *A. hydrophila*. The results of the experiment showed that the fermented medium had a toxic effect and was able to inhibit the growth of the tested microorganisms (Fig. 5).

Haemolytic test

To demonstrate the hemolytic activity on blood agar, beta hemolysis surrounding the bacterial colonies was observed in all 4 samples (Fig. 6).

Emulsification test

The isolated samples were tested for their emulsifying activity with petrol, kerosene, and diesel. It was found that the E24 value was highest, at 70% with petrol, 62.5% with kerosene, and 65% with diesel in the culture samples

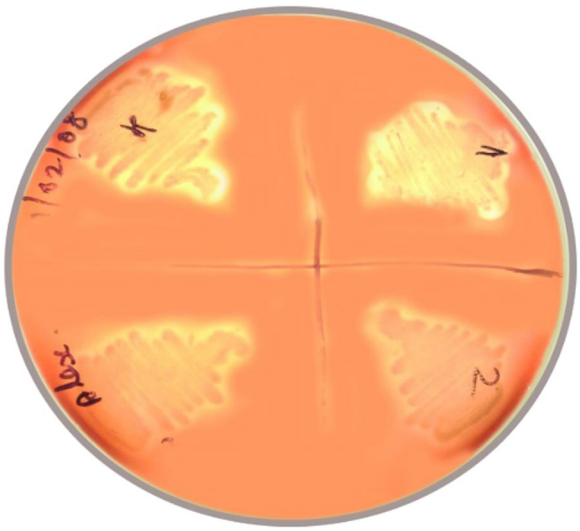


Fig. 6 Haemolytic activity

Table 4 Showing emulsification index E_{24} of the samples with kerosene, petrol and diesel

S.no	Sample	Emulsification index e_{24}		
		Kerosene	Petrol	Diesel
1	Tween 80	90	100	95
2	PS03	24	42.8	38
3	PS04	30	50	40
4	PS08	22.2	35	25
5	PS10	62.5	70	65

PS10, and lowest in the sample PS08, with 35% emulsification with petrol, 22.2% with kerosene, and 25% with diesel (Table 4).

Out of the ten soil samples, four samples were confirmed to be *P. aeruginosa*. All strains showed positive results for antagonistic tests, haemolytic assays, and emulsification tests. In general, all strains were effective against *B. subtilis*, *S. aureus*, and *E. coli*, but were less effective against *A. hydrophila*. The PS10 strain showed the highest emulsification index, while the PS08 strain showed the lowest emulsification index. Based on microscopic and biochemical examination, *P. aeruginosa* was determined to be the most effective isolate.

Discussion

The screening of biosurfactant-producing microbes from environmental sources, especially hydrocarbon-contaminated locations, has gained significant attention due to their potential applications in various industries and environmental remediation. The extraction of biosurfactant from *P. aeruginosa* requires a two-step process,

including the cultivation of the bacteria and the extraction of the biosurfactant. The cultivation of *P. aeruginosa* is typically done in a nutrient-rich medium that allows the bacteria to produce and secrete the biosurfactant. After cultivation, the biosurfactant can be extracted using various methods, including solvent extraction, precipitation, and centrifugation.

Several studies have reported the successful isolation of *P. aeruginosa* from oil-spilled soils and their subsequent use in the production of biosurfactants. For instance, a study conducted by Das and Mukherjee [36] reported the isolation of *P. aeruginosa* from crude oil-contaminated soil and demonstrated the production of a biosurfactant with excellent emulsification activity. Furthermore, another study by El-Sheshtawy [37] reported the isolation of *P. aeruginosa* from oil-polluted soil samples and demonstrated the potential application of its biosurfactant in the bioremediation of oil-polluted sites. A study conducted by Singh [38] demonstrated the extraction of biosurfactant from *P. aeruginosa* inhabiting oil-spilled soils. The researchers used a nutrient-rich medium containing peptone, yeast extract, and glucose to culture *P. aeruginosa*. The bacteria were grown for five days at 30 °C, and the biosurfactant was extracted using a solvent extraction method with chloroform and methanol. Another study by Gupta [39] used a similar method for the extraction of biosurfactant from *P. aeruginosa*. The researchers used a nutrient-rich medium containing tryptone, yeast extract, and glucose to culture the bacteria, which were grown for seven days at 30 °C. The biosurfactant was extracted using a precipitation method with ammonium sulfate. *P. aeruginosa* is a well-known bacterial species that has been widely studied for its ability to produce biosurfactants and degrade hydrocarbons in oil-contaminated environments [40]. In the study, we screened ten soil samples for biosurfactant-producing bacteria, and four were confirmed to be *P. aeruginosa*. The strains exhibited positive results for antagonistic, haemolytic, and emulsification tests, indicating their ability to produce biosurfactants. The results of the study are consistent with previous research demonstrating the potential of *P. aeruginosa* as a biosurfactant producer. *P. aeruginosa* isolated from oil-contaminated soil samples was found to produce biosurfactants with excellent emulsification and surface tension reduction properties. Another study investigated the antimicrobial activity of *P. aeruginosa* strains isolated from soil and found that all strains showed antimicrobial activity against at least one of the tested microbial species [41]. This is consistent with the findings of this study, where all *P. aeruginosa* strains were effective against *B. subtilis*, *S. aureus*, and *E. coli*. Similarly, a study by Banat [16] reported that *P. aeruginosa* was able to produce a biosurfactant with strong emulsification and foaming properties.

The effectiveness of *P. aeruginosa* strains against different bacterial species is also noteworthy. The strains were effective against *B. subtilis*, *S. aureus*, and *E. coli*, which are commonly found in soil environments, but less effective towards *A. hydrophila*, which is an aquatic bacterium [42]. These results suggest that *P. aeruginosa* strains may be more effective in soil-based bioremediation applications compared to aquatic environments. The fact that *P. aeruginosa* strains were less effective towards *A. hydrophila* is consistent with previous studies that have shown that the effectiveness of biosurfactants produced by *P. aeruginosa* strains can vary depending on the target microbe [43]. The emulsification test used in this study is a common method for screening biosurfactant-producing microbes. Several studies have used this method to isolate and identify biosurfactant-producing microbes from various sources, including soil contaminated with petroleum hydrocarbons [44, 45].

Overall, the results of this study suggest that *P. aeruginosa* strains isolated from oil-contaminated soil samples have the potential to be used as biosurfactant producers for various industrial and environmental applications. However, further studies are needed to fully understand the biosurfactant production potential of these strains and to optimize the conditions for biosurfactant production.

Conclusion

This study emphasizes the significance of screening biosurfactant-producing microbes from hydrocarbon-contaminated locations, given their adaptive mechanisms for biosurfactant production. Specifically, our investigation focused on isolating biosurfactant-producing bacteria from oil-contaminated soil samples, with an emphasis on *P. aeruginosa*. The results revealed that all *P. aeruginosa* strains isolated from the soil samples exhibited positive results in antagonistic, haemolytic, and emulsification tests, with strain PS10 demonstrating the highest emulsification index. These findings underscore the potential of *P. aeruginosa* as a biosurfactant producer for various industrial and environmental applications.

Recommendations for future studies

P. aeruginosa is a well-known bacterium in the scientific community and has been widely studied for its potential in bioremediation applications. Future studies should focus on exploring and optimizing the conditions for biosurfactant production by *P. aeruginosa* strains. This includes investigating the effects of different carbon sources, nutrient concentrations, pH levels, and temperature on biosurfactant production. By optimizing these conditions, we can maximize biosurfactant yields and enhance the efficiency of production

processes. To gain a deeper understanding of biosurfactant synthesis pathways in *P. aeruginosa*, future studies should employ gene expression analysis techniques. By examining the expression patterns of genes involved in biosurfactant production, we can elucidate the regulatory mechanisms and identify key genes responsible for biosurfactant synthesis. This knowledge can guide further optimization strategies and potentially lead to genetic engineering approaches for enhanced biosurfactant production. It is crucial to evaluate the industrial applications of *P. aeruginosa*-derived biosurfactants. Future studies should investigate their effectiveness in various applications, such as enhanced oil recovery, environmental remediation, and as green alternatives to chemical surfactants. Conducting comparative studies with existing commercial surfactants can provide insights into the advantages and limitations of *P. aeruginosa* biosurfactants, paving the way for their potential commercialization. Given the potential of *P. aeruginosa* biosurfactants, it is essential to explore their effectiveness in the bioremediation of oil spills. Future studies should assess their ability to solubilize and degrade hydrocarbon pollutants, enhance microbial degradation rates, and promote the removal of oil from contaminated environments. This research can contribute to the development of efficient and sustainable bioremediation strategies. The study highlights the importance of exploring microbial diversity in contaminated environments for the discovery of new biosurfactant-producing bacteria. Future studies should continue to investigate diverse habitats and target different oil-contaminated environments to identify novel bacterial strains capable of producing biosurfactants. This approach can expand our understanding of microbial ecology, promote biodiversity conservation, and uncover unique biosurfactant-producing microbes.

Abbreviation

P. aeruginosa	*Pseudomonas aeruginosa*
B. subtilis	*Bacillus subtilis*
S. aureus	*Staphylococcus aureus*
E. coli	*Escherichia coli*
A. hydrophila	Aeromonas hydrophila
CFU	Colony forming unit
TSI	The Triple Sugar Iron
MSM	Mineral salt medium

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The idea for this article was conceived and worked out by AY and VJ who also wrote the manuscript. The data analysis was carried out by both of them. All the authors read and approved the manuscript for final communication.

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Data and materials have been provided with the manuscript as tables and figures. The datasets are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable for this work.

Consent for publication
All authors have approved the publication of this study.

Competing interests
All authors hereby declare that there is no conflict of interest.

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References

1. National Oceanic and Atmospheric Administration (2021) Oil spills. Retrieved from https://oceanservice.noaa.gov/facts/oil-spills.html
2. United Nations Environment Programme (2021) Oil spills. Retrieved from https://www.unep.org/explore-topics/oceans-seas/what-we-do/addressing-ocean-pollution/oil-spills
3. Environmental Protection Agency (2021) Oil spills prevention and preparedness regulations. Retrieved from https://www.epa.gov/oil-spills-prevention-and-preparedness-regulations
4. The Guardian (2020) Mauritius declares state of environmental emergency after oil spill from grounded ship. Retrieved from https://www.theguardian.com/world/2020/aug/08/mauritius-declares-state-of-environmental-emergency-after-oil-spill-from-grounded-ship
5. Prasad S, Manjunath BR (2017) Biosurfactants and their applications. In: Green chemistry for environmental sustainability, pp 39–56. Springer, Cham
6. Urum K, Pekdemir T (2004) Evaluation of biosurfactants for crude oil contaminated soil washing. Chemosphere 57(9):1139–1150. https://doi.org/10.1016/j.chemosphere.2004.07.023
7. Perfumo A, Smyth T J, Marchant R, Banat IM (2018) Production and roles of biosurfactants and bioemulsifiers in accessing hydrophobic substrates. In: Handbook of hydrocarbon and lipid microbiology (pp. 1–27). Springer, Cham
8. Vatsa P, Sanchez L, Clement C, Baillieul F, Dorey S, Riollet F (2010) Identification of a gene required for the biosynthesis of a lipopeptide surfactant produced by Bacillus subtilis. J Bacteriol 192(17):4529–4537. https://doi.org/10.1128/JB.00291-10
9. Cameotra SS, Singh P (2008) Bioremediation of oil-effluent contaminated soil slurry by a bacterial consortium amended with poultry litter: a laboratory study. J Environ Manage 86(3):489–496. https://doi.org/10.1016/j.jenvman.2006.12.019
10. Das P, Yang X P, & Ma L Z (2014) Microbial biosurfactants and their environmental and industrial applications. In: Microbial biosurfactants, pp 13–34. Elsevier. https://doi.org/10.1016/B978-0-12-408043-5.00002-8
11. Chen J, Song X, Zhang H, Lu X (2019) Progress and prospects of biosurfactant-enhanced oil recovery technology. J Pet Sci Eng 181:106240. https://doi.org/10.1016/j.petrol.2019.106240
12. Saikia R, Deka S, Deka M (2019) An overview of biosurfactants and their applications in enhancing oil recovery. J Petrol Sci Eng 173:682–694. https://doi.org/10.1016/j.petrol.2018.09.015
13. Palleroni NJ (2010) Family I Pseudomonadaceae. In: Bergey's manual of systematic bacteriology, 2nd ed., Vol. 2, pp. 323–442. Springer
14. Wang W, Gao S, Li Q, Wang S, Ma F (2019) Bio-surfactant production by Pseudomonas aeruginosa isolated from oil-spilled soil and its potential

application in enhanced oil recovery. Front Microbiol 10:2627. https://doi.org/10.3389/fmicb.2019.02627
15. Zeng G, Liu Y, Wang R, Song B, Liu Y, Zhou M (2016) Biosurfactant-enhanced phytoremediation of petroleum-contaminated soil using Suaeda heterophylla. Ecol Eng 91:356–362. https://doi.org/10.1016/j.ecoleng.2016.02.013
16. Banat IM, Satpute SK, Cameotra SS, Patil R, Nyayanit NV (2014) Cost-effective technologies and renewable substrates for biosurfactants' production. Front Microbiol 5:697. https://doi.org/10.3389/fmicb.2014.00697
17. Al-Tahhan RA, Sandrin TR, Bodour AA, Maier RM (2000) Rhamnolipid-induced removal of lipopolysaccharide from Pseudomonas aeruginosa: effect on cell surface properties and interaction with hydrophobic substrates. Appl Environ Microbiol 66(8):3262–3268. https://doi.org/10.1128/AEM.66.8.3262-3268.2000
18. Gudina EJ, Rodrigues AI, Alves E, Domingues MR, Teixeira JA (2010) Batch and fed-batch production of biosurfactant by Lactobacillus paracasei ssp. paracasei A20 in a micro-aerated bioreactor. Biotechnol Progress 26(1):124–130. https://doi.org/10.1002/btpr.320
19. Liu J, Wang X, Sun Y, Li Y, Zhang Y (2016) Screening and optimization of biosurfactant production by Pseudomonas aeruginosa HYS in fermentation process. Colloids Surf, B 146:8–14. https://doi.org/10.1016/j.colsurfb.2016.05.036
20. Sharma D, Saharan BS, Yadav RK (2015) Biodegradation of diesel by Pseudomonas aeruginosa NCIM 5514 and optimization of biosurfactant production using Box-Behnken design. Environ Sci Pollut Res 22(7):5193–5205. https://doi.org/10.1007/s11356-014-3891-8
21. Varjani SJ, Upasani VN (2016) Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactant. Bioresour Technol 215:51–62. https://doi.org/10.1016/j.biortech.2016.04.053
22. Abdel-Mawgoud AM, Lépine F, Déziel E (2010) Rhamnolipids: diversity of structures, microbial origins and roles. Appl Microbiol Biotechnol 86(5):1323–1336. https://doi.org/10.1007/s00253-010-2498-2
23. Singh P, Cameotra SS, Chopra HK (2010) Biosurfactants from Pseudomonas aeruginosa: structural characterization and their efficacy in reducing the surface tension of hydrocarbons. J Petrol Sci Eng 71(1–2):23–30. https://doi.org/10.1016/j.petrol.2009.11.002
24. Chen YG, Chen XY, Jiang X, Zhang YX, Li SP (2018) Advances in the microbial production of rhamnolipids for industrial applications. Appl Microbiol Biotechnol 102(17):7369–7382. https://doi.org/10.1007/s00253-018-9160-3
25. Atlas RM, Bartha R (2018) Microbial ecology: fundamentals and applications. Pearson Education.
26. Bailey MJ, Lilley AK, Timms-Wilson TM (2018) Microbial ecology of soils. Biol Environ Proc R Irish Acad 118B(1):1–10. https://doi.org/10.3318/bioe.2018.01
27. Beveridge TJ, Breznak JA (2018) Methods for general and molecular microbiology. American Society for Microbiology Press, Washington, DC
28. Mnif I, Ghribi D, Mnif S (2015) Screening and characterization of biosurfactant-producing bacteria isolated from wastewaters in Tunisia. J Basic Microbiol 55(6):738–748. https://doi.org/10.1002/jobm.201400658
29. Das P, Mukherjee S, Sen R (2008) Antimicrobial potential of a lipopeptide biosurfactant derived from a marine Bacillus circulans. J Appl Microbiol 104(6):1675–1682. https://doi.org/10.1111/j.1365-2672.2007.03686.x
30. Kosaric N, Fontana FV, Roger R (1991) Biosurfactants: research trends and applications. CRC Press
31. Sivaraj R, Aravindhan SP, Sivaramakrishnan S (2012) Screening methods for the isolation of biosurfactant producing microorganisms: a review. Process Biochem 47(2):165–176. https://doi.org/10.1016/j.procbio.2011.11.008
32. Jatinder S, Anita P (2015) A review on screening, detection and characterization of biosurfactant producing microorganisms. Int J Environ Res Dev 5(1), 1–16. https://doi.org/10.24294/ijerd.v5i1.204
33. Bodour AA, Drees KP, Maier RM, Jeffries TW (2003) Isolation and characterization of a novel Aspergillus nidulans mutant deficient in poly(r-3-hydroxybutyric acid) synthesis. FEMS Microbiol Lett 218(1):39–45. https://doi.org/10.1111/j.1574-6968.2003.tb11535.x
34. Navarro-Noya YE, Hernández-Rodríguez CS, Morillo-Sánchez R, Zenteno-Savín T (2014) Comparison of three bacterial enumeration methods in a soil contaminated with crude oil. Int J Environ Res Public Health 11(7):7393–7403. https://doi.org/10.3390/ijerph110707393
35. Li L, Li Q, Li H, Chen Y, Wang J (2015) Enumeration of petroleum hydrocarbon-degrading bacteria in soil: comparison of MPN and CFU methods. Annals of Microbiology 65(2):715–721. https://doi.org/10.1007/s13213-014-0925-6
36. Das P, Mukherjee S (2019) Isolation and characterization of Pseudomonas aeruginosa from crude oil-contaminated soil and assessment of its biosurfactant production potential. J Basic Microbiol 59(2):143–152. https://doi.org/10.1002/jobm.201800457
37. El-Sheshtawy HS, El-Kady MY, El-Moghazy AY, Mostafa YS (2020) Potential application of a bio-surfactant produced by Pseudomonas aeruginosa in the bioremediation of oil-polluted sites. Environ Sci Pollut Res 27(23):29423–29435. https://doi.org/10.1007/s11356-020-09031-4
38. Singh P, Cameotra SS, Chopra HK (2018) Extraction and characterization of biosurfactabnt produced by Pseudomonas aeruginosa PN1 and its application in emulsification of crude oil. Colloids Surf, B 170:530–540. https://doi.org/10.1016/j.colsurfb.2018.06.051
39. Gupta R, Sharma R, Beg QK, Lorenz P (2016) An optimized method for the extraction of biosurfactant from Pseudomonas aeruginosa strain HBP1 using respons surface methodology. Front Microbiol 7:1226. https://doi.org/10.3389/fmicb.2016.01226
40. Sriram MI, Gayathri S, Banat IM (2011) Studies on the production of halophilic biosurfactant by α-proteobacterium, Kocuria marina MBRS10. J Chem Technol Biotechnol 86(9):1193–1201. https://doi.org/10.1002/jctb.2625
41. Bodour AA, Guerrero-Barajas C, Jiorle BV, Malati MA, Elbahloul YM (2003) Pseudomonas aeruginosa isolated from soil in the vicinity of Jeddah Refinery, Saudi Arabia, showed a diversity of quorum sensing signal molecules. EcoSal Plus 1(1):1–14. https://doi.org/10.1128/ecosalplus.6.2.1.2.9
42. Papadopoulou M, Voidarou C, Vavias S, Bezirtzoglou E, Alexopoulos A, Tsigarida E, Eotzekidou P (2014) Antimicrobial activity of Pseudomonas aeruginosa strains isolated from different environmental and clinical sources against bacterial fish pathogens. Braz J Microbiol 45(2):613–620. https://doi.org/10.1590/S1517-83822014000200039
43. Gudina EJ, Rocha V, Teixeira JA, Rodrigues LR (2016) Antimicrobial and anti-adhesive activities of cell-bound biosurfactant from Pseudomonas aeruginosa PA1. RSC Adv 6(67):62986–62995. https://doi.org/10.1039/C6RA10964F
44. Kiran GS, Hema TA, Gandhimathi R, Selvin J, Thomas TA, Ravji TR (2010) Optimization and production of a biosurfactant from the sponge-associated marine fungus Aspergillus ustus MSF3. Colloids Surf, B 79(2):239–248. https://doi.org/10.1016/j.colsurfb.2010.03.008
45. Youssef NH, Duncan KE, Nagle DP, Savage KN, Knapp RM, McInerney MJ (2017) Comparison of methods to detect biosurfactant production by diverse microorganisms. J Microbiol Methods 139:99–104. https://doi.org/10.1016/j.mimet.2017.06.013

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