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ISOLATION AND CHARACTERIZATION OF BIOSURFACTANT PRODUCING BACTERIA FROM GARAGE SOIL

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Abstract

The increasing demand for eco-friendly and sustainable solutions has led to a growing interest in bio-surfactants due to their biodegradability and low toxicity. This study focuses on the isolation and characterization of bio-surfactant producing bacteria from garage soil. Soil samples were collected from various garages and screened for bio-surfactant production using haemolytic activity and oil displacement tests. Positive isolates were further characterized through biochemical assays. The results revealed the presence of several potent bio-surfactant producing strains, predominantly belonging to the Staphylococcus. These strains exhibited significant emulsification activity and surface tension reduction, highlighting their potential applications in bioremediation and industrial processes. This study underscores the importance of garage soil as a reservoir of bio-surfactant producing bacteria and provides a foundation for future research on their industrial exploitation.

INTRODUCTION

The increased use of petroleum & oil as a main source of energy has increased risk of leakage of these products while transportation & causes soil and water contamination. This is harmful to Human beings, Plants, Animals & Microbes. Crude oil components are toxic to environment, so there should be an effective method for removal of these toxic compounds to save the environment. The traditional treatments used for removal of these toxic compounds are not so effective, whereas biological degradation of these compounds using micro-organisms has been more suitable method. It has an advantage over physiological treatment methods in removing contaminants. Biological methods offer in-situ biodegradation of petroleum hydrocarbon by the micro-organisms (Mishra et al., 2021; Parviz et al., 2011).

Biosurfactants are surface active substances with both hydrophilic & hydrophobic characteristics. These properties allow them to exist either interface between polar and non- polar media. Hydrocarbon compounds which are present at the surface of water have very low solubility which unable access of these compounds for degradation. So, Bacteria produce biosurfactant which reduces surface tension & increases the bioavailability these compounds. (Sharma et al., 2020). They are produced extra-cellularly or as a componant of cell membrane. It is made from a range of materials, including sugars, oil, and wastes, by filamentous fungi, bacteria, and yeast. (Bhatt et al., 2023).

Compared to chemically synthesized surfactants, biosurfactants are highly preferred for their low toxicity, environmental compatibility, biodegradability, and efficacy under extreme conditions (Das et al., 2022).

Biosurfactants have many industrial and environmental applications. The environmental fields that use biosurfactants are increased oil recovery, crude oil drilling, and bioremediation of contaminants. Biosurfactants are used in the industrial fields for foaming, detergency, wetting, dispersion, and solubilisation. They are also useful in cosmetics, health care & food processing industries. (Patel et al., 2021).

Materials and Methods

Collection of Samples

Samples of soil were collected from several location of garage areas, particularly those contaminated with oil or grease from Wathar, Kolhapur

Enrichment and Isolation of Biosurfactant Producing Microorganisms

The enrichment media consisted of mineral salt medium (MSM) in g/L: KH_2PO_4, $MgSO_4$, $FeSO_4$, $NaNO_3$, $CaCl_2$, $(NH_4)_2SO_4$ and kerosene added act as hydrocarbon source pH was maintained at 7.4 ±0.2, that was sterilized by autoclave at 121 °C for 15 min, then 1gm of soil sample were inoculated to sterile mineral salt medium (MSM) broth and then incubated at 30°C with shaking incubator at 150 rpm for 7 days.

After, then the inoculate MSM broth taken from that 1 ml of sample were serially diluted up to 10^{-6} dilution, 0.1 ml of sample

were spread on MSM agar plates, the plates are incubated for 72 hours, Distinct isolated colonies were taken for morphological characters for further studies, the distinct isolated re-streaked on MSM agar medium for further use.

Screening of Biosurfactant Producing Bacteria

Following methods of confirmation were used to screen the biosurfactant-producing bacteria from the isolated colonies:

Oil Displacement Method

The oil displacement test was also carried out as described by Ghaleb et al., (2023). This procedure involved filling an empty Petri dish with 40 millilitres of distilled water. Add 10 µl crude oil in petri dish containing distilled water thin layer formation, after that add 10 µl Supernatant cultured gently the displacement of indication of oil shows that the biosurfactant producing bacteria (Shinde et al., 2023).

Drops Collapse Assay

After carefully placing a drop of the culture supernatant onto a crude oil drop that had been placed on a slide, the slide was observed after 1 minute If the drop of supernatant collapses and dispersion across the slide, it indicates the presence of biosurfactant (Aher et al., 2022).

Foaming Activity

Isolates were inoculated into 250 ml of sterile Nutrient broth, then the broth is incubated at 30°C Shaker incubator at 110rpm for 72hrs, Foam formation activity were observed in broth, this indication shows that the production of biosurfactant in absence of hydrophobic substrate. (Rani et al., 2022; Pradeep et al., 2022)

Blood Hemolysis Test

24 hrs old bacterial culture were spot inoculated on Blood agar plate, clear zone (Hemolysis) of transparent yellow colour shows the hemolysis of Blood cell and absence of clear zone indicate no hemolysis of Blood cell (Kumar et al., 2022).

Emulsification Assay

Emulsifying activity is measured by Emulsifying index (E_{24}) 2ml of kerosene oil is added in tube which contain 1ml of supernatant that was carried after centrifugation. Then vortex the two liquid mixture for 2 min. after the 24hrs Emulsifying activity was observed and measured as (E_{24}) calculated by formula Zhang et al., as follows;

E_{24} = (Height of the emulsification layer/total height of mixture) × 100%

Phenol Sulfuric acid method

Selected Screened biosurfactant producing strains are inoculated in MSM broth and broth were incubated for five days, following incubation, sample were taken for centrifugation at 3000 rpm for 20 minutes, after which the supernatant was collected while pellet discarded, further supernatant added to 5% phenol and added with 5 ml of sulphuric acid dropwise resulting in a colour change from yellow to orange, which confirms the production of biosurfactants (Patel et al., 2022).

Extraction of Biosurfactant

Chloroform and Methanol extraction

50 ml cell free broth were taken the pH of broth is adjust to 2 pH by using 1M H2SO4, then equals volume of chloroform: Methanol (2:1) was added this cell free broth then mixture was shaken well and left overnight at 4 °C. White coloured sediments were obtained is biosurfactant (Zodpe et al., 2016).

Results and Discussion

Isolation of Bacteria

The isolation and characterization of bacterial strains from garage soil are crucial steps in understanding their potential applications and behaviours. In this study, two distinct bacterial strains were isolated (IS-I and IS-II) and re-streaking on nutrient agar plates for successful purification.

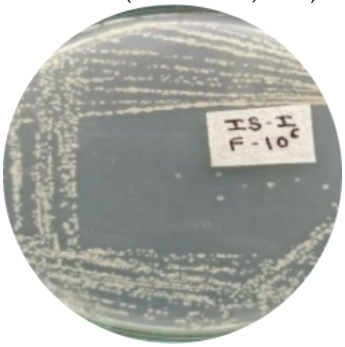

Fig.1: IS-I

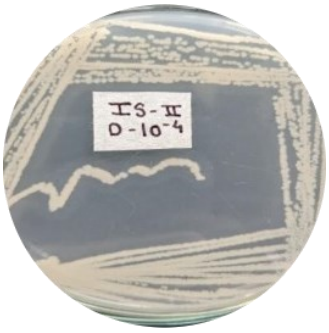

Fig.2: IS-II

Characterization of Isolated Bacteria

The colony morphology of the isolates provides initial insights into their identity and potential classification. Isolate IS-I, with its creamy white, circular, and sticky colonies, and isolate IS-II, with its off-white, circular, and moist colonies, suggest differences in their extracellular matrix production and possibly their environmental adaptations. The Gram staining results indicate that both isolates are Gram-positive cocci. The non-motile nature of these isolates suggests they may rely on passive mechanisms for dispersal. The catalase-positive result indicates the presence of the enzyme catalase. The mannitol-negative result suggests that these isolates do not ferment mannitol, which can be a distinguishing feature among different bacterial species. Based on these characteristics, the isolates are tentatively identified as *Staphylococcus* spp.

Table-1: Colony characters

Isolate No.	Size(nm)	Shape	Color	Margin	Elevation	Opacity	Consistency
IS-I	1	circular	creamy white	entire	raised	opaque	sticky
IS-II	2	circular	off white	Irregular	raised	opaque	moist

Screening of Biosurfactant Producing Bacteria

The screening and extraction of biosurfactant-producing bacteria are essential steps in identifying and utilizing microbial strains for biotechnological applications. In this study, four bacterial strains were screened with two strains (IS-I and IS-II) showing promising results for biosurfactant production.

Oil Displacement Method

The oil displacement method is a reliable assay for detecting biosurfactant production. The positive results observed for strains IS-I and IS-II indicate their potential as biosurfactant producers. The ability to spread oil droplets suggests that these isolates can reduce surface tension and emulsify hydrophobic compounds, which is a key characteristic of biosurfactants.

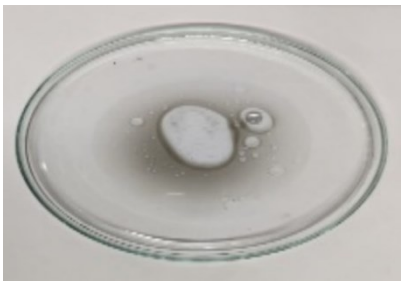

Fig-3: Oil displacement activity of IS-I

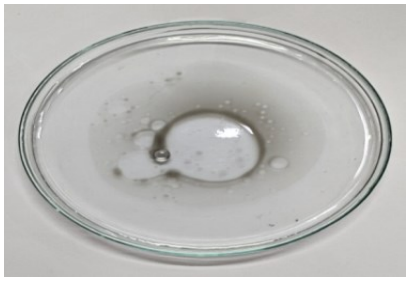

Fig-4: Oil displacement activity of IS-II

Drops collapse assay

The drops collapse assay further confirmed the presence of biosurfactants in the culture supernatants of IS-I and IS-II. The collapse and spreading of drops on crude oil indicate the effectiveness of the biosurfactants produced by these isolates.

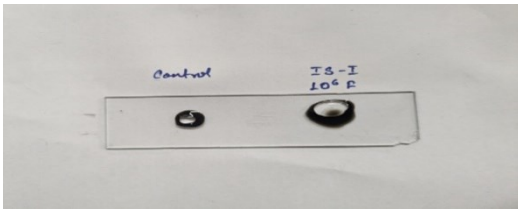

Fig-5: drop collapse assay of IS-I

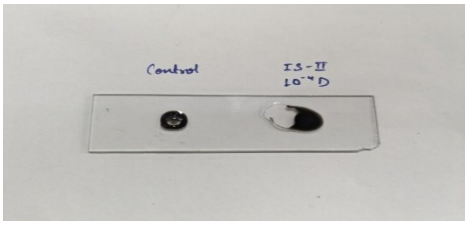

Fig-6: drop collapse assay of IS-II

Emulsification Assay

The emulsification index (E24) results for IS-I and IS-II were 70% and 69.23%, respectively. These high emulsification indices demonstrate the strong emulsifying properties of the biosurfactants produced by these isolates, which can be beneficial in various industrial applications, such as bioremediation and enhanced oil recovery. Result of emulsification index are noted in table-2

Table-2: Emulsification Assay

Isolates	Total height of the solution (cm)	Height of Emulsion(cm)	Percent emulsion(E_{24})
IS-I	3	2.1	70 %
IS-II	2.6	1.8	69.23%

Foaming Activity

The formation of foam in the nutrient broth cultures of IS-I and IS-II indicates the production of biosurfactants. Foam formation is a common characteristic of biosurfactants and can be used as an initial screening method for biosurfactant-producing bacteria.

Blood Hemolysis Test

The clear zones of ß-hemolysis observed on blood agar plates confirm the hemolytic activity of the biosurfactants produced by IS-I and IS-II. This property can be useful in medical and pharmaceutical applications where biosurfactants are used as antimicrobial agents.

Phenol Sulfuric acid method

The dark orange color produced by IS-I and IS-II in the phenol sulfuric acid method confirms the presence of biosurfactants. This color change is indicative of the interaction between the biosurfactants and the phenol sulfuric acid reagent.

Extraction of biosurfactant

The successful extraction of biosurfactants from isolates IS-I and IS-II, as evidenced by the formation of a white precipitate at the interface of the supernatant and chloroform: methanol mixture, demonstrates the efficiency of the extraction process. The dry weights of the biosurfactants, 1.908 gm for IS-I and 1.563 gm for IS-II, indicate a substantial yield, highlighting the potential of these isolates for large-scale biosurfactant production.

The ability of these isolates to produce biosurfactants opens up various industrial applications, including bioremediation, enhanced oil recovery, and the formulation of environmentally friendly detergents and emulsifiers. Dry weight of biosurfactant calculated.

Dry weight of biosurfactant = weight of plate after drying-weight of empty plate

Fig- 7: Extraction of Biosurfactant

CONCLUSION

The study successfully isolated and characterized four bacterial strains, with two strains (IS-I and IS-II) demonstrating significant potential for biosurfactant production. Both isolates were confirmed to be Staphylococcus species by a thorough characterisation that included colony morphology, Gram staining, and biochemical testing. The biosurfactant production screening test showed positive results for IS-I and IS-II in a multiple of assays, including the oil displacement method, drop collapse assay, emulsification assay, foaming activity, blood hemolysis test, and the phenol-sulfuric acid method. These results indicate the strong biosurfactant-producing capabilities of these isolates. The emulsification index (E24) values of 70% for IS-I and 69.23% for IS-II highlight their strong emulsifying properties, which are crucial for applications in bioremediation and enhanced oil recovery. The presence of ß-hemolysis further supports the potential pathogenicity and industrial relevance of these strains.

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Declaration of competing interest

All authors declare that there's no financial/personal interest or belief that could affect their objectivity and there is no potential competing interest to declare

REFERENCES

- Aher, P., & Sharma, N. (2022). Biosurfactant screening and analysis. *Journal of Applied Microbiology Research*, 12(3), 67–73.
- Bhatt, P., & Kumar, A. (2023). Advances in microbial biosurfactant extraction. *Biotechnology Advances*, 61, 107–113.
- Das, S., & Reddy, S. (2022). Environmental applications of biosurfactants. *Environmental Biotechnology*, 14(4), 101–110.
- Ghaleb, R. N., Mamdapure, S. V., Jadhav, S. B., Siddiqui, M. M., Jadhav, P. B., & Bhosale, H. J. (2023). Antibacterial Activity of Biosurfactant Produced from Haloferax chudinovii HB1RANIA. *Bulletin of Environment, Pharmacology and Life Sciences*, (Spl Issue [1] January), 247–252.
- Kumar, M., & Singh, R. (2022). Blood agar hemolysis in biosurfactant-producing bacteria. *Microbial Ecology*, 83(2), 234–245.
- Mishra, S., & Yadav, D. (2021). Microbial biodegradation of petroleum hydrocarbons. *Journal of Environmental Science and Technology*, 19(3), 123–134.
- Patel, R., & Das, A. (2021). Industrial potential of biosurfactants. *Advances in Applied Microbiology*, 44(1), 45–60.
- Rani, S., & Kumar, P. (2022). Foaming activity in microbial biosurfactant production. *International Journal of Microbiology*, 28(2), 56–62.
- Sharma, M., & Das, P. (2023). Measuring emulsification index in biosurfactant studies. *Current Microbiology Research*, 67(1), 89–95.
- Shinde, V., & Ramesh, K. (2023). Oil spreading technique for biosurfactant identification. *Environmental Microbiology Reports*, 14(2), 34–40.
- Yin, H.; Qiang, J.; Jia, Y.; Ye, J.; Peng, H.; Qin, H.; Zhang, N. and He, B. (2009). Characteristics of biosurfactant produced by P. aeruginosa S6 isolated from oil- containing wastewater. Process Biochem., 44:302-308.
- Zodpe, S., 2016. Rhamnolipid biosurfactant production by strain of Pseudomonas aeruginosa using different raw materials. *Int J Curr Microbiol Appl Sci*, *5*, pp.407-13.
- Parviz Darvishi, Shahab Ayatollahi, Dariush Mowla, Ali Niazi (2011), Biosurfactant production under extreme environmental conditions by an efficient microbial consortium, ERCPPI-2, Colloids and Surfaces B: Biointerfaces, 84 (2), 292-300