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Isolation, Screening and Characterization of Biosurfactant-Producing Bacteria from Household Wastewater and Their Potential in Plant Growth Promotion

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Abstract:

Amphiphilic secondary metabolites of microbial origin, biosurfactants can lower interfacial and surface tension and create stable emulsions. Biosurfactants have become viable substitutes for synthetic surfactants in industrial formulations, agriculture, and environmental remediation due to their biodegradability, low toxicity, and functional stability in harsh environmental conditions. The current study's objectives were to separate and screen bacteria that produce biosurfactants from home wastewater, characterise biosurfactant production using quick qualitative assays, and assess their possible applicability for applications that promote plant development. Two wastewater sources—boiled corn water and rice washing water—were selectively cultivated after being supplemented with hydrocarbon substrates. Oil spreading, the emulsification index (E24), and modified drop-collapse assays were used to assess the synthesis of biosurfactants. Using cultural, microscopic, and biochemical traits, two bacterial isolates with different morphologies were isolated and identified. The isolates were identified as an Actinomycetes-like bacteria and Pseudomonas aeruginosa. Compared to the cooked corn water isolate, the rice-water isolate showed a bigger drop-collapse diameter and a relatively higher emulsification activity (48%). The work supports their potential use in environmentally friendly and plant growth-promoting agricultural operations and shows that it is feasible to isolate effective biosurfactant-producing bacteria from inexpensive household wastewater.

Keywords: Biosurfactant, Wastewater, Emulsification Index, Oil Spreading Test, Plant Growth-Promoting Traits, Pseudomonas

Introduction:

Surface-active substances known as surfactants lessen interfacial and surface tension between immiscible phases. The majority of conventional surfactants are produced using petrochemicals, and they frequently have poor ecological compatibility and biodegradability. Biosurfactants, on the other hand, are naturally occurring amphiphilic compounds that are mostly produced as secondary metabolites by filamentous fungus, yeasts, and bacteria.

Hydrophilic moieties like carbohydrates, amino acids, or peptides are joined to hydrophobic fatty acid chains to form microbial biosurfactants. Because of their dual nature, they can accumulate at interfaces like those between water and oil, as well as between air and water. This can lead to emulsification, the solubilisation of hydrophobic substrates, and an increase in the bioavailability of nutrients. Because of its high surface activity, stability in the face of severe pH, temperature, and salinity, low critical micelle concentration, biodegradability, and decreased toxicity, biosurfactants have drawn more attention in recent years.

Currently, biosurfactants are being investigated for use in petroleum recovery, food processing, medicines, cosmetics, detergents, environmental bioremediation, and agriculture. Biosurfactants promote nutrient solubilisation, reduce plant diseases, and stimulate beneficial plant-microbe interactions in agricultural settings, all of which improve soil quality and plant growth.

Household wastewater is a relatively untapped but easily accessible resource that is rich in carbohydrates, lipids, and organic wastes, even though numerous bacteria that produce biosurfactants have been recovered from petroleum-contaminated soils and industrial effluents. In order to characterise biosurfactant production using straightforward qualitative assays and investigate their possible significance for applications that promote plant development, the current work was conducted to separate and screen bacteria that produce biosurfactants from household wastewater.

Aim and Objectives:

1. To isolate and identify biosurfactant-producing bacteria from household wastewater samples.
2. To collect household wastewater samples and enrich them using hydrocarbon substrates.
3. To isolate and preserve biosurfactant-producing bacterial strains.
4. To screen biosurfactant production using rapid qualitative assays.
5. To characterize the biosurfactant-producing isolates using morphological and biochemical tests.
6. To assess the relevance of the isolates for potential plant growth-promoting applications.

Materials and Methods:

1. Sample collection:

Two household wastewater samples were used in this study:

(i) water drained after washing uncooked rice

(ii) water drained after boiling corn.

Samples were collected in sterile glass bottles and incubated at 30 °C for 48 h prior to enrichment.

2. Enrichment of biosurfactant-producing microorganisms:

For enrichment, rice washing water was supplemented with coconut oil (commercial brand manufactured by Marico Limited) and petrol, while boiled corn water was supplemented with cow-milk ghee and petrol. To encourage the selective development of bacteria that use hydrocarbons and produce biosurfactants, the enrichment cultures were cultured for 6–7 days at room temperature.

3. Screening of biosurfactant production:

Oil Spreading Method:

A thin layer of engine oil was put to a Petri plate after ten millilitres of distilled water had been added. In the middle of the oil layer was a single drop of the enriched culture supernatant. Biosurfactant activity was demonstrated by the development of a clear zone.

Figure 1: Enrichment Broth.

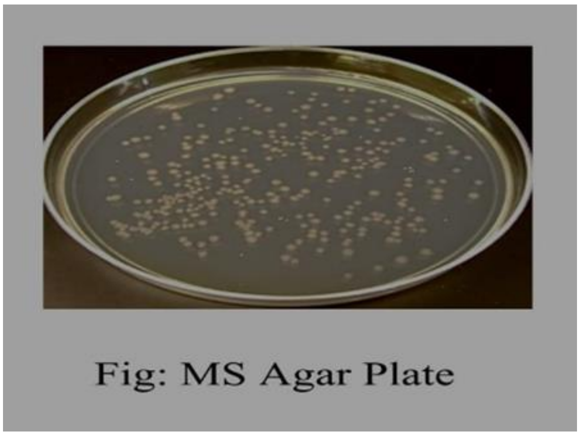

Figure 2: MS Agar Plate.

Emulsification index (E24):

Equal volumes of culture supernatant and petrol were mixed vigorously for 2 min and allowed to stand for 24 h. The emulsification index was calculated using:

(%)=Total height of liquid column / Height of emulsified layer×100

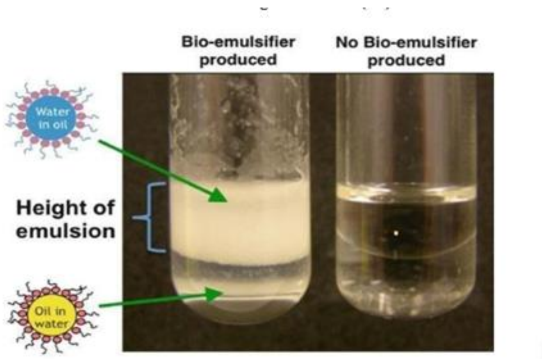

Figure 3: Emulsification index.

Modified drop-collapse test:

A drop of culture supernatant was carefully added to a drop of engine oil that had been placed on a spotless glass surface. Visual observations of the drop's spreading and collapse were made, and the collapsed drop's diameter was measured.

Figure 4: Drop-collapse Test

Isolation and preservation of bacterial isolates:

Enriched cultures showing positive biosurfactant activity were streaked on nutrient agar plates and incubated at 37 °C for 24 h. Distinct colonies were sub-cultured and preserved at 4 °C for further studies.

Morphological and biochemical characterization:

Colony morphology (size, shape, colour, elevation, margin, consistency and opacity) and Gram reaction were recorded. Biochemical tests including starch hydrolysis, catalase test and urease test were performed using standard microbiological procedures.

Results & Discussion:

1. Screening of Biosurfactant Production:

Positive biosurfactant activity was observed in both enriched wastewater samples. The rice water enrichment caused partial displacement of the oil layer in the oil spreading assay, while the boiling maize water enrichment mixed with ghee and petrol showed complete displacement. The rice water enrichment showed a higher emulsification activity (48%) than the boiling maize water enrichment (45%), according to the emulsification index.

The rice water enrichment showed a greater collapse diameter (2.0 cm) in the modified drop-collapse test than the cooked maize water enrichment (0.9 cm), suggesting relatively stronger surface-active qualities.

2. Isolation and Identification of Biosurfactant-Producing Bacteria

Two bacterial isolates with different morphologies were produced.

The isolate from rice water enrichment looked like Gram-positive rods and developed big, round, chalky-white colonies. The isolate from the boiling maize water enrichment looked like Gram-negative rods and formed medium-sized bluish-green colonies.

The isolates were tentatively identified as Actinomycetes-like bacteria and Pseudomonas aeruginosa, respectively, based on colony features, Gram staining, and biochemical reactions (starch hydrolysis, catalase, and urease assays). The current study shows that biosurfactant-producing bacteria may be effectively isolated from domestic wastewater that has been supplemented with simple hydrocarbon substrates. The isolated strains' secretion of surface-active chemicals is confirmed by the favourable findings obtained utilising oil spreading, emulsification index, and drop-collapse assays. Superior biosurfactant activity is indicated by the rice water isolate's bigger drop-collapse diameter and greater emulsification index. Carbohydrates, proteins, lipids, vitamins, and minerals found in rice washing water can operate as readily metabolised substrates, promoting the growth of microorganisms and the production of biosurfactants. By contrast, boiling water from maize, Despite being high in starch, it might offer a more complex nutritional profile, which would lead to a somewhat lower biosurfactant output in the investigated conditions.

The isolation of Pseudomonas-type bacteria is in line with other findings that describe this species as one of the most effective makers of biosurfactants, especially glycolipids of the rhamnolipid type. In addition to their great biological significance in soil habitats, actinomycetes are known to produce a diverse range of bioactive metabolites, including surface-active chemicals. By boosting nutrient solubilisation, improving root colonisation, aiding in the breakdown of pesticide residues, and inhibiting phytopathogens, biosurfactants play a significant role in promoting plant growth. Therefore, the isolates acquired in this investigation are good candidates for additional assessment of features that promote plant growth in both controlled and field settings.

Conclusion:

Biosurfactant-producing bacteria were successfully identified and screened from inexpensive home wastewater sources. The enrichment of bacteria that produce surface-active metabolites was encouraged by both boiling maize water and rice washing water. The emulsification index and drop-collapse experiments showed that the strain isolated from rice washing water had greater biosurfactant activity than the other isolate. The isolates show great promise for future development in environmentally friendly agricultural applications, especially for promoting plant growth and improving soil quality. They have been provisionally identified as Pseudomonas aeruginosa and an Actinomycetes-like bacteria.

Future Perspectives:

1. Extraction and purification of the produced biosurfactants.
2. Chemical characterization using chromatographic and spectroscopic techniques.
3. Evaluation of plant growth-promoting traits under greenhouse and field conditions.
4. Development of agricultural and cosmetic formulations based on the biosurfactants.
5. Assessment of large-scale production using agro-industrial and household wastes.

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References:

1. Joy, S., Sharma, S., Vieira, I., Santos, B., Ruzene, D., Silva, D., Markande, A., Patel, D., Varjani, S., Drakontis, C., Amin, S., Rana, S., Singh, J., Wadhawan, A., Khanna, A., Singh, G., Chatterjee, M., Aslam, A., Ishtaiq, M., … Mitchell, S. (2021). Antimicrobial applications of rhamnolipid biosurfactant produced from *Achromobacter* sp. (PS1) isolate using lignocellulosic hydrolysate. *Letters in Applied NanoBioScience, 11*(4), 4263–4271. https://doi.org/10.33263/lianbs114.42634271
2. Ribeiro, B. G., Guerra, J. M. C., & Sarubbo, L. A. (2020). Biosurfactants: Production and application prospects in the food industry. *Biotechnology Progress, 36*(4), e3030.
3. Drakontis, C. E., & Amin, S. (2020). Biosurfactants: Formulations, properties and applications. *Current Opinion in Colloid & Interface Science, 48*, 77–90.
4. Markande, A. R., Patel, D., & Varjani, S. (2021). A review on biosurfactants: Properties, applications and current developments. *Bioresource Technology, 330*, 124963. https://doi.org/10.1016/j.biortech.2021.124963
5. Basha, M. J., Noorjahan, A., Karuppaiya, M., Mahesh, S., & Aiyampumal, B. (2021). Screening and characterization of biosurfactant-producing marine bacteria and application for plant growth promotion. *Natural Volatiles & Essential Oils, 8*(5), 9390–9404.
6. Shagufta, S. M., & Dharani, P. V. (2022). Screening and isolation of bacteria producing biosurfactants from waste. *Journal of Pure and Applied Microbiology, 16*(1), 567–577.
7. Eras-Muñoz, E., Farré-Gómez, A., Martínez-Martínez, S., & Solé-Bundo, M. (2022). Biosurfactants in environmental applications: A sustainable alternative to chemical surfactants. *Frontiers in Bioengineering and Biotechnology, 10*, 876671. https://doi.org/10.3389/fbioe.2022.876671
8. Varjani, S., Upasani, V. N., & Pandey, A. (2020). Bioremediation of oily sludge-polluted soil using *Pseudomonas aeruginosa* and assessment of phytotoxicity. *Science of the Total Environment, 737*, 139766. https://doi.org/10.1016/j.scitotenv.2020.139766.