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SCREENING AND CHARACTERIZATION OF BIOSURFACTANTS FROM SOIL-DERIVED *PSEUDOMONAS AERUGINOSA* USING KEROSENE

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Abstract–Microorganisms produce surface-active substances called biosurfactants, which are used in industrial processes, pharmaceuticals, and bioremediation. The screening and isolation of *Pseudomonas aeruginosa* from soil samples to extract biosurfactants is the main objective of this study. To isolate microorganisms using selective media, soil samples were gathered from hydrocarbon-contaminated locations and serially diluted. Based on molecular, biochemical, and morphological characterization, presumed *P. aeruginosa* colonies were identified. Oil displacement, drop collapse, and emulsification index tests were used to evaluate the production of biosurfactants. Surface tension reduction and possible industrial uses of the extracted biosurfactants were further examined. The findings demonstrate *P. aeruginosa* environmentally friendly biotechnological applications and validate its effectiveness as a promising biosurfactant producer.

INTRODUCTION

Biosurfactants enjoy endless benefits compared with compound surfactants, particularly in regard to biodegradability, similarity with the climate, low poisonousness, high selectivity, and their action even in outrageous temperature, pH, and saltiness conditions (Banat *et al.,* 2010). These mixtures are surfactants and have amphipathic particles, with hydrophobic and hydrophilic segments that demonstrate liquids of various polarities (oil/ endlessly water/oil), permitting admittance to hydrophobic substrates and causing changes like surface pressure decrease, and expanding the area of contact of insoluble mixtures (like hydrocarbons), their portability, bioavailability and, later, its biodegradation (Aparna *et al*., 2011).

Microorganisms produce bio surfactants, safe substances used in environmental cleaning, oil recovery, and bioremediation. An adaptable soil bacterium called *Pseudomonas aeruginosa* is well-known for its capacity to generate bio surfactants, especially in reaction to hydrocarbon sources such as kerosene. The purpose of this study is to identify and separate *Pseudomonas aeruginosa* from soil samples with an emphasis on the bacteria's capacity to generate biosurfactants. Through the extraction and characterization of these bio surfactants, we hope to evaluate their efficacy in environmental applications, particularly in the bioremediation of soils contaminated by kerosene, and offer insights into long-term approaches to pollution control.

Bio surfactants have lipophilic gatherings of proteins as well as peptides with hydrophobics parts or carbonated chains of 10e18 carbons, and hydrophilic gatherings described by esters, hydroxyl, phosphate, carboxyl, or starches. They are typically created in the dramatic stage or fixed period of microbial development when there is a high cell thickness (Suwansukho *et al.,* 2008).

MATERIALS AND METHODS

Collection and preparation of sample

The sample is collected from the oil spilled areas to

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facilitate the production of Bio surfactant by using microorganisms present in the soil that degrade the oil spills. By separating the *Pseudomonas aeruginosa* from the soil, we can extract the bio surfactants. First, homogenize the soil and mix it with distilled water to create slurry. Then dry the soil for the serial dilution process. Create a soil suspension by mixing 10 g of prepared soil with 90 ml of sterile distilled water in a sterile container. Shake well and incubate at 37 °C for 24 hours to allow microbial activity. Perform serial dilutions of the soil suspension to isolate microorganisms that produce bio surfactants. Prepare a series of sterile dilution tubes with 9 ml of sterile distilled water. Transfer 1 ml of the soil suspension to the first tube, mix well, and then transfer 1 ml from the first tube to the second tube, and so on, to achieve several dilution levels (e.g., 10^-1, 10^-2, 10^-3, etc.) in Figure 1.

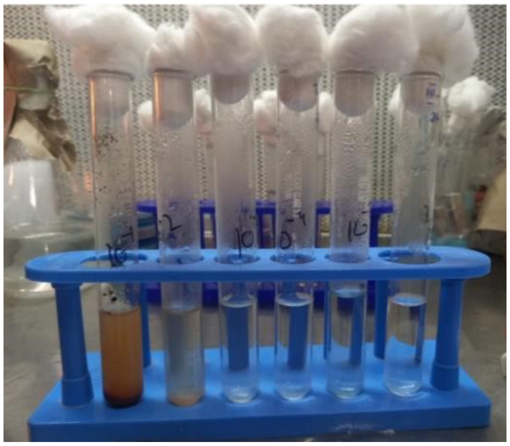

Fig. 1. Serial Dilution of soil sample

Isolation and screening of *Pseudomonas aeruginosa*

Plate 100 microfibers from each dilution onto a nutrient agar plate. Incubate the plates at 37 °C for 24-48 hours. After incubation, examine the plates several number of colonies have been grown. In this case, the specified microorganism *Pseudomonas aeruginosa* was used to extract the biosurfactant. To obtain the specific growth of *Pseudomonas aeruginosa* the culture has been streaked on the MacConkey agar and incubated at 37 °C for 24-48 hours. After the incubation period the MacConkey agar suppressed the gram-positive bacteria and promoted the gram-negative bacteria and the *Pseudomonas aeruginosa* was identified with their specific morphological characters.

Morphological identification of *Pseudomonas aeruginosa*

To identify whether the isolated microorganism is *Pseudomonas aeruginosa*, we run some morphological characteristics;

Gram Staining

A thin layer of the bacterial culture was applied to a glass slide to get it ready for analysis. The slide was coated with crystal violet reagent, left to soak for a minute, and then rinsed with tap water. The slide was then rinsed off after a few drops of Gram's iodine were added and allowed to sit for 30 seconds. After applying 95% ethanol to decolorize the slide, it was carefully rinsed with tap water once more. The slide was then exposed to safranin, cleaned with water, allowed to air dry, and examined using an oil immersion objective. Depending on the color of the stain retained, bacterial isolates that produce bio surfactants can be categorized as either gram-positive (purple) or gram-negative (pink).

Extraction of Bio surfactants

Inoculate the *Pseudomonas aeruginosa* into a liquid growth medium (e.g., nutrient broth) and incubate under optimal conditions for biosurfactant production. After growth, transfer the culture to a separatory funnel and add kerosene in a 1:1 ratio (v/v). Shake gently to mix and then allow the phases to separate in Figure 2. The kerosene is extracted from the liquid medium after the biosurfactant layer has developed. The resulting layer is centrifuged for 15 minutes at 12,000 rpm. After removing the supernatant from the centrifuge tube, biosurfactant is the result.

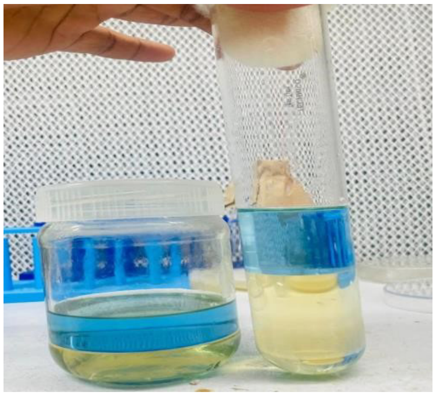

Fig. 2. Bisurfactant Layer formed.

Thin Layer Chromatography

Three separate solvent samples (water, ethanol, and n-hexane) have been collected and placed in three Eppendorf tubes. Three TLC sheets are taken, and using a toothpick, spots are inserted into each sheet 1-2 cm from the bottom until a fine visible circle (roughly 30-40 tiny dots) is formed. Give the area time to fully dry. A suitable solvent is made in the following ratio 3:2:2:1 (water, acetic acid, methanol, and chloroform). After adding the solvent to the TLC chamber, put the TLC sheet inside. To stop evaporation, make sure the sample spot stays above the solvent level and covers it. After removing the sheet, mark the solvent front right away and let the sheets dry. Determine the Rf value for each location by measuring the distance that each compound travels in Figure 3: TLC Analysis.

Fig. 3. TLC Analysis.

$$\text{Rf} = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

RESULTS AND DISCUSSION

Using kerosene as the only carbon source, the biosurfactant was effectively extracted from *Pseudomonas aeruginosa* in this investigation. For seven days, the bacterium was cultivated in a minimal medium that was enhanced with 2% v/v kerosene. A reduction in surface tension and the development of an oily layer on the culture medium's surface were two obvious indicators of biosurfactant production during this incubation. Following the incubation period, a solvent mixture consisting of methanol and chloroform (2:1) was used to extract the biosurfactant. The solvent was then evaporated to produce a viscous, oily substance. To verify its identity and evaluate its characteristics, this biosurfactant was subsequently put through several characterization procedures.

The extracted bio surfactant's composition was examined using Thin Layer Chromatography (TLC). The biosurfactant sample was applied to a TLC plate coated with silica gel. Chloroform and methanol (2:1), a solvent system frequently used to separate lipid and glycolipid compounds, were used to develop the plate. Following development, a single, distinct spot was visible when the TLC plate was visualized under UV light. The observed spot's retention factor (Rf) value was comparable to established rhamnolipid standards, indicating that *Pseudomonas aeruginosa* produced a glycolipid biosurfactant. A single spot on the TLC plate indicated the bio surfactant's purity, indicating that the extraction procedure produced a comparatively pure compound.

Bio surfactants have lipophilic gatherings of proteins as well as peptides with hydrophobics parts or carbonated chains of 10e18 carbons, and hydrophilic gatherings described by esters, hydroxyl, phosphate, carboxyl, or starches. They are typically created in the dramatic stage or fixed period of microbial development when there is a high cell thickness (Suwansukho *et al*., 2008).

CONCLUSION

In summary, this study effectively showed that *Pseudomonas aeruginosa* can use kerosene as a carbon source to produce a glycolipid biosurfactant. Thin Layer Chromatography (TLC), surface tension measurements, and emulsification assays were used to isolate and characterize the biosurfactant. The glycolipid nature of the biosurfactant was confirmed by TLC analysis, which showed a single spot with a retention factor resembling rhamnolipids. The biosurfactant solution's strong surfactant qualities were demonstrated by the significant reduction in surface tension to 28mN/m. Furthermore, the biosurfactant demonstrated outstanding emulsification activity, creating a stable emulsion with kerosene-an essential property for applications like oil recovery and spill cleanup. These findings demonstrate *Pseudomonas aeruginosa*'s capacity to generate potent biosurfactants from hydrocarbons such as kerosene, providing a sustainable substitute for synthetic surfactants.

The bio surfactant's potential uses in the industrial and environmental sectors, especially in the bioremediation of petroleum-based contaminants, are suggested by its capacity to lower surface tension and emulsify hydrophobic compounds. With an emphasis on its potential for sustainable biotechnology solutions, this study offers insightful information about the viability of employing *Pseudomonas aeruginosa* for the large-scale production of bio surfactants for a range of applications.

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Conflicts of Interest: The authors declare that they have no conict of interest.

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