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Effective Biosurfactants production by *Pseudomonas aeruginosa* and its efficacy on different Oils

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Abstract: A rhamnolipid producing bacterium, *Pseudomonas aeruginosa* was isolated from contaminated soil with oily wastes. The *Pseudomonas aeruginosa* has grown with glucose and corn oil as a carbon source produced biosurfactant. This biosurfactant was purified by procedures that included chloroform-ethanol extraction and 0.05M bicarbonate treatments. The active compound was identified as rhamnolipid by using thin layer chromatography. The biosurfactant efficacy was tested on coconut oil, groundnut oil, sunflower oil and olive oil. The coconut oil responded better and gave a maximum level of 1cm than the olive oil, groundnut oil and sunflower oil.

Keywords: Biosurfactant production, *Pseudomonas aeruginosa*, Rhamnolipid, Test efficacy.

1. Introduction

Biosurfactants are amphiphilic compounds produced on living surfaces, mostly microbial cell surfaces or excreted extracellularly. It contains hydrophobic and hydrophilic moieties that reduce surface tension and interfacial tensions between individual molecules at the surface and interface respectively. Well known synthetic surfactants are used for a wide variety of purposes, such as emulsification, foaming, detergency, solubilization, wetting and spreading. Almost all surfactants currently in use are chemically derived from petroleum. However, the interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environment friendly characteristics, the possibility of their production through fermentation and their potential applications in such areas such as the environmental protection, surface crude oil recovery, health care and food processing industries (Zajic & Steffens, 1984). Biosurfactants have the following advantages compared with chemical surfactants; lower toxicity, higher biodegradability (Swisher, 1970), better environmental compatibility (Parkinson, 1985), higher foaming (Haferburg *et al.*, 1986), higher selectivity and specific activity at extreme temperature, pH levels and salinity. Most biosurfactant studies have used bacteria grown on hydrocarbon substrates. Few workers have utilized carbohydrates (Suzuki *et al*., 1974; Cooper *et al*., 1981). *Pseudomonas aeruginosa* has been one of the most widely studied biosurfactants. Several rhamnolipid biosurfactants produced by *Pseudomonas aeruginosa* strains have been characterized (Edwards and Hayashi, 1965; Hirayama and Kato, 1982; Hisatsuka *et al*., 1971). Reiling *et al*., (1986) developed a continuous production of *Pseudomonas aeruginosa* to achieve 2.25g rhamnolipids as an L-rhamnose for scientific and industrial purposes. In this paper, we consider the production and test for the efficacy of extracting biosurfactants on different oil samples.

2. Materials and Methods

Pseudomonas aeruginosa was isolated from soil contaminated with oily wastes. The organism was maintained in nutrient agar slants in an atmosphere of 37^0C. The organisms were grown in 50ml sterile nutrient broth in a 250ml flask at 37^0C. Erlenmeyer

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flasks of 1000ml capacity containing 250ml of the mineral salt medium with glucose and corn oil as the carbon source were individually inoculated with 5ml of particular inoculums. The mineral salt medium (gl^{-1}) contained: $(NH_4)_2SO_4$ (0.5945); $FeSO_4.7H_2O$ (0.0001); $CaCl_2.2H_2O$ (0.002); $MgSO_4.7H_2O$ (0.061); NaCl (0.124); Glucose (0.112); Corn oil (1.25ml) and phosphate buffer (250ml). The phosphate buffer contained: NaH_2PO_4 (0.39) and Na_2HPO_4 (0.35). The flasks were incubated in a rotatory shaker incubator at 30^0C for 72 hours. The culture so obtained was used for the extraction of biosurfactants.

Rhamnolipid was recovered from the culture supernatant after the removal of cells by centrifugation at 6,800rpm for 20 minutes. The precipitate was dissolved in 25ml of 0.05M bicarbonate (pH 8.6) re-acidified and re-centrifuged at 12,000rpm for 20 minutes. The precipitate was then extracted with chloroform-ethanol (2:1) three times and it is then concentrated by evaporation at room temperature to obtain a biosurfactant concentrate. Following the evaporation, the biosurfactant concentrate is dissolved in 25ml of 0.05M bicarbonate (pH 8.6).

The homogenecity was checked and the identification was made by thin layer chromatography (TLC). Precoated TLC plates spotted with samples were developed with the following solvent systems chloroform: methanol: acetic acid (65:15:2 by volume). The compounds on the plates were visualized by heating after spraying with 2% anthrone solution in 0.2% H_2SO_4. Identification was performed by running rhamnose standards simultaneously.

Equal volumes of extracted biosurfactant in 0.05M *Pseudomonas aeruginosa* bicarbonate and the oil samples i.e., olive oil, coconut oil, groundnut oil and sunflower oil being tested (5ml+5ml) were taken in screw cap tubes. These tubes were then vortexed well and incubated at room temperature. The tubes were examined periodically for a decrease in the thickness of the oil layers and hence an increase in the biosurfactant layer. Observations were made every 1 hour to 8 hours.

3. Results

Using an enrichment culture technique a biosurfactant producing soil bacteria was isolated and identified as *Pseudomonas aeruginosa* on the basis of various physiological and biochemical tests as described in Bergey's manual of determinative bacteriology (1974). The bacterium is gram-negative, motile, rod-like and grows aerobically. The enrichment of organism in the mineral salt medium incorporated with trace amounts of glucose and 0.05% corn oil act as a carbon source as well as to induce the biosurfactant production. Rhamnolipid was recovered from culture supernatant by centrifugation, precipitation with hydrochloric acid, purified by re-centrifugation and bicarbonate treatment. The purified samples were detected as one spot of the thin layer chromatography with the above-mentioned solvent systems (for detailed see the materials and methods section). The concentrate of biosurfactant obtained following extraction from the production medium was tested with 4 different samples; coconut oil, olive oil, groundnut oil and sunflower oil. When tested with coconut oil, the level of biosurfactant was found to increase by 1cm in 8 hours which was found to be greatest among four samples tested. Following incubation with olive oil, groundnut oil and sunflower oil, the thickness of the biosurfactant layer was found to rise by 0.5cm, 0.3cm and 0.2cm in 8 hrs respectively (Fig. 1; Table 1).

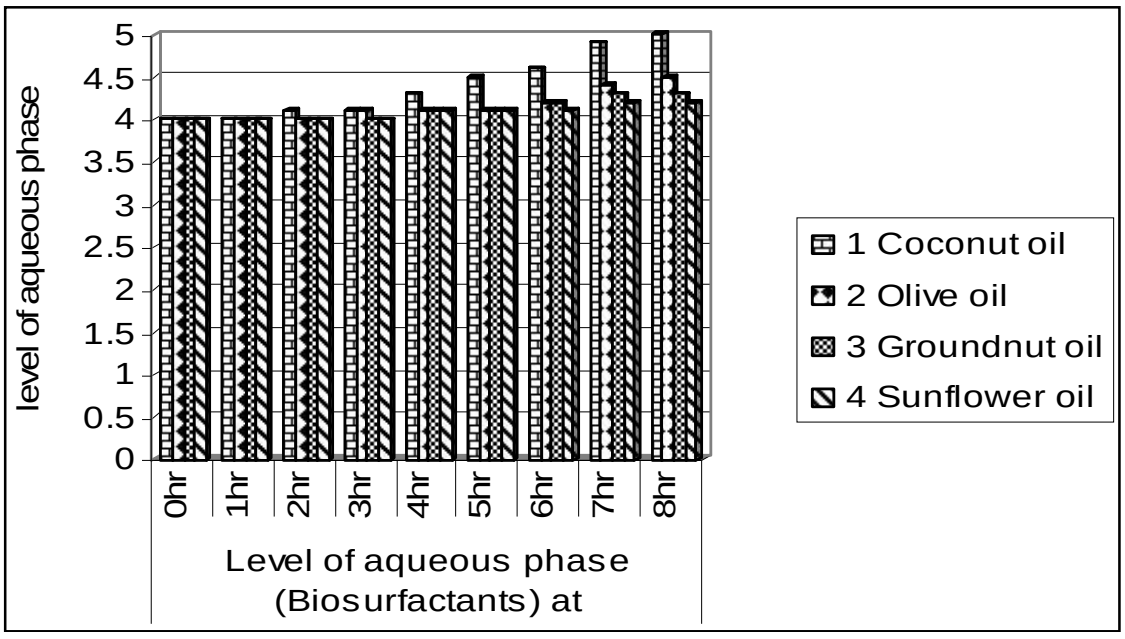

Fig. 1. Test efficacy of four different oils.

Table 1. Test efficacy of four different oils

S. No.	Name of sample	Level of aqueous phase (Biosurfactants) at								
		0 hour	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours	8 hours
1	Coconut Oil	4	4	4.1	4.1	4.3	4.5	4.6	4.9	5.0
2	Olive Oil	4	4	4	4.1	4.1	4.1	4.2	4.4	4.5
3	Groundnut Oil	4	4	4	4	4.1	4.1	4.2	4.3	4.3
4	Sunflower Oil	4	4	4	4	4.1	4.1	4.1	4.2	4.2

4. Discussion

The result obtained from the present investigation revealed the ability of *Pseudomonas aeruginosa* in producing biosurfactant in minimal media incorporating glucose and corn oil. During growth on *Pseudomonas aeruginosa* undergoes two distinct types of metabolism; exponential growth linked with amino acid catabolism and stationary growth linked with glucose metabolism. This behavior is known as reverse diauxic (Hamilton and Dawes, 1960). At this transition point, biosurfactant production was initiated. Rhamnolipids are also produced by *Pseudomonas aeruginosa* in media containing glucose or glycerol as a carbon source, particularly when the cells become limited for nitrogen (Guerra-Santos *et al*., 1986; Mulligan and Gibbs, 1989; Wagner *et al*., 1983), indicating that they may serve other roles besides being involved in solubilizing hydrophobic substrates. Like that of our study, primary isolation was made in nutrient broth followed by enrichment in minimal medium containing traces of glucose and 0.05% corn oil. Corn oil serving as the sole carbon source produces significantly increased levels of both cellular mass and rhamnolipids.

The increase in rhamnose cannot be attributed solely to the presence of more cells since cell mass was 2-3 times greater when corn oil was used. In addition to corn oil, other vegetable oils including soya bean oil, coconut oil and cottonseed oil result in similar cell growth and rhamnolipid production (Linhardt *et al*., 1989). Several types of organisms are involved in the production of biosurfactant, but *Pseudomonas aeruginosa* has been reported by other workers as excellent biosurfactant producer and have been widely studied. Corn oil is readily available at low cost and its use as a carbon source in the absence of glucose avoids potential sugar contamination of rhamnose product and produces significantly increased levels of both cellular mass and rhamnolipid. This enrichment induced the production of biosurfactants. For the detection of rhamnolipids, four independent tests were carried out. These included the lowering of the interfacial tension (IFT) by rhamnolipids (Guerra-Santos *et al*., 1984), the detection of thin layer chromatography (Koch *et al*., 1988), the haemolysis of erythrocytes by rhamnolipids (Johnson and Boese-Marrazzo *et al*., 1980) and the growth inhibition of *Bacillus subtilis* exerted by rhamnolipids (Itoh *et al*., 1971).

In our present investigation, the complete separation of rhamnolipids was performed by TLC. The efficacy of biosurfactant concentrates extracted was tested with four different oil samples viz; coconut oil, groundnut oil, sunflower oil and olive oil. The greatest activity was found in coconut oil followed by olive oil, groundnut oil and sunflower oil in the above sequence. In coconut oil, the production got increased 1cm, emulsify. The thickness also increased in olive oil, this may be due to saturated fatty acids in olive oil and is hence relatively easy for the organism to act upon. The complete emulsification of sunflower oil would hence take a long time compared to the above mentioned saturated oils due to the longer time is taken for the organism to break down the unsaturated fatty acids. The addition of hydrocarbon forced the existing cells to produce high concentrations of biosurfactants.

5. Conclusion

The present study demonstrated the biosurfactant producing potential of *Pseudomonas aeruginosa* isolated from oil contaminated soil using corn oil as a sole carbon source. Extracted biosurfactant showed the emulsifying property. The ability of the produced biosurfactant to emulsify different oils is an important feature that can be used for bioremediation of oil spills. Due to efficient emulsification properties, it can be used in industrial and environmental applications.

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