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Studies on biosurfactant production by *Pseudomonas aeruginosa* R2 isolated from oil contaminated soil sample

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A biosurfactant producing strain was isolated from soil sample obtained from coconut oil mill and identified as *Pseudomonas aeruginosa* R2 based on physiological and biochemical test together with 16s rRNA sequence analysis. Primary screening for biosurfactant producer was carried out by observing hemolysis on Superimposed blood agar and zone of clearance on Tributyrin agar. Optimization of culture conditions involved use of various vegetable oils as carbon source and different organic as well as inorganic compounds as nitrogen source, of which 1 per cent coconut oil and 0.4 per cent ammonium nitrate at pH 7 when kept at 30^0C for 120 rpm/96 hours showed maximum biosurfactant yield. The biosurfactant was partially purified using chloroform and ethanol mixture (2:1) and quantitatively estimated by Anthrone assay which was found out to be 1.7 g/L. The biosurfactant could reduce the surface tension up to 35 mN/m with 70 per cent emulsification index (E24) in 36hrs. TLC analysis of biosurfactant demonstrated rhamnose as a sugar moiety and FT-IR results confirmed it to be rhamnolipid type of biosurfactant. It also exhibited antimicrobial activity and showed stability on exposure to high temperature (up to 100^0C). Emulsification activity found with the biosurfactant against hydrocarbons shows its possible application in bioremediation of environments polluted with oils.

Key words : Biosurfactant, Rhamnolipid, Emulsification activity, CTAB- Cetyl-trimethylammonium bromide, SIBA- Superimposed blood agar.

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INTRODUCTION

Microbial surfactants are low molecular weight surface-active metabolites which facilitate the diffusion of insoluble substrates like hydrocarbons into the cell (Panesar *et al.,* 2011) reducing the surface tension and interfacial tension in both aqueous solutions and hydrocarbon mixtures (Desai and Banat 1997; Zhang and Miller, 1995) They are made up of diverse group of chemical structures such as glycolipids, lipopeptides, lipoproteins, fatty acids, neutral lipids and phospholipids (Banat *et al.,* 2010). The features that make them commercially promising alternatives to chemically synthesized surfactants are their lower toxicity, higher biodegradability, greater environmental compatibility, better foaming properties and stability at extremes of pH, salinity and temperature (Desai and Banat, 1997). They possess anti-bacterial, antifungal and antiviral activity. They also exhibit biomedical properties like inhibition of fibrin clot and display anti-adhesive action against several pathogenic microorganisms (Banat *et al.,* 2000; Cameotra and Makkar, 2004; Gautam and Tyagi, 2005; Rodrigues *et al.,* 2006).

The genus *Pseudomonas* is capable of using different substrates such as glycerol, mannitol, fructose, glucose, n-paraffins and vegetable oils to produce rhamnolipid type of biosurfactant (Desai and Banat, 1997; Koch *et al.,* 1991). Unlike chemical surfactants, which are mostly derived from petroleum feedstock, biosurfactant molecules can be produced by using cheaper agro based substrates and waste materials (Abouseoud *et al.,* 2008).

The present study focuses on the screening, isolation and identification of the biosurfactant producing bacterium from soil obtained from coconut oil mill. We report optimization of media and growth conditions for biosurfactant production by *Pseudomonas aeruginosa* strain R2. The properties and initial chemical characterization of the biosurfactant are also presented.

RESEARCH METHODOLOGY

Sample collection, enrichment and screening of biosurfactant producer :

Soil sample from the vicinity of coconut oil mill was collected and 1 g of it was inoculated in Nutrient Broth (100ml)