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Production of Biosurfactant by *Pseudomonas aeruginosa*

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Abstract: *Surfactants are chemically and biologically amphiphilic compounds that have hydrophilic and hydrophobic domain. Microbes produce biosurfactants in relatively large quantities. Biosurfactants have wide applications in industries including petroleum , food, agriculture. This study is based on potential biosurfactant production by Pseudomonas aeruginosa. BHMS (Bushnell Hass Mineral Salt) medium with glucose as substrate is used as production medium for Pseudomonas aeruginosa. The crude biosurfactant is extracted from supernatant. Different confirmatory tests were performed including oil displacement test. Gas chromatography was performed for qualitative estimation of rhamnolipids.*

Keywords: Biosurfactants, *Pseudomonas aeruginosa,* Rhamnolipids

I. INTRODUCTION

Surfactants are chemically and biologically amphiphilic compounds that have hydrophilic and hydrophobic domain. Most of the bio-surfactants produced by microbes are synthesized extracellularly and many microbes are known to produce bio-surfactants in large relative quantities. Biosurfactants enhance the surface area of hydrophobic water insoluble substrates. Biochemical nature of biosurfactants is glycolipids, lipopeptides, phospholipids, neutral lipids or fatty acids and polymeric biosurfactants. Majority of known biosurfactants are synthesized by microorganisms grown on water immiscible hydrocarbons, but some are produced on water soluble substrates such as glucose, glycerol, and ethanol. Up to now, the most commonly isolated and best studied groups of biosurfactants are those of rhamnolipids, glycolipid compounds and phospholipids [14]. Rhamnolipids are glycolipid compounds produced by *Pseudomonas* sp. which could reduce water surface tension and emulsify oil. *Pseudomonas aeruginosa* can be isolated from oil contaminated soil, petroleum contaminated soil. And by directed evolution *P. aeruginosa* and other organisms can be developed for biosurfactant production. These compounds are biodegradable and hence are no threat to environment.

Classification of Biosurfactants is based on their molecular weight. Glycolipids, rhamnolipids, trehalolipids and sophorolipids are low molecular weight biosurfactants.

High molecular weight biosurfactants are commonly known as emulsans. Ron and Rosenberg reported that these are exopolymers composed of polysaccharides, lipids, proteins, lipopolysaccharides lipoproteins or complex mixtures of these biopolymers.

Biosurfactants are also classified depending on their chemical structure.

- **Glycolipids**: Most known biosurfactants are glycolipids. They are carbohydrates whose constituents include mono, di, tri, tetrasaccharides. Best known are rhamnolipids, trehalopipids and sophorolipids [Desai and Banat 1997]
- **Lipopeptides:** Bacteria like *Bacillus brevis* and *Bacillus polymyxa* produce antibiotics gramicidin and polymxin that are lipopeptides. These lipopeptides have surface active properties. [1]
- **Fatty Acids, phospholipids and neutral lipids:**
- Fatty acids produced from alkanes have surface active properties. They are produced by several bacteria and yeast which use alkanes as substrate.
- **Particulate Biosurfactant**: These are microemulsions produced by various bacteria eg. *Acinetobacter*.

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1.1 Applications of Biosurfactants

Mukherjee et. Al. elucidated applications of biosurfactants in medicinal field. HanifYuliani, MekaSaimaPerdaniet. alexplained Antimicrobial activity ofbiosurfactants against 6 human pathogens eg. *E. coli , S. aureus*, *Pseudomonas*etc . According to Krishnaswamy M, Subbuchettiar G et al biosurfactants exhibit anticancer activity. Some extracellular glycolipids induce cell differentiation in stead of cell proliferation. Hence have promissing role in anticancer activity. [15]

As per Rodrigues *et al.* 2006a biosurfactants act as anti-adhesive agents against pathogens. Adsorption of biosurfactants to a substratum surface modifies its hydrophobicity, interfering in the microbial adhesion and desorption processes. [16] Biosurfactants can be used as a preventive strategy to delay the onset of pathogenic biofilm growth on catheters and other medical insertional materials, reducing the use of synthetic drugs and chemicals[12]

According toJ. Vater, B. Kablitz biosurfactants are used for cleaning or treating, agents of food contact surfaces, and as food ingredients. They can act as antioxidants and emulsifiers. [10]

1.2 Role in Bioremediation

Bioremediation is a **branch of biotechnology** that employs the use of living organisms, like microbes and bacteria, in the removal of contaminants, pollutants, and toxins from soil, water, and other environments. Bioremediation is used to clean up oil spills or contaminated groundwater.

II. Material and Method

2.1 Bacterial Strain

Pseudomonas aeruginosa was isolated from petroleum contaminated soil. The culture was maintained on nutrient agar slant. The two loopful culture was inoculated in 25ml nutrient broth and incubated on rotary shaker at 30^0C for 8 – 12 hrs. An aliquot of 2 ml was transferred into 100 ml of Bushnell Haas Mineral Salt medium as inoculum. The medium contained (g/l):dipotassium phosphate 1.0, calcium chloride 0.02, magnesium sulphate 0.2, potassium dihydrogen phosphate 1.0, ammonium nitrate 1.0, ferric chloride 0.05. Glucose was used as carbon source. Production media was incubated on shaker for 96 hrs at 30 ^{0}C.

2.2 Extraction of Biosurfactant

After incubation for 4 days , crude biosurfactant was extracted from production media . The cells were removed by centrifugation at 10000 rpm for 20 min. Biosurfactant were then precipitated by acidification at pH 2.0. It was incubated overnight at 4^0C. After that centrifugation carried out at 10000 rpm for 15 min. Then precipitate was dissolved in sodium bicarbonate (pH 8.6). Followed by reacidification& centrifugation at 12000 rpm for 20 min. the precipitate was extracted with chloroform methanol mixture. Organic solvent evaporated.

2.3 Oil Displacement Test

This method is used to determine the surface activity. The diameter of clear zone is measured after 96 hrs of incubation. 40ml of distilled water was added in petriplate of diameter 10 cm. After that, 15µl of crude oil was added to form thin layer on surface of water & 10 µl of solution was dropped onto the surface of oil. The test was conducted at room temperature. The maximum diameter of clear zone was observed.

2.4 Gas Chromatography

Gas chromatography was performed at analytical laboratory at Mitcon Institute Pune for qualitative estimation.

III. Result and Discussion

Biosurfactant producing bacteria *Pseudomonas aeruginosa*were isolated from petroleum contaminated soil. Referring the work of researchers*P. aeruginosa* was selected for production process. Rhamnolipid synthesis is regulated by QS, a mechanism controlling the production of most virulence factors in P. aeruginosa [17]

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Bacterial cells were inoculated in nutrient broth and incubated at 30^0C for 8-12 hrs on rotary shaker to obtain higher biomass. The production process was carried out as described previously. After *P. aeruginosa*was grown under optimum conditions, the recovery was done using cell free extract. The method in recovery process include solvent extraction and purification. The yield of biosurfactant was quite less that is 1.5 gm/ lit. According to Rodrigues et al. alternate media can be used for biosurfactant production. Rice wash water from brewery can be alternate source for biosurfactant production.

3.1 Oil Displacement Test

Name of Organism	Oil Displacement (cm)
Pseudomonas aeruginosa	7

Table 1: Zone of oil displacement test

3.2 Gas Chromatography

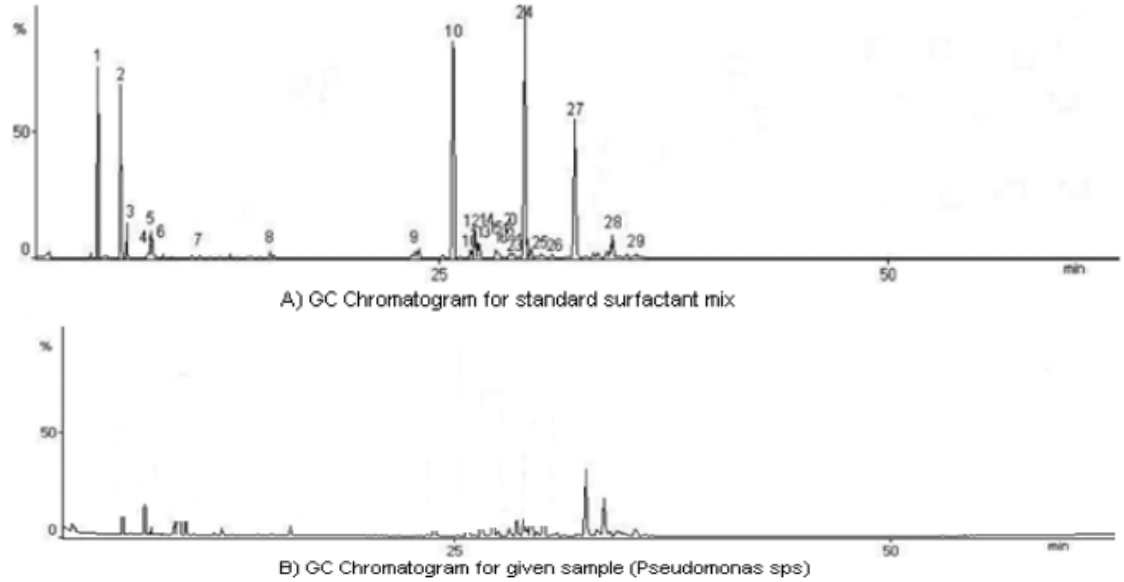

Figure 1: Chromatogram of standard mix (A) and of sample (B)

Gas chromatography was carried out for qualitative estimation of biosurfactant in analytical laboratory of Mitcon Institute Pune. Standard mixture was run along with crude sample. The peaks obtained in chromatogram of sample (B) were compared with peaks in chromatogram of standard mixture (A). Hence the components in sample are identified as sulphonates. Also some new picks were obtained. For that further characterization is required.

IV. Conclusion

Biosurfactants can be produced using various substrates and different producers. Water soluble sources like glucose are of primary importance. Also waste from agriculture industry can be used as substrate for biosurfactant production. Microorganisms isolated from oil or petroleum contaminated soil can be good producers as they get adapted to the environment. Biosurfactants have wide applications in medicines, environmental sciences etc. further study can be carried out in optimization of production process and applications of biosurfactants can be studied.

References

[1]. Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Micro and Mole Bio Revi. 1997;61:47-64.

[2]. N.K. Bordoloi, B.K. Konwar, Colloids Surf. B Biointerfaces 63 (2008) 73–82.

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[3]. Y. Zhang, R.M. Miller, Appl. Environ. Microbiol. 58 (1992)
[4]. F. Ahimou, P. Jacques, M. Deleu, Enzyme Microbial Technol. 27 (2000) 749–754.
[5]. L.M. Prieto, M. Michelon, J.F. Burkert, S.J. Kalil, C.A. Burkert, Chemosphere 71 (2008) 1781–1785.
[6]. Fijan, S. Microorganisms with claimed probiotic properties: An overview of recent literature. Int. J. Environ.Res. Public Health 2014, 11, 4745–4767. [CrossRef] [PubMed]
[7]. Gasbarrini, G.; Bonvicini, F.; Gramenzi, A. Probiotics History. J. Clin. Gastroenterol. 2016, 50, S116–S119.[CrossRef] [PubMed]
[8]. M. Nakano, M. Marahiel, P. Zuber, J. Bacteriol. 170 (1988) 5662–5668.
[9]. N. Roongaswang, J. Thaniyavarn, S. Thaniyavarn, T. Kameyama, M. Haruki, T. Imanaka, M. Morikawa, S. Kanaya, Extremophiles 6 (2002) 499–506.
[10]. J. Vater, B. Kablitz, C. Wilde, P. Franke, N. Mehta, S. Cameotra, Appl. Environ. Microbiol. 68 (2002) 6210–6219.
[11]. J.-Y. Wu, K.-L. Yeh, W.-B. Lu, C.-L. Lin, J.-S. Chang, Bioresour. Technol. 99 (2008) 1157–1164.
[12]. Rodrigues, L.R., Van der Mei, H.C., Teixeira, J.A. and Oliveira, R. (2004) Influence of biosurfactants from probiotic bacteria on formation of biofilms on voice prosthesis. *Appl Environ Microbiol* 70, 4408– 4410.
[13]. De Almeida et al., 2016 D.G. De Almeida, R. Soares Da Silva, C.F. de, J.M. Luna, R.D. Rufino, V. A. Santos, I.M. Banat, L.A. Sarubbo Biosurfactants: Promising molecules for petroleum biotechnology advances Frontiers in Microbiology, 7 (2016), pp. 1 14, 10.3389/fmicb.2016.01718
[14]. Mata-Sandoval JC, Karns J, Torrents A. Influence of rhamnolipids and Triton X100 on the biodegradation of three pesticides in aqueous phase and soil slurries. J Agric Food Chem 2001;49:3296–303
[15]. Krishnaswamy M, Subbuchettiar G, Ravi TK, Panchaksharam S (2008) Biosurfactants properties, commercial production and application. Current Science 94: 736-747.
[16]. Rodrigues L, Banat IM, Teixeira J, Oliveira R (2006) Biosurfactant; potential applications in medicine. J AntimicrobChemother 57: 609-618.
[17]. Smith RS, Iglewski B (2003) P. aeruginosa quorum-sensing systems and virulence. CurrOpinMicrobiol 6:56–60