International Journal of Research in Computer Science
eISSN 2249-8265 Volume 2 Issue 4 (2012) pp. 19-21

www.ijorcs.org

STUDIES ON THE PRODUCTION OF RHAMNOLIPIDS BY PSEUDOMONAS PUTIDA

Harikrishna Yadav Nanganuru[1], Narasimhulu Korrapati[2]
[1 2] *Department of Biotechnology, National Institute of Technology Warangal, Andhra Pradesh, India*
Email: harikrishyadav@gmail.com
Email: simha_bt@nitw.ac.in

Abstract: *Rhamnolipid as a potent natural biosurfactant has a wide range of potential applications, including enhanced oil recovery, biodegradation, and bioremediation. Observation of tensio-active indicated that biosurfactants were produced by the newly isolated and promising strain Pseudomonas putida. The biosurfactants were identified as rhamnolipids, the amphiphilic surface-active glycolipids usually secreted by Pseudomonas sp. In addition, the ability to generate rhamnolipids by placement of the right microbes might help overcome rhamnolipid adsorption during flow through reservoir rocks and the resultant degradation that would decrease the rhamnolipid concentrations. Their production was observed when the strain was grown on soluble substrates, such as glucose or on poorly soluble substrates. Maximum value 1.13 mg/ ml was occurred on the second day. Production of biosurfactants depends on the nutrient media. The surface tension was decreased with increasing time and growth.*

Keywords: Biosurfactants, Rhamnolipids, *Pseudomonas putida.*

I. INTRODUCTION

Pseudomonas putida is an environmental bacterium that can be survived in many different habitats, including water, soil, and plants [1, 2]. This bacterium produce the rhamnolipids (biosurfactant), which are amphiphilic molecules composed of a hydrophobic fatty acid moiety and a hydrophilic portion composed of one or two rhamnose [3]. Rhamnolipids belong to the glycolipid class. Rhamnolipids have been identified predominantly from *Pseudomonas sp.* [4, 5 and 6]. Because of their tensioactive Properties, Rhamnolipids have several potential industrial and environmental applications [7, 8]. These are used in the production of fine chemicals, the characterization of surfaces and surface coatings, and usage as additives for environmental remediation, and they have even been reported to be useful as a biological control agent [9]. Rhamnolipids are "secondary metabolites", and as such, their production coincides with the onset of the stationary phase [10, 11]. Their exact role is not clear may be they assist the colonization of host tissues or participate in increasing the bioavailability and degradation of hydrophobic organic contaminants by the host bacteria [12, 13 and 14]. Biosurfactants can improve the bioavailability of hydrocarbons to the microbial cells by increasing the area at the aqueous-hydrocarbon interface. This increases the rate of hydrocarbon dissolution and their utilization by microorganisms [15].We shown here that *Pseudomonas putida* produces a surfactant which substantially changes the surface tension of the culture medium with respect to time.

II. MATERIALS AND METHODOLOGY

Pseudomonas putida Cultures were obtained from Microbial Type Culture Collection. In this study, the cultures were grown in water soluble medium containing Beef extract, Yeast extract, Peptone, and NaCl. 2% glucose was added to the medium. The cultures were grown in flasks with 100ml of medium. Then this culture was incubated at 30^{o}C for 80 hrs. Samples of the culture media of selected strain were centrifuged at 8000rpm for 20 min. Samples were observed after 3, 6, 12, 24, 36, 48, 60, 72 and 80 hrs. Surface tension of the supernatant fluid of the culture was measured by the Wilhelmy method with filter paper as a sensing element on a surface tensiometer. Prior to the measurements calibration was done against clean water. Haemolysis of erythrocytes was done for the detection of rhamnolipids. Then the culture supernatants were concentrated by the addition of $ZnCl_2$ at pH 6.5. The precipitated material was dissolved in sodium phosphate buffer at same pH and extracted twice with equal volumes of diethyl ether. The pooled organic phases were evaporated to dryness and the pellets dissolved in 100 ml of methanol. 10 µl of the concentrated culture supernatants were spotted on paper filter discs and then put onto agar plates containing 5% sheep blood. The blood agar plates were incubated at room temperature for 2 days and *Pseudomonas putida* plates were put at 30^{o}C for 1 night and then the zones of haemolysis and growth inhibition were measured. Extracellular glycolipids

concentration was evaluated in triplicate by measuring the concentration of rhamnose: 333 ml of the culture supernatant was extracted twice with 1 ml diethyl ether. The ether fractions were evaporated to dryness and 0.5 ml of H2O was added. To 100 ml of each sample 900 ml of a solution containing 0.19% orcinol was added. After heating for 30 min at 80^0C the samples were cooled at room temperature and the OD421 was measured. The rhamnolipid concentrations were calculated from a standard curves prepared with l-rhamnose and expressed as rhamnose equivalents (RE).

III. RESULTS AND DISCUSSION

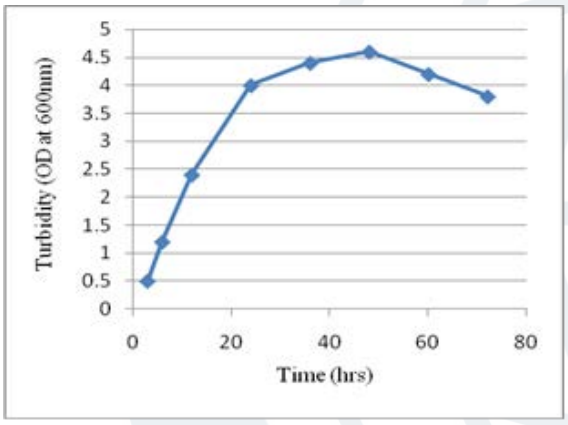

Figure 1: Turbidity of the biosurfactant was calculated by optical density at 600nm with respect to Time (hrs)

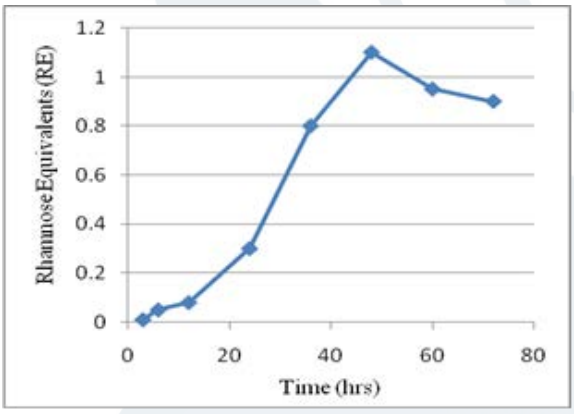

Figure 2: Production of biosurfactants (Rhamnose equivalents[RE]) by P. putida in medium with glucose with respect to Time (hrs)

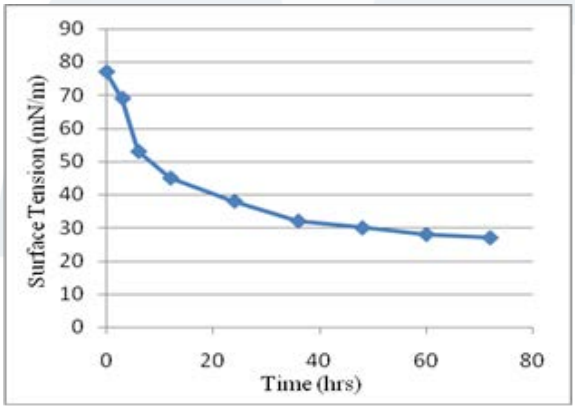

Figure 3: Surface tension of the biosurfactant with respect to the Time (hrs)

There are three indepent test to detect and quantify the biosurfactant. These included detection by thin-layer chromatography [16], haemolysis of erythrocytes by rhamnolipids [17] and growth inhibition of *Pseudomonas putida* by rhamnolipids [18]. Blue agar plates containing ethyltrimethylammonium Bromide and methylene blue were used to detect extracellular glycolipid production [19]. Biosurfactants were observed by the formation of dark blue halos around the colonies. This test was used for the detection of rhamnolipids by the haemolysis of erythrocytes by rhamnolipids [17]. This result suggests that *Pseudomonas putida* produced a mixture of rhamnolipids, the surface-active glycolipids which are usually secreted by *Pseudomonas putida*. These findings were further analysed using the fact that rhamnolipids possess haemolytic properties. The culture supernatant contained abundant amounts of haemolysin as the diameter of the haemolytic zone was 11 mm. A clear growth inhibition zone with a diameter of 36 mm was quantified when the concentrated culture supernatant of *P. putida* was spotted. However, enough rhamnolipids were secreted to cause a drop in the surface tension from 78 to 38 mN/m even after 24 h of incubation. The Surface tension reached a minimum of 27 mN/ m in the stationary growth phase and did not decline further on. Biosurfactant production increased progressively and maximal values of 1.13 mg/ ml were reached in the stationary phase again. Rhamnolipid production started more rapidly and the Surface tension of the medium started to decrease at 3 hrs of incubation. This was expected, since growth limiting conditions are required for rhamnolipid production [10]. The Surface tension decreased further on to 52 mN/ m, coincidently with the transition to the stationary growth phase. Moreover, the inoculums culture fluid may have contained diffusible autoinductors which regulate rhamnolipids synthesis in *P. putida* [20]. Rhamnolipid production seems possible from most carbon sources supporting bacterial growth. Nevertheless, oil of vegetable origin, such as soybean, corn, canola, and olive, provides the highest productivity [21, 22, 23 and 24].

IV. CONCLUSION

These strains represent a valuable source of new compounds with surface-active properties, and potential application for bioremediation. Maximal rhamnolipid production 1.2 mg/ ml was occurred in the medium, in stationary growth, indicating its characteristics as a secondary metabolite at 48 hrs. This was expected, since growth limiting conditions are required for rhamnolipid production.

V. REFERENCES

[1] Costerton JW (1980) Pseudomonas aeruginosa in nature and disease. In: Sabath CD (ed) Pseudomonas aeruginosa: the organism, diseases it causes and their treatment. Hans Huber Publishers, Bern, Switzerland pp 15–24

[2] Lyczak JB, Cannon CL, Pier GB (2000) Establishment of Pseudomonas aeruginosa infection: lessons from a Versatile opportunist. Microbes Infect 2:1051–1060. doi:10.1016/S1286-4579(00)01259-4

[3] Jarvis FG, Johnson MJ (1949) A glycolipide produced by Pseudomonas aeruginosa. J Am Chem Soc 71:4124–4126. doi:10.1021/ja01180a073

[4] Burger MM, Glaser L, Burton RM (1966) Formation of rhamnolipids of Pseudomonas aeruginosa. Methods Enzymol 8: 441–445. doi:10.1016/0076-6879(66)08082-0

[5] Zhang Y, Miller RM (1995) Effect of rhamnolipid (biosurfactant) structure on solubilisation and biodegradation on n-alkanes. Appl Environ Microbiol 61:2247–2251

[6] Beal R, Betts WB (2000) Role of rhamnolipid biosurfactants in the uptake and mineralization of hexadecane In Pseudomonas aeruginosa. J Appl Microbiol 89: 158–168. doi:10.1016/0076-6879(66)08082-0

[7] Lang S, Wullbrandt D (1999) Rhamnose lipids—biosynthesis, microbial production and application potential. Appl Microbiol Biotechnol 51:22–32. doi:10.1007/s002530051358

[8] Maier RM, Soberón-Chávez G (2000) Pseudomonas aeruginosa rhamnolipids: biosynthesis and potential applications. Appl Microbiol Biotechnol 54:625–633

[9] Stanghellini ME, Miller RM (1997) Biosurfactants: their identity and potential efficacy in the biological control of Zoosporic plant pathogens. Plant Dis 81:4–12. doi:10.1094/PDIS.1997.81.1.4

[10] Venkata Ramana K, Karanth NG (1989) Factors affecting biosurfactant production using Pseudomonas aeruginosa CFTR-6 under submerged conditions. J Chem Technol Biotechnol 45: 249–257. doi:10.1002/jctb.280450402

[11] Déziel É, Paquette G, Villemur R, Lépine F, Bisaillon J-G (1996) Biosurfactant production by a soil Pseudomonas strain growing on polycyclic aromatic hydrocarbons. Appl Environ Microbiol 62:1908–1912

[12] Finnerty W. R. (1994), Biosurfacatants in environmental biotechnology. Curr. Opin. Biotechnol. 5, 291D295. doi:10.1016/0958-1669(94)90031-0

[13] Rosenberg E. (1986), Microbial surfacatants. Crit. Rev. Biotechnol. 3, 109D132.

[14] Rouse J. D., Sabatini D. A., Suflita G. M., and Harwell J. H. (1994), Influence of surfactants on microbial de gradation of organic compounds. Crit. Rev. Environ. Sci. Technol. 24, 325D370. doi:10.1080/10643389409388471

[15] Gerson D. F. (1993), The biophysics of microbial surfactants: growth on insoluble substrates. In: Surfactant Science Series, Biosurfactants: Production, Properties, Applications (N. Kozaric ed.). Marcel Dekker, New York, USA, pp. 269D286.

[16] Koch A. K., Reiser J., Kappeli O. and Fiechter A. (1988), Genetic construction of lactose-utilizing strains of Pseudomonas aeruginosa and their application in bio surfactant production. Bio/Techn. 6, 1335D1339. doi:10.1038/nbt1188-1335

[17] Johnson M. K. and Boese-Marrazzo D. (1980), Pro duction and properties of heat-stable extracellular he molysin from Pseudomonas aeruginosa. Infect. Im mun. 29, 1028D1033.

[18] Itoch S., Honda H., Tomita F. and Suzuki T. (1971), Rhamnolipid produced by Pseudomonas aeruginosa Grown on n-paraffin. J. Antibiot. 24, 855D859.

[19] Siegmund I. and Wagner F. (1991), New method for detecting rhamnolipids excreted by Pseudomonas species during growth on mineral agar. Biotechnol. Tech. 5, 265D268. doi:10.1007/BF02438660

[20] Ochsner A. R. and Reiser J. (1995), Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in Pseudomonas aeruginosa. Proc. Natl. Acad. Sci. USA 92, 6424D6428. doi:10.1073/pnas.92.14.6424

[21] Lang S, Wullbrandt D (1999) Rhamnose lipids—biosynthesis, microbial production and application potential. Appl Microbiol Biotechnol 51:22–32. doi:10.1007/s002530051358

[22] Linhardt RJ, Bakhit R, Daniels L, Mayerl F (1989) Microbially produced rhamnolipid as a source of rhamnose. Biotechnol Bioeng 33:365–368. doi:10.1002/bit.260330316

[23] Sim L, Ward OP, Li Z-Y (1997) Production and characterization of a biosurfactant isolated from Pseudomonas aeruginosa UW-1. J Ind Microbiol Biotechnol 19:232–238. doi:10.1038/sj.jim.2900450

[24] Robert M et al (1989) Effect of the carbon source on biosurfactant production by Pseudomonas aeruginosa 44T1. Biotechnol Lett 11: 871–874. doi:10.1007/BF01026843

How to cite

Harikrishna Yadav Nanganuru, Narasimhulu Korrapati, "Studies on the Production of Rhamnolipids by Pseudomonas Putida". *International Journal of Research in Computer Science*, 2 (4): pp. 19-21, July 2012. doi:10.7815/ijorcs.24.2012.035