Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 10	No. 2	pp. 305-308	2011

Original Research Paper

Screening of Biosurfactant Producing *Pseudomonas aeruginosa* from Petroleum Contaminated Sites of Akola City

Prasad M. Deshmukh
Department of Microbiology, Shri Shivaji College of Arts, Commerce and Science, Akola-444 001, Maharashtra, India

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 9-9-2010
Accepted: 27-10-2010

Key Words:
Biosurfactants
Surface tension
Pseudomonas aeruginosa
Petroleum contaminated soil

ABSTRACT

Biosurfactants are amphiphilic compounds produced on living cell surfaces mostly microbial cell surfaces or excreted extracellularly, and contain hydrophobic and hydrophilic moieties that reduce the surface tension. Biosurfactants have wide application in microbial enhanced oil recovery, agriculture, lower toxicity, higher biodegradability, pharmaceuticals and therapeutics. Total eight biosurfactant producing *Pseudomonas aeruginosa* were isolated from petroleum contaminated sites. The biosurfactant properties of these isolates were further confirmed by haemolysis test and measurement of surface tension by using Troub's stalagnometer. The initial surface tension of medium was found to be 70 dynes/cm. The reduction of surface tension of medium was studied using enriched inorganic salt medium with 0.03 % of glucose and paraffin as a sole source of carbon. The lowest surface tension of medium was found to be 38.50 dyne/cm.

INTRODUCTION

Biosurfactants are surface active agents with wide range of properties including the lowering of surface and interfacial tensions of liquids. Surface tension is defined as the change in free surface enthalpy per unit area and is the force acting on the surface of a liquid leading to minimization of the area of that substrate (OECD 1995). Biosurfactants are compounds of microbial origin which exhibit surface activity. At present, biosurfactant synthesis has been studied extensively (Fiechter 1992, Banat et al. 2000). The biosurfactants are amphiphatic molecules consisting of hydrophobic and hydrophilic domains. Due to their amphiphatic nature, biosurfactants can act as partition at the interfaces between different fluid phases such as oil/water or water/air interfaces (Karanth et al. 1999).

Recently biosurfactant production have drawn attention of researchers throughout the world because of their higher biodegradability, reduced toxicity compared to synthetic surfactants and their application in enhanced oil recovery and food emulsification (Zajic & Panchal 1976, Zajic & Steffens 1984). Rapid advances in biotechnology over the past few decades have led to considerable interest in the development of biological methods for manufacturing biosurfactants on the industrial scale. Various types of biosurfactants are synthesized by a number of microbes particularly during their growth on water immiscible substrates (Makkar & Cameotra 1997). A majority of biosurfactants are produced by bacteria. Among the bacteria *Pseudomonas* species are well known for their capability to produce rhamnolipid biosurfactant with potential surface active properties when grown on different carbon substrates (Parra et al. 1989, Koch et al. 1991, Mercade et al. 1993). *Pseudomonas aeruginosa* produces two types of glycolipids both containing rhamnose as the carbohydrate moiety (Itoh et al. 1972).

The objective of this study was to screen out biosurfactant producing *Pseudomonas aeruginosa* by measuring the surface tension of medium using Troub's stalagnometer. The effects of different salt concentrations, pH and temperature on the biosurfactant production were also evaluated.

MATERIALS AND METHODS

Isolation of biosurfactant producing *Pseudomonas aeruginosa*: Total 20 soil samples were collected from different motor garages and petrol pump area from Akola city.

One gramme of soil sample was added to 10mL of sterile distilled water and vortexed thoroughly. The supernatant was serially diluted and spread on the surface of nutrient agar and incubated at 37°C for 24h. Green pigment colour colonies were randomly selected and inoculated on cetrimide agar and incubate at 37°C for 24h. Identification of *Pseudomonas aeruginosa* was done by using standard biochemical reactions.

Enrichment of bacteria: The enrichment was carried out using 100mL Inorganic Salt Medium (ISM) (g/L) $NaNO_3$: 4.0, KCl : 0.1, KH_2PO_4 : 0.5, K_2HPO_4 : 1.0, $CaCl_2$: 0.01, $MgSO_4.7H_2O$: 0.5, $FeSO_4.7H_2O$: 0.01, Yeast extract : 0.1, glucose : 0.03% and paraffin in 250mL Erlenmeyer flask. For enrichment of bacteria, the pH of the medium was adjusted to 7.2 using 0.1 N HCl and NaOH. The flasks were

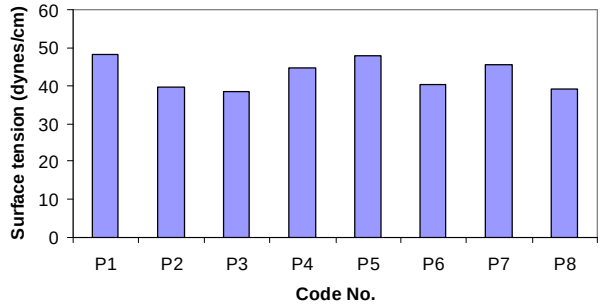

Fig. 1: Measurement of surface tension (dynes/cm).

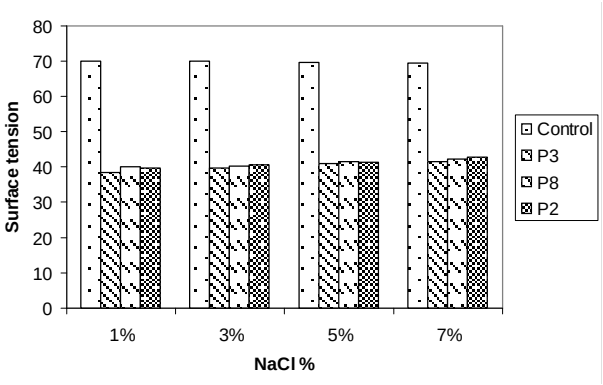

Fig. 2: Effect of different salt concentrations on the surface tension.

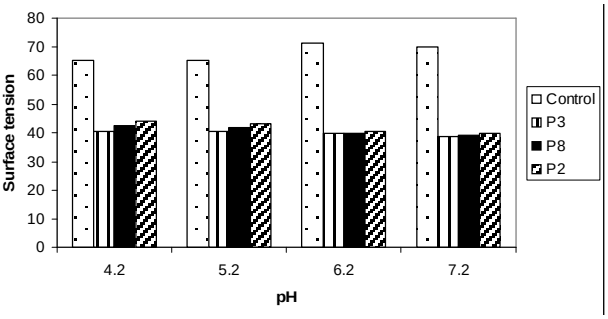

Fig. 3: Effect of different pH values on surface tension.

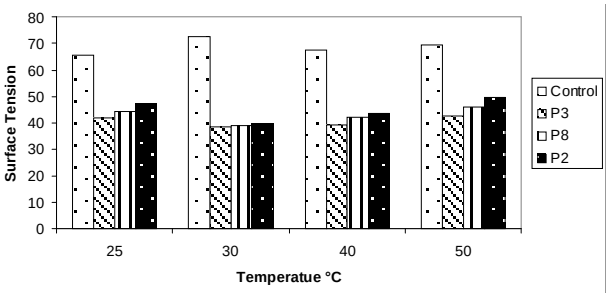

Fig. 4: Effect of different temperatures on surface tension.

inoculated with isolated colonies of *Pseudomonas aeruginosa* and incubated on rotary shaker (150 rpm) at room temperature for 3 days.

Screening of the biosurfactant producing *Pseudomonas aeruginosa*

a. Haemolysis test: The first screening for identification and isolation of biosurfactant producing bacteria was done by haemolysis test. The bacterial colonies were inoculated on blood agar plate and incubated at 37°C for 24h.

b. Measurement of surface tension: The cell free broth was obtained by centrifugation (5000 rpm, 30 min). The surface tension of cell free broth was measured by using Troub's stalagnometer (Shete et al. 2006).

c. Effect of different salt concentrations on surface tension: The effect of salt concentration on surface tension was determined by adding different concentrations (1% to 7%) of NaCl to the Nutrient broth. The mixtures were incubated at 30°C on rotary shaker at 150 rpm for 3 days.

d. Effect of different pH on surface tension: The effect of pH on surface tension was carried out by changing pH (4.2 to 7.2) of Nutrient broth (pH = 7.2). The broths were incubated at 30°C on rotary shaker at 150 rpm for 3 days.

e. Effect of different temperatures on surface tension: The effect of different temperatures on surface tension (25-50°C) was investigated by using nutrient broth. The broths were placed on rotary shaker at 150 rpm for 3 days in selected temperatures.

RESULTS AND DISCUSSION

The 14 aerobic Gram negative biosurfactant producing *Pseudomonas aeruginosa* were isolated from different soil samples. All of the isolated *Pseudomonas aeruginosa* were tested for haemolytic activity. Among the isolates, 11 *Pseudomonas aeruginosa* showed haemolytic activity. Selected isolates were used for further screening. Out of the 11 isolates of *Pseudomonas aeruginosa,* eight isolates showed the ability to reduce surface tension of cell free broth to values below 50 dynes/cm (Table 1, Fig. 1). Surface tension reduces in 1%-7% salt concentration, but suitable concentrations for reducing surface tension were 1% and 3% (Table 2, Fig. 2). The surface tension of whole cell free broth of selected isolates maintained nearly constant at all tested pH (4.2-7.2) at 30°C, indicating that the pH variation has no appreciable effect on surface tension. But, maximum of surface tension reduction was at pH range from 6.2-7.2 (Table 3, Fig. 3). All of the isolates reduced surface tension in tested temperatures but the best temperature for selected isolates were between 30 and 40 (Table 4, Fig. 4).

Table 1: Measurement of surface tension (dynes/cm).

Sr. No.	Code No. of the isolate	Surface tension (dynes/cm)
1	P_1	48.3
2	P_2	39.7
3	P_3	38.5
4	P_4	44.6
5	P_5	47.8
6	P_6	40.1
7	P_7	45.5
8	P_8	38.95

Table 2: Effect of different salt concentrations on the surface tension.

Sr. No.	NaCl %	Control	P_3	P_8	P_2
1	1%	70.0	38.5	39.9	39.7
2	3%	70.0	39.7	40.2	40.5
3	5%	69.7	41.0	41.5	41.1
4	7%	69.3	41.5	42.3	42.9

Table 3: Effect of different pH values on surface tension.

Sr. No.	pH	Control	P_3	P_8	P_2
1	4.2	65.10	40.43	42.3	44.1
2	5.2	65.10	40.40	41.7	42.9
3	6.2	71.20	39.60	39.8	40.2
4	7.2	70.00	38.50	38.9	39.7

Table 4: Effect of different temperatures on surface tension.

Sr. No.	Temperature (°C)	Control	P_3	P_8	P_2
1	25	65.40	41.7	44.3	46.94
2	30	72.70	38.5	38.9	39.70
3	40	67.70	39.3	42.1	43.59
4	50	69.33	42.4	45.9	49.70

The present work demonstrated that *Pseudomonas aeruginosa* P3, P8 and P2 produced rhamnolipid biosurfactant when grown on a medium using paraffin carbon source. Similar results were observed by Itoh et al. (1972), Syldatk et al. (1985), Baruah (1997) and Shete et al. (2006), while studying rhamnolipid biosurfactant producing *Pseudomonas aeruginosa* when grown on medium containing paraffin as a carbon source. All bacterial isolates of *Pseudomonas aeruginosa* were tested for haemolytic activity, which is regarded by some authors as indicative property of biosurfactant production and used as a rapid method for bacterial screening (Brenheimer & Avigad 1970, Banat 1995a, Lin 1996). Identification of biosurfactant producing bacteria can be further confirmed by measurement of surface tension of medium using Troub's Stalagnometer (Shete et al. 2006). Reduction of surface tension of medium measured by isolated bacteria from petroleum contaminated sites indicates the production of surface active compounds like biosurfactants. Similar results were obtained by (Khade & Dasgupta 2008). They isolated several bacteria and yeast, which show ability to reduce the surface tension of the medium to values below 50dyne/cm. For the characteristics of biosurfactants mostly they were under moderate conditions (Lang & Wullbrandt 1999, Ron & Rosenberg 2001) but very little information has been reported from extreme environment (Yakimov et al. 1995).

The present work showed that the isolation of biosurfactant producing *Pseudomonas aeruginosa* P_3, P_8, P_2 from paraffin oil medium has good activity and stability in wide ranges of pH, temperature and NaCl concentrations, indicating that it will have high potential applications in various environments.

ACKNOWLEDGEMENT

The author is thankful to Dr. Mohd. Mussadiq, Head of the Department of Microbiology and Biotechnology, Shri Shivaji College of Arts, Commerce and Science, Akola and Dr. Arti Deshpande, Head and Dr. Dnyansagar Bhokare, Department of Microbiology, Shri Shankarlal Khandelwal College, Akola for their continuous inspiration, encouragement, valuable suggestions during the study.

REFERENCES

Baruah, A., Saini, V. S., Adhikari, D.K. and Sista, V.S. 1997. Production of biosurfactants by *Pseudomonas* and *Bacillus* strains. Indian Journal of Microbiology, 37: 145-148.

Banat, I. M. 1995a. Biosurfactants production and possible uses in microbial-enhanced oil recovery and oil pollution remediation: A review. Bioresource Technology, 51: 1-12.

Banat, I.M., Makkar, R.S. and Comeotra, S.S. 2000. Potential commercial applications of microbial surfactants. Appl. Microbiol. Biotechnol., 53: 459-508.

Bernheimer, A.W. and Avigad, L.S. 1970. Nature and properties of a cytolytic agent produced by *Bacillus subtilis*. Journal of General Microbiology, 61: 361-369.

Fiecthter, A. 1992. Biosurfactant-Moving towards industrial application. Trends. Biotechnol., 10: 208-217.

Itoh, S. and Suzuki, T. 1972. Effect of rhamnolipids on growth of *Pseudomonas aeruginosa* mutant deficient in n-paraffin-utilizing ability. Agric. Biol. Chem., 36: 2233-2235.

Karanth, N.G.K. Deo, P.G. and Veenanadig, N.K. 1999. Microbial production of biosurfactants and their importance. Ferment. Sci. Technol., 77: 116-126.

Khade, R.G. and Dasgupta, D.D. 2008. Screening of biosurfactant/ biomulsifier producing yeasts and bacteria from petroleum contaminated sites. J. Microb. World, 10(1): 71-79.

Koch, A.K., Kappeli, O., Fiechter, A. and Reiser, J. 1991. Hydrocarbon assimilation and biosurfactant production in *Pseudomonas aeruginosa* mutants. J. Bacteriol., 173: 4212-4219.

Lang, S. and Wullbrandt, D. 1999. Rhamnose lipid-biosynthesis, microbial production and application potential. Appl. Microbiol. Biotechnol., 51: 22-32.

Lin, S. 1996. Biosurfactants: Recent reviews. J. Chem. Tech. Biotechnol., 66: 109-120.

Makkar, R. S. and Cameotra, S. S. 1997a. Biosurfactant production by thermophilic *Bacillus subtilis* strain. J. Ind. Microbiol. Biotechnol., 18: 37-42.

Mercade, M.E., Manresa, M.A. Robert, M., Epsuny, M.J., de Anes C. and Guinea, J. 1993. Olive oil mill effluents (OOME). New substrate for biosurfactant production. Biores. Technol., 43: 1-6.

OECD 1995. Surface tension of aqueous solutions, OEDC guideline. Organization for Economic Co-operation and Development, Paris.

Parra, J.L. Guinea, J. M., Manresa, A.M., Robert, M.E. Mercade, F. Comelles and Bosch, M.P. 1989. Chemical characterization and physicochemical behaviour of biosurfactants. Journal of Am. Oil Chem. Sco., 66: 141-145.

Ron, E. Z. and Rosenberg, E. 2001. Natural roles of biosurfactant. Environ. Microbiol., 3: 229-236.

Shete, H.G., Chitanand M.P. and Joshi P. S. 2006. Study of biosurfactant production using bacterial isolates from soil samples of petrol pump and oil mill area. J. Microb. World, 8(1): 136-139.

Syldatk, C., Lang, F., Wanger, V. Wray and Witte L. 1985. Chemical and physical characterization of four interfacial-active rhamnolipids from *Pseudomonas* sps. DSM 2874 grown on n-alkanes. *Z.* Naturforsch., 40: 51-60.

Yakimov, M., Kenneth, M., Timmis, Wray, V. and Fredrickson, L. 1995. Characterization of a new lipopeptide surfactant produced by thermotolerant and halotolerant subsurface *Bacillus licheniformis* BAS50. Applied Environmental Microbiology, pp. 1706-1713.

Zajic, J.E. and Panchal, C.J. 1976. Bioemulsifiers-Critical Reviews in Microbiology, 5: 39-66.

Zajic, J.E. and Steffens, W. 1984. Biosurfactants-Critical Reviews in Biotechnology, 1: 87-107.