ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(2):321-325

ORIGINAL RESEARCH ARTICLE

Isolation and Screening of Biosurfactants Produced by *Pseudomonas aeruginosa* from Oil Spilled Soils

Sneha K.S, Padmapriya B* and T.Rajeswari

Department of Microbiology, School of Life science, Karpagam University, Coimbatore-1, Tamilnadu, India

Received 12 Dec 2011; Revised 19 Mar 2012; Accepted 28 Mar 2012

ABSTRACT

Biosurfactant is a structurally diverse group of surface-active molecule, synthesised by microorganisms. It has the capability of reducing surface and interfacial tension with low toxicity and high specificity and biodegradability. The objective of this study was to isolate and identify the biosurfactant producing bacteria from the oil spilled areas. The samples were serially diluted and pour plated in the nutrient agar plates. Three organisms were isolated and screened for the biosurfactant production. To confirm the ability of isolates in biosurfactant production, different screening methods including Emulsification test, emulsification index E24, drop collapse method, oil spreading test and blood haemolysis test were assessed. This study suggested that *Pseudomonas sps* showed the maximum biosurfactant production.

Key words: Biosurfactant, *Pseudomonas aeruginosa*, Emulsification, oil spreading.

INTRODUCTION

Naturally occurring surface-active compounds derived from micro organismsare called biosurfactants. Biosurfactants are amphiphilic biological compounds produced extracellularly or as part of the cell membrane by a variety of yeast, bacteria and filamentous fungi [1].

The ability to reduce surface tension is a major characteristic of surfactant. Surfactants are key ingredients used in detergents, shampoos, toothpaste, oil additives, and a number of other consumer and industrial products. The total surfactant production has exceeded 2.5 million tons in 2002 for many purposes such as polymers, lubricants and solvents. The growth rate is related to the world demand in detergents since this sector uses over 50% of surfactant production [2]. The biosurfactants are complex molecules covering a wide range of chemical types including peptides, fatty acids, phospholipids, glycolipids, antibiotics, lipopeptides, etc.

Biosurfactants, lead to an increasing interest on these microbial products as alternatives to chemical surfactants [3].There are numbers of reports on the synthesis of various types of biosurfactants by microorganisms using water-soluble compounds such as glucose, sucrose, ethanol or glycerol as substrates [4]. Petroleum related industry was found to be one of the industries that have a greater potential to produce biosurfactants producing microorganism. In the present study, the biosurfactant producing microorganism was screened and characterized for the production of biosurfactants.

MATERIALS AND METHOD

Sample collection

For the isolation of biosurfactant producing bacteria, the sample was collected from automobile workshop in Coimbatore; The sample was taken in sterile polythene bag and was taken to the laboratory and analyzed for the isolation of dye decolorizing bacteria. The temperature of the soil sample during sample collection was 300ºC. The pH of the sample during sample collection was 7.

Isolation of hydrocarbon producing bacteria

The collected sample was serially diluted and plated on nutrient agar and using pour plate technique. From this organisms were isolated and identified using different preliminary techniques.

Identification of microorganisms

The isolated microorganisms were identified by Gram staining and Biochemical Tests [5].

Screening of biosurfactant producing organisms

The isolated colonies were tested for their biosurfactant production by following methods.

***Corresponding Author:** B.Padmapriya**, Email:** pmcbe22895@gmail.com

Emulsification test [6]

For emulsification test cultures were grown on Bushnell Haas broth with fried oil as the carbon source, incubated in a shaker for 7 days. Broth cultures were centrifuged at 6000 rpm for 20 minutes and emulsification factor was precipitated using chilled acetone and vacuum dried. Then the precipitate was dissolved and diluted with Tris Buffer (pH8.0) in 30 mL screw capped test tubes. 0.1 mL of fried oil was added and kept in shaker for 20 minutes at 150 rpm and the mixture was allowed to stand for 20 minutes. Then the values were read at 620nm in a spectrophotometer.

Emulsification index E24[7]

For measuring the emulsification capacity, two equal volume of supernatant and substrate is added in a test tube. The mixture is vortexed at high speed for 2 minutes. After 24 hours, the height of the stable emulsion layer is measured. The emulsion index E_{24} is calculated as the ratio of the height of the emulsion layer and the total height of liquid:

$$E_{24} = \frac{h_{emulsion}}{h_{total}} \times 100\%$$

Blood Haemolysis Test [8]

The fresh single colonies from the isolated cultures were taken and streaked on Blood agar plates. The plates were incubated for 48-72 hours at 37 ºC. The bacterial colonies were then observed for the presence of clear zone around the colonies. This clear zone indicates the presence of biosurfactant producing organisms.

Oil spreading method [9]

30ml of distilled water was taken in the pertiplates. 1 ml of used frying oil was added to the centre of the plates containing distilled water. Now add 20µl of the c supernatant of the cultures isolated from the soil to the centre. The biosurfactant producing organism can displace the oil and spread in the water.

Drop collapse method [10]

This assay relies on the destabilization of liquid droplets by surfactants. Therefore, drops of a cell suspension or of culture supernatant are placed on an oil coated, solid surface. If the liquid does not contain surfactants, the polar water molecules are repelled from the hydrophobic surface and the drops remain stable. If the liquid contains surfactants, the drops spread or even collapse because the force or interfacial tension between the liquid drop and the hydrophobic surface is reduced. The stability of drops is dependent on surfactant concentration and correlates with surface and interfacial tension.

RESULTS

Petroleum contaminated soil samples were collected and the organisms *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella sp*., were isolated from the soil. Then the preliminary tests were done to identify the organisms (**Figure1(a),(b) & Table 1**).In another study the biosurfactant producing organisms were isolated from the oil spilled soils of automobile of workshop *Pseudomonas sp* were isolated [11].

In this study, *Pseudomonas aeruginosa* showed the highest production of biosurfactant in both cell free culture and pellets. For the emulsification of the present study among the cell free cultures, *Pseudomonas aeruginosa* showed the highest production (0.113%) of biosurfactant and also in the pellets *Pseudomonas aeruginosa* showed the highest production of biosurfactant(0.044). These results showed (**Table 2**) that highest extracellular biosurfactant production, compared to intracellular biosurfactants by isolates.

In this test among the three cultures *Pseudomonas aeruginosa* produce the highest value of biosurfactant than the *Staphylococcus aureus* and *Klebsiella sp*. The values are shown in (**Table 3**). For the emulsification activity test for present study among *Pseudomonas aeruginosa* showed the highest production of biosurfactant (70%).Emulsification activity of D_{610} was found to be 1.4 crude oil degradation was studied for 10 days and degradation activity was increased on incubation time and maximum degradation was observed in 168hrs (72%) where as 59% degradation activity was observed at 48hrs at temperature 35^{o}C by Proteus *inconstans*[12]. The studies which both proved that strains isolated from areas with permanent contamination had better emulsification activity [13].

The isolated organisms *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella* were streaked in the blood agar plates. The plates streaked with the culture *P.aeruginosa* showed the β haemolytic activity. *S. aureus* showed the β haemolytic activity. *Klebsiella* showed the α haemolytic activity. Also 15 Gram positive and 15 Gram negative spherical bacteria were isolated. Among these, 59 strains had beta-hemolytic activity, 46 were able to collapse oil and 20 could spread oil [14].In addition, other microbial products such as virulence factors lyses blood agar and biosurfactants that are poorly diffusible may not lyses blood cells [15]. Thus, it is not clear whether blood agar lyses should be used to screen for

biosurfactant production. However, such screening can be used as a rapid method, in which samples with the positive result are subsequently subjected to biosurfactant-activity tests in liquid media.

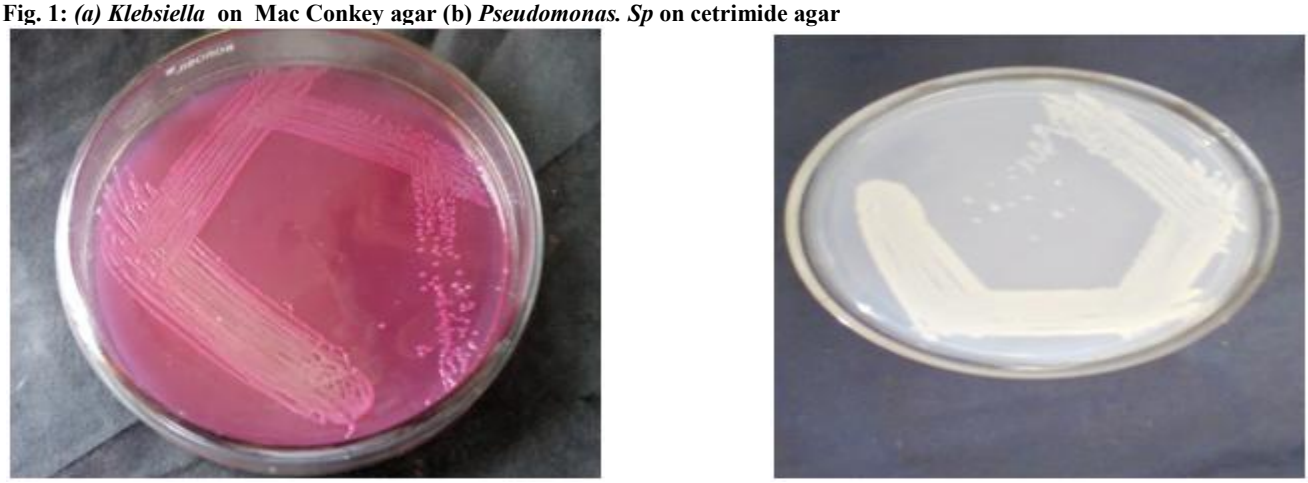
The drop collapse test and oil displacement tests are indicative of the surface and wetting activities [9].Drop collapse test and oil displacement test were highly positive for crude biosurfactant of *Trichosporon asahii* than commercial surfactants, Tween 80 and SDS, which indicated high surface activity [16]. In the present study oil spreading and drop collapse methods are used for screening of biosurfactant. In oil spreading test *P.aeruginosa* was produced a clear zone in the maximum level and in the drop collapse collapse method is used for the screening of biosurfactant. In drop collapse method the three organisms *Pseudomonas aeruginosa, Staphylococcus aureus*, *Klebsiella* were collapsed. This indicated that the three organisms were positive for biosurfactant production. The growth of bacteria on hydrocarbons is usually accompanied by the production of surfactants that helps in the adherence of the cells to oil droplets [17].

Table 1: Biochemical characteristics of isolated organisms

S.No	Grams stain & Biochemical test	Microorganisms		
		Pseudomonas aeruginosa	***Staphylococcus aureus***	***Klebsiella***
1	Gram stain	Gram -ve, Rod, Motile	Gram +ve, cocci, Non-Motile	Gram –ve, Rod, Non- Motile
2	Indole	-	-	-
3	Methyl Red	-	+	-
4	Voges proskauer	-	+	+
5	Citrate utilization test	+	-	+
6	Oxidase	+	-	-
7	Urease	-	-	+
8	Catalase	+	+	+
9	Nitrate	-	+	-
10	Gelatin liqufication	+	+	+
11	Triple Sugar Iron	A/AG	A/AK	A/A
12	Coagulase test	-	+	-

+Positive, - negative

Fig. 1: *(a) Klebsiella* on Mac Conkey agar (b) *Pseudomonas. Sp* on cetrimide agar



(a) **(b)**

Fig 2: (a) β haemolysis - *S . aureus* (b) β haemolysis- *P. aeruginosa* (c) α haemolysis - *Klebsiella Sp*

(a) **(b)** **(c)**

Fig 3: Oil drop method- *P. aeruginosa*

Table 2: Emulsification of hydrocarbons by biosurfactant from isolated organisms

S. No	Microorganism	Emulsification (D_{620})	
		Cell free culture	Intracellular
1	*Pseudomonas aeruginosa*	0.113	0.044
2	*Staphylococcus aureus*	0.071	0.035
3	*Klebsiella sp*	0.028	0.023

Table 3: Percentage of emulsification activity of isolated organisms

S. No	Microorganisms	% of Emulsification activity
1	*Pseudomonas aeruginosa*	70%
2	*Staphylococcus aureus*	60%
3	*Klebsiella sp*	40%

REFERENCE

1. Chen SY, Wei YH and Chang JS. Repeted pH- stat fed-batch fermentation for rhamnolipid production with indigenous *Pseudomonas aeroginosa S2*. Appl J Microbiol Biotechnol; 76: 67-74.
2. Deleu M and Paquot M. From Renewable Vegetables Resources to Microorganisms: New Trends in Surfactants. C.R.Chimie 2004; 7: 641-646.
3. Banat IM, Makkar RS and Cameotra SS. Potential commercial Application of Microbial Surfactants. Appl Microbiol Biotechnol 2000; 53:495-508.
4. Desai JD and Banat IM. Microbial production of Surfactant and their Commercial. Microbiol Mol Biol Rev 1997; 61: 47-64.
5. National Committee for Clinical Laboratory Standards .Performance standards for antimicrobial disc susceptibility tests (7th ed.) NCCLS, Wayne, Pennsylvania, USA (2000) M2–A7
6. Rosenberg E, Zuckerberg A, Rubinovitz C, Gutnick DL. Emulsifier of *Arthrobacter* RAG-1:isolation and emulsifying properties. Appl Env Microbiol 1979; 37: 402–408.
7. Iqbal S, Khalid ZM and Malik KA. Enhanced biodegradation and emulsification of crude oil and hyperproduction of biosurfactants by a gamma ray-induced mutant of *Pseudomonas aeruginosa*. Lett. Appl Microbiol 1995; 21: 176-179.
8. Mulligan CN, Cooper DG and Neufeld RJ. Selection of microbes producing biosurfactants in media without hydrocarbons. J Ferment Technol 1984; 62: 311-314.
9. Jain DK, Thompson DC and Lee H. A drop collapsing test for screening surfactant producing microorganisms. J Microbiol met 1991; 13: 271-279.
10. Youssef NH, Duncan KE, Nagale DP, Savage KN, Knapp RM and McInerney MJ. Comparison of methods to detect biosurfactant production by diverse microorganism. J Microbiol Met 2004; 56:339-347.
11. Thavasi R, Nambaru VRMS, Jayalakshmi S, Balasubramanian T, Ibrahim M. Biosurfactant Production by *Azotobacter chroococcum* isolated from the Marine Environment. Marine Biotechnol 2009; 11: 551 – 556.
12. Padmapriya B, Rajeswari T, Suganthi S, Rajeswari T and Jayalakshmi S. Biosurfactant Production And Plasmid Isolation From Newly Isolated Hydrocarbonoclastic Bacteria *Proteus inconstans* .Int J Pharm Biol Arc 2011; 2: 784-790.
13. Okerentugba PO and Ezerony OU. Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluentin Nigeria. Afr. J. Biotechnol 2003; 2: 288-292.
14. Ghayyomi Jazeh M, Forghani F and Deog-Hwan OH. Biosurfactan Production by *Bacillus sp*. isolated from Petroleum Contaminated Soils of Sirri Island. American Journal of Applied Sciences 2012; 9: 1-6.
15. Safary M, Ardakani AR, Suraki MS, Khiavi A and H. Motamedi. Isolation and Characterization of Biosurfactant Producing Bacteria from Caspian Sea. Biotechnology 2010; 9: 378-382.

16. Preethy C and Nilanjana D. Biosurfactant production and diesel oil degradation by yeast species *Trichosporon asahii* isolated from petroleum hydrocarbon contaminated soil. Int J Eng Sci Tech 2010; 2: 6942-6953.

17. Rosenberg M, Rosenberg E. Role of adherence in growth of *Acinetobacter calcoaceticus* RAG-1 on hexadecane. J Bacteriol 1998; 148: 51- 57.