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Antibacterial effect of Surface active agent produced by *Pseudomonas* sp. isolated from Saltpan

T. Murugan*, M. Murugan

Centre for Biological Science, Noorul Islam Centre for Higher Education, Kumaracoil - 629 180, Tamil Nadu, India

Abstract

The intention of the study was to isolate the efficient antibacterial potent biosurfactant producing bacteria. Eight morphologically distinct bacteria isolated from saltpan soil were screened for biosurfactant activity by oil spreading and haemolysis assay. Four isolates showed positive result in oil spreading assay and three in haemolysis activity. Among these, one isolate (BS-6) showed better activity in both tests was considered as potential strain and identified as *Pseudomonas* sp. by studying cultural, morphological and biochemical characteristics. The biosurfactant produced by *Pseudomonas* sp. was extracted and studied for their antibacterial effect on six selected bacterial pathogens. In this study, the biosurfactant exhibited good antibacterial activity against tested pathogens and Gram negative pathogens were highly susceptible. This present study substantiates the antibacterial potential of biosurfactant produced by *Pseudomonas* sp. It could be gain more important in future for various pharmaceutical applications.

Keywords : Saltpan, Biosurfactant, *Pseudomonas* sp., Antimicrobial activity, Pharmaceutical application.

INTRODUCTION

Surfactants are surface active chemical agents, which are used for many purposes in food, agricultural, industrial, cosmetic and pharmaceutical applications [1]. These compounds are amphiphilic agent with both lipophilic and hydrophilic in nature [2]. Most of surfactants are chemically synthesized, mainly from petrochemical origin [3] and cause toxic problems to the environment. To rectify these problems, the alternative source for the surfactant is biological origin *i.e.* biosurfactant.

Biosurfactants are surface active chemical metabolites produced by microorganisms such as bacteria and fungi. They have compensation over their chemicals counterparts because they are biodegradable, have low toxicity, effective at high temperatures or pH values and have better environmental compatibility [4, 5] Biosurfactants constitute a diverse group of surface active molecules such as glycolipids, lipoproteins, fatty acid**s,** neutral lipids, phospholipids and polymeric structures [6, 7].

Biosurfactants have diverse applications includes biocontrol agent in agricultural field, health and beauty products in the cosmetic industries, *etc*. It can be used as emulsifiers, de- emulsifiers, wetting and foaming agents, functional food ingredients, cosmetics and pharmaceuticals [8, 9]. They possess antibacterial, antifungal and antiviral properties; and they have anti-adhesive action against several pathogenic microorganisms [10, 11]. This present study was undertaken to isolate antimicrobial potent biosurfactant producing bacteria from halophilic soil.

MATERIALS AND METHODS

Sample

The soil samples were collected from polluted sites at saltpan of Puthalam, Kanyakumari District, Tamil Nadu. Three samples were collected in a clean sample container and transported immediately to the laboratory for the isolation of bacteria.

Isolation of bacteria

The collected samples were subjected serial dilution using test tubes containing 9 ml of saline. The dilution was made upto 10^{-6} and about 0.1 ml of diluted sample from 10^{-3} and 10^{-4} were inoculated into sterile Nutrient agar supplemented with 5% sodium chloride plates. The inoculumn was spread evenly with the help of clean glass L-rod and the plates were incubated at 37°C for 24 to 48 h. After incubation, the morphologically distinct bacterial colonies were selected and made pure culture by subsequent subculturing into fresh agar medium.

Screening for biosurfactant activity

The selected organisms were subsequently subjected to preliminary screening for biosurfactant production by oil spreading technique and haemolytic activity [11].

Oil spreading techniques

The bacteria were grown into liquid media and the supernatants were collected by centrifugation. About 30 ml of distilled water was added to a large petridish followed by the addition of 20 ml of Oil to the surface of water and 10 ml of culture supernatant.

Haemolytic activity

The isolated bacterial colonies were streaked onto blood agar plates containing 5% sheep blood and incubated at 37°C for 24 to 48 h. Hemolytic activity was detected as the presence of clear zone around bacterial colonies.

Characterization of potential isolate

The potential isolate which has good biosurfactant activity was identified by studying cultural, morphological and biochemical characteristics include growth appearance on agar plate, Gram staining, motility, catalase, oxidase and nitrate reduction test.

Biosurfactant Production

The selected potential candidate was grown in 250 ml Erlenmeyer flasks containing 50 ml of mineral salt medium. The flask was incubated at 37°C on a shaker incubator for 5 days. After incubation, to isolate the biosurfactant, the bacteria were removed by centrifugation and the remaining supernatant liquid was filtered through a 0.22 mm milipore filter. Biosurfactant was obtained by adjusting the supernatant pH 2.0 using 6N HCl and keeping it at 4°C for overnight. The precipitate thus obtained was collected by centrifugation and dried.

Antibacterial activity

Antibacterial activity of the biosurfactant was determined by agar well diffusion method against six bacterial pathogens *viz. Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia* and *Vibrio cholera*. 0.1ml of 18 h fresh bacterial culture was spread evenly on Nutrient agar plate using sterile cotton swab and allowed to dry for few min. after, wells of 6 mm in diameter were punched off into medium with sterile cork borer and filled with 30 µl of biosurfactant suspension prepared in methanol (1mg/ml). All the plates were incubated at 37°C for 24 h and then the zone of inhibition was measured**.**

RESULT AND DISCUSSION

A total of eight morphologically distinct bacterial colonies were isolated from the collected soil samples and were screened for biosurfactant activity. In this study, four isolates showed positive for oil spreading assay (BS-1, BS-3, BS-6 and BS-7) and one isolate (BS-6) showed higher zone formation of 4.2 mm, 4.0 mm and 3.6 mm in petrol, diesel and kerosene respectively. In haemolyis assay, three isolates (BS-1, BS-6 and BS-7) produced zone of clearance on blood agar (Table 1). Among these, two isolates (BS-1 and BS-6) showed complete heaemolysis. Based on the assay, the isolate BS-6 was selected for further study as potential isolate.

Table 1: Screening for biosurfactant activity

Colony code	Oil spreading assay	Haemolytic activity
BS-1	+ve	+ve
BS-2	-ve	-ve
BS-3	+ve	-ve
BS-4	-ve	-ve
BS-5	-ve	-ve
BS-6	+ve	+ve
BS-7	+ve	+ve
BS-8	-ve	-ve

Oil spreading assay is frequently used for a preliminary screening of isolates for biosurfactant activity. Displacement oil by the addition of culture supernatant on the water surface was positive result and it indicates the presence of biosurfactant in the culture filtrates. In oil spreading techniques *Bacillus* and *Pseudomonas* sp. showed higher zone formation when petrol, diesel and kerosene were used [12]. Haemolytic activity method is often used for screening of microorganisms for the ability to produce biosurfactants on hydrophilic media [13]. In the present study, three isolates will cause lysis of the blood cells and exhibit a colorless zone around the colonies.

The potential isolate (BS-6) was identified by studying cultural, morphological and biochemical characteristics. The isolate produced light yellowish green, irregular, transparent, flat colonies. It was Gram negative, rod shaped, motile, oxidase, catalase and nitrate reduction positive (Table 2). Based these results, the isolate was confirmed as *Pseudomonas* sp. *Pseudomonas* sp. was efficient producers of biosurfactants [14, 15] and this result was supported by Patil *et al.* [16], they isolated rhamnolipid biosurfactant producing *P.aeruginosa* strain from soil.

Table 2. Characterization of potential isolate

Sl.No.	Characters	Results
1	Colony morphology	Transparent, mucoid, yellowish green colonies
2	Gram stain & Morphology	Gram -ve, Rods
3	Motility test	Motile
4	Oxidase test	Positive
5	Catalase test	Positive
6	Nitrate reduction	Positive

The biosurfactant was produced by the potential isolate was extracted from the cell filtrate by cold-precipitation using HCl and the extracted pellets were dried and used. In the antibacterial activity assay, the biosurfactant produced the isolate showed highest inhibition activity on *E.coli* (15 mm) followed by *K.pneumoniae* (13 mm), *B.cereus, S.aureus* (12 mm), *S.aureus* and *V.cholerae* (10 mm) (Table 3).

Table 3: Antibacterial activity of biosurfactant

Sl.No.	Bacterial pathogens	Zone of inhibition (mm)
1	*B.cereus*	12
2	*S.aureus*	10
3	*E.faecalis*	12
4	*E.coli*	15
5	*K.pneumoniae*	13
6	*V.cholerae*	10

In this study, Gram negative pathogenic organisms were highly inhibited when compared to Gram positive organisms. The biosurfactant produced by the strain *P.aeruginosa* was found to be effective against wide range of test pathogenic organisms such as *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Proteus vulgaris* and *Proteus mirabilis* [16]. Also, Karkera *et al.* [17] found that, the biosurfactant produced by *P.aeruginosa* was effective against Gram positive organisms.

CONCLUSION

The isolated strain of *Pseudomonas* sp. has the ability to produce good surface active agents and the biosurfactant produced by *Pseudomonas* sp. have good antibacterial effect and it could gain more important in future for various foods, cosmetic and pharmaceutical applications.

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