



CODEN (USA): IAJPBB

ISSN: 2349-7750

**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.200636>Available online at: <http://www.iajps.com>

Research Article

**SCREENING OF BIOSURFACTANT PRODUCTION BY
BACILLUS SP ISOLATED FROM COASTAL REGION IN
CUDDALORE TAMILNADU****Bhuvanewari. M* and P. Sivagurunathan**

Department of Microbiology, Faculty of Sciences, Annamalai University, Tamil Nadu, India.

Abstract:

Marine microorganisms produce extracellular or membrane associated surface-active compounds (bio surfactants). Biosurfactant are organic compounds belonging to various classes including glycolipids, lipopeptides, fatty acids, phospholipids that reduce the interfacial tension between immiscible liquids. This study deals with production and characterization of biosurfactant from Bacillus sp. The efficiency of Bacillus sp strain isolated from a marine sediments soil sample from coastal region -Cuddalore.

Methods: *Bushnell Haas (BH) liquid medium was used as the enrichment medium with 1 %(v/v) diesel as the sole carbon source to select diesel degrading bacteria. Serial dilutions (1/10) from the third enrichment process were plated out into BH agar plates, which were covered with 100 µl of diesel oil and incubated at 30°C for approximately one week. The single colonies were streaked into nutrient agar plates incubated at 30°C overnight and stored at 4°C until further use. To elute the biosurfactant from TLC and screen the antimicrobial activity of biosurfactant against clinical pathogens by agar well diffusion method.*

Result: *The results showed the Bacillus sp strains could be effective for crude oil biodegradation.*

Conclusion: *Bacillus spp produces bio surfactant which helps in conversion of the hydrophobic layer into small micelles which it can easily engulf as a carbon source which is the basic nutritional requirement.*

Key words: *Isolation, Bacillus sp, Biosurfactant, TLC and Agar well method.*

Corresponding author:**Bhuvanewari. M,**

Department of Microbiology,

Faculty of Sciences,

Annamalai University, Tamil Nadu,

India.

Email: bhuva31087@gmail.com

QR code



Please cite this article in press as Bhuvanewari. M and P. Sivagurunathan, Screening of Biosurfactant Production by *Bacillus Sp* Isolated From Coastal Region in Cuddalore Tamilnadu, *Indo Am. J. P. Sci*, 2016; 3(11).

INTRODUCTION:

Surface-active compounds produced by microorganisms are of mainly two types, those that reduce surface tension at the air-water interface (bio surfactant) and those that reduce the interfacial tension between immiscible liquids, or at the solid liquid interface (bio emulsifiers). Bio surfactant usually exhibit emulsifying capacity but bio emulsifiers do not necessarily reduce surface tension. Because of the presence of hydrophobic and hydrophilic groups, surfactants partition preferentially at the interface between fluid phase of different degrees of polarity and hydrogen bonding [1]. These amphiphilic compounds have functional properties like surface and interface activity, emulsification, wetting, foaming, detergency, phase dispersing, solubilisation and density reduction of heavy hydrophobic compounds and find wide applications in industries [2].

The total surfactant production has exceeded 2.5 million tons in 2010 for many purposes such as polymers, lubricants and solvents. From the total surfactants output, about 54% of them is consumed as household or laundry detergents, with only 32% destined for industrial use [3].

Interest in microbial surfactants has been progressively escalating in recent years due to their diversity, ecofriendly nature, possibility of large-scale production, selectivity, performance under intense circumstances and their impending applications in environmental fortification [4].

Extracellular membrane vesicles partitioned hydrocarbons to form a micro emulsion, which plays an important role in alkane uptake by microbial cells. Vesicles of *Acinetobacter* sp. having a diameter of 20–50 nm and a buoyant density of 1.158 cubic g/cm, consists of protein, phospholipids and lipopolysaccharide. The cellular lipid content of *Pseudomonas nautica* increased in eicosanoid-grown cells up to 3.2 fold, compared with acetate-grown cells. Phospholipids, mainly phosphatidylethanolamines and phosphatidyl glycerides, were accumulated in eicosanoid-grown cells [5]. The present investigation was conducted by following objectives: production of bio surfactant by *Bacillus* sp using the carbon sources such as glucose, fructose, maltose, sorbitol, Xylose, mannitol and screening the bio surfactant and their microbial activity.

MATERIALS AND METHODS:

Collection of sample

Bacillus sp was isolated from coastal region – Cuddalore using Bushnell Haas agar supplemented with 0.1% (v/v) of crude oil and identified to species

by following Bergey's Manual of Determinative Bacteriology [6].

Isolation and screening of potential bacteria

Bacillus sp

Isolation, screening and identification of biosurfactant producing marine bacteria from sea sediment samples were collected from coastal region – Cuddalore, Tamil Nadu, India. These samples were transported to the laboratory in 250 ml pre-sterilized bottles and stored at 4°C until further processing. The central portions of the samples were serially diluted using pre-sterilized seawater and spread plated on Bushnell Haas agar prepared with sea water and supplemented with 1% (v/v) crude oil. After 4 days incubation, morphological distinct colonies were isolated, and sub cultured 4-5 times on Zobell marine agar plates to obtain axenic cultures and were preserved as lyophilized stocks stored at 4°C for further studies [7].

Characterization of biosurfactant-producing isolates

The selected biosurfactant-producing bacteria were characterized morphologically and biochemically.

Screening of biosurfactant producing microorganism

Biosurfactants production is detected by various techniques.

Drop Collapsing technique

Hemolytic activity

CTAB Agar Plate method

Drop collapsing technique

The isolates were grown in BH medium with diesel as carbon source, incubated with shaking for 48 hours at 37°C and 200 rpm. The glass slides used was rinsed with hot water, ethanol and distilled water, and dried. The slides were coated with diesel and equilibrated for 24 hours to ensure a uniform oil coating. 1µl of supernatant sample was then applied to the center of the oil drops using 10µl micropipette. The results were monitored visually after 1 hour. If the drop remained beaded, the result was scored as negative. If the drop collapsed, the result was scored as positive.

Heamolysis test

Fresh colonies were prepared by streaking on nutrient agar and incubate at 37°C for 24hrs. These fresh single colonies of culture are restreaked into blood agar plates and the plates were incubated at 37°C 48-72hrs. The bacterial colonies were then observed for the presence of clear zones around the colonies.

These clear zones were used as qualitative method for biosurfactant production.

CTAB Agar Plate method

The CTAB agar plate method is a semi-quantitative assay for the detection of extra cellular glycolipids or other anionic surfactants. It was developed by Siegmund and Wagner. The microbes of interest are cultivated on a light blue mineral salts agar plate containing the cationic surfactant cetyltrimethylammonium bromide and the basic dye methylene blue. If anionic surfactants are secreted by the microbes growing on the plate, they form a dark blue, insoluble ion pair with cetyltrimethylammonium bromide and methylene blue. Thus, productive colonies are surrounded by dark blue halos.

Biosurfactant production on MSM supplemented with different carbon source in the form of oil

The *Bacillus* sp isolate was inoculated in Mineral salt liquid medium and is used for production of biosurfactant. The pH was adjusted to 7 before autoclaving at 121°C for 15 minutes. After sterilization, 2 % of the different carbon sources such as glucose, fructose, xylose, maltose, sorbitol, mannitol and sucrose separately were added. Then a bacterium was inoculated in to the mineral salt medium and it was placed on a reciprocal shaker at 100rpm at 37°C for 3 days.

Biosurfactant recovery and Antibacterial activity

The culture broth was centrifuged (10000 rpm, 15min) to remove the cells and there after sterilized with membrane filter. The clear sterile supernatant served as the source of the crude biosurfactant that recovered from the cell free culture supernatant by acid precipitation method. The culture supernatant

was acidified with 6N HCl to obtain pH of 2.0. The extraction is performed twice with an equal volume of ethyl acetate. Pooled solvent extract were concentrated using an evaporator under reduced pressure and antibacterial activity by using Agar well diffusion method against selected human pathogens such as *Escherichiacoli*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Bacillus cereus* and *Staphylococcus aureus* [8].

RESULT:

In this study, marine sediment samples were gathered from coastal region – Cuddalore (Dt), TN, India. The examples were handled for the isolation of biosurfactant bacteria and among ten colonies of bacteria were acquired on Bushnell Haas agar plate. Among them, only one bacteria produced biosurfactant. So examined the selected bacteria by morphologically unmistakable colony was separated and named as NA-1 (Table-1).

Screening of Biosurfactant isolate-NA-1

10 µl cell suspension of each strain was placed on the polystyrene coated glass plate that was coated by immersion oil. If the cell suspension contains biosurfactant then the drop collapses or spread due to the reduction of hydrophobic surface and if there is no biosurfactant in the cell suspension then the drops remain stable as the polar water molecules are repelled from the hydrophobic surface. Stability of the drop depends on biosurfactant concentration. Only one strain NA-1 gave positive result and also that forming a clear zone around the colonies on blood agar plate, it positive result in CTAB Agar Plate method. According to the screening, the selected

NA-1 confirmed biosurfactant producing bacteria (Table-2).

Table 1: Characterization of biosurfactant-producing isolate-NA-1

S. No	Test	Result
1.	Grams staining	Gram positive rods, central or terminal, ellipsoidal or cylindrical spores
2.	Motility test	Positive
3.	Catalase test	Positive
4.	Oxidase test	Positive
5.	Indole	Negative
6.	Methyl red	Negative
7.	Voges proskauer	Positive
8.	Citrate utilization	Positive

Table 2: screening of Biosurfactant isolate-NA-1

S. No	Screening techniques	Result
1.	Drop collapse test	Positive (Droplet collapse with the hydrocarbon)
2.	Haemolysis Test	Positive
3.	CTAB Agar Plate method	Positive

The utilization of 7 carbon sources by NA-1 isolates was studied. Among the carbon sources, glucose, fructose, maltose, sorbitol, Xylose and mannitol were utilized by isolate where NA1 sucrose was not utilized (Table 3).

Antibacterial activity

20 ml of Mueller-Hinton agar was poured on Petri plates and overnight of bacterial pathogens broth

culture was swabbed on the surface of the agar media and further well was prepared by using well cutter. To each well 20µl, 40µl, 60µl and 80µl of crude extract from NA-1 was added and incubated at 37° C for 24hrs kept in a thermostat incubator. After incubation, the zone of inhibition around the well was measured and indicated mm in diameter **Tabl-4** and **Fig: 1**.

Table 3: Utilization of carbon sources

S.No	Test	NA-1
1	Glucose	+
2	Fructose	+
3	Xylose	+
4	Maltose	+
5.	Sorbitol	+
6	Mannitol	+
7	Sucrose	-

+: Presence of growth; -: Absence of growth

Table 4:Anti-bacterial activity

S.No	Samples	Zone of inhibition (mm)				
		<i>P. aeruginosa</i>	<i>E. coli</i>	<i>K.pneumoniae</i>	<i>Staphylococcus aureus.</i>	<i>Bacillus cereus</i>
1	20µl	NZ	8	NZ	NZ	NZ
2	40 µl	10	11	08	09	NZ
3	60µl	13	15	12	11	08
4	80	17	19	14	14	11

NZ – No zone of inhibition

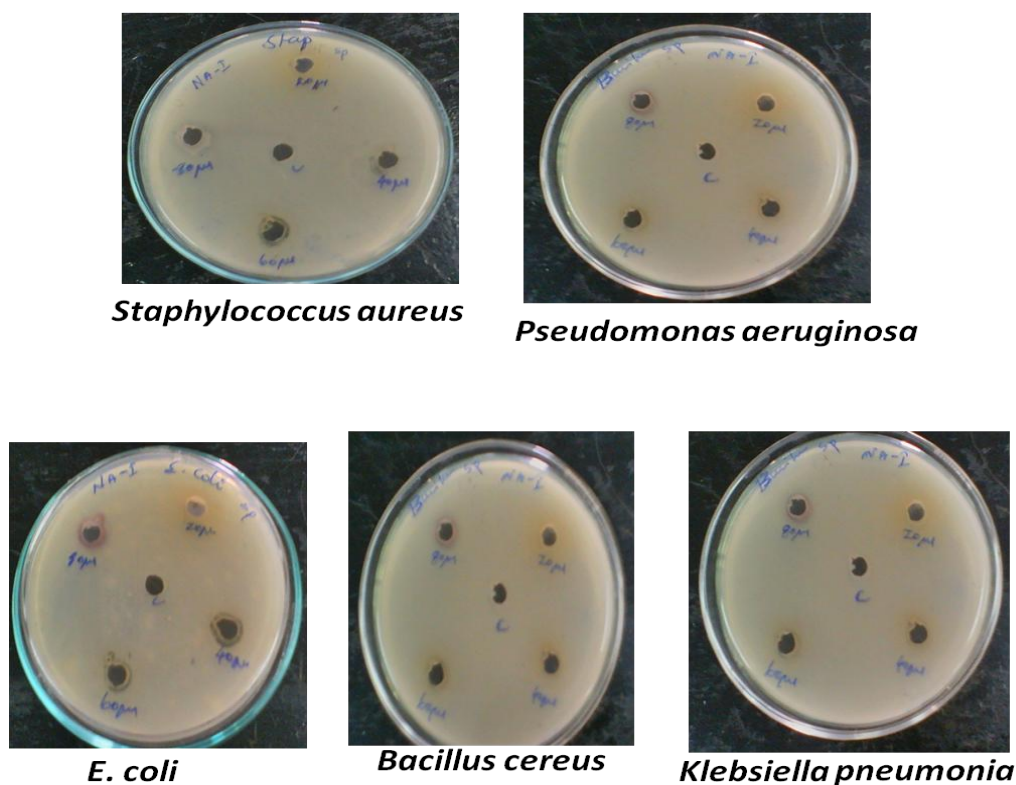


Fig 1: Anti-bacterial activity of biosurfactant- NA-1

DISCUSSION:

Ten bacterial strains were isolated from coastal region of Cuddalore a marine sediments and streaked in Bushnell Haas agar for several times and maintained at pure culture. These bacterial strains were screened to check the biosurfactant production using Drop collapse test, haemolysis test and CTAB Agar Plate method [9].

The screening of biosurfactant producing *Bacillus sp* by was investigated by hemolytic assay, drop collapse test, emulsification index, oil displacement test, and results shows similar to the studies as reported by Saravanan, 2012[1].

Our results illustrated antimicrobial activity and thus can be useful in many domestic and commercial uses. The isolated biosurfactant non-selectively showed activity against both Gram-positive and Gram negative bacterial strains. This is quite contrasting to earlier reports on antimicrobial actions of the bio surfactants where the lipopeptide biosurfactants have been reported to be active mostly against Gram-positive bacteria [3].

The antimicrobial properties of the bio surfactants have been widely reported. However, the bio surfactants with antimicrobial properties reported till

date is produced mostly by the micro-organisms of terrestrial origin [10]. However, the number of reports on marine antimicrobial biosurfactant molecules is negligible, so their antimicrobial potential have not been explored in details. This problem was identified in the present work and the bio surfactants isolated from marine bacteria as well as petrochemical wastes were tested for antimicrobial action against a battery of pathogenic test organisms. Five pathogenic strains named as *Escherichia coli*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Bacillus cereus* and *Staphylococcus aureus* were taken for antimicrobial test. Among these ten strains, bio surfactants produced from NA-1 showed antimicrobial activity against selected pathogens. The previous study antimicrobial activities of the clinical isolates from diabetic foot ulcer pathogens were performed and are reported by Andrea *et al.*, (2007)[9].

CONCLUSION:

Now-a-days the production of biosurfactant is increasing due its properties like low toxicity, biodegradability, digestibility and eco-friendly technologies must be used to clean the environment

such as degradation by microorganisms. Biological methods are the processes that use plants (phytoremediation) or microorganisms (bioremediation) to remove these pollutants from soil. The present study focused on studying the production of biosurfactant by bacteria isolated from marine sediment sample selectively *Bacillus* sp. which is assumed to be potent biosurfactant producer and also used as antibacterial activity against selected pathogens.

REFERENCES:

1. Saravanan, V and Vijayakumar, S., 2012. Isolation and Screening of biosurfactant producing microorganism from oil contaminated soil. *Acad. Indus. Res.* 1 (5).
2. Anantharaj, B and Thivakaran, P. (2010). Isolation and production of biosurfactant producing organism from oil spilled soil. *JBiosci Tech*, 1 (3):120-126
3. Singh, P., Cameotra, S.S., (2004). Enhancement of metal bioremediation by use of microbial surfactants. *Biochem. Biophys. Res. Commun.*, 319, 291-297.
4. Gholamreza Dehghan-Noudeh, Mohammad Hasan Moshafi, Effat Behravan, Saeed Torkzadeh and Masoud Ahmadi Afzadi. 2009. Screening Three Strains of *Pseudomonas aeruginosa*: Prediction of Biosurfactant-Producer strain *American Journal of Applied sciences* 6(8):1453-1457.
5. Husain, D. R., Goutx, M., Acquaviva, M., Gilewicz, M., Bertrand, J.C., (1997). The effect of temperature on eicosane substrate uptake modes by a marine bacterium *Pseudomonas nautica* strain 617: relationship with the biochemical content of cells and supernatants. *World J. Microbiol. Biotechnol.*, 13: 587- 590.
6. Buchanan RE, Gibbons NE, Cowan ST, Holt TG, Liston J, Murry RG, Niven CF, Ravin AW, RY Stainer (1974) *Bergey's manual of determinative bacteriology*. Williams and Wilkins Co., Baltimore, 1246 p.
7. Thavasi R (2006) Biosurfactants from marine hydrocarbonoclastic bacteria and their application in marine oil pollution abatement. Ph.D. Thesis, Annamalai University, Chidambaram, 162 p.
8. Rosenberg, E. and Ron, E.Z. (1999) High and low molecular mass microbial surfactants. *Applied Microbiology and Biotechnology* 52, 154-162.
9. Andrea Giuliani Pirri, Silvia Fabiole Nicolette. 2007. Antimicrobial peptides an overview of a promising class of therapeutics. *Central European Journal of biology* 5(2): 21-22.
10. Youssef, N.H., Dunacn, K.E., Nagle, D.P., Savage, K.N., Knapp, R.M., McInerney, M.J., 2004. Comparison of methods to detect biosurfactant production by diverse microorganisms. *J. Microbiol. Methods* 56, 339-347.