International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 3, Issue 12 - 2016

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2016.03.12.029

Isolation and identification of Biosurfactant producing *Pseudomonas aeruginosa* from marine sediment samples and its Antimicrobial Properties

R.Sumathi[1]* and N.Yogananth[2]
[1]Department of Microbiology, Kanchi shri Krishna College of Arts and Science, Kilambi -631 551, Kancheepuram, Tamil Nadu, India
[2]Department of Biotechnology, Mohamad Sathak College of Arts and Science, Chennai, Tamil Nadu, India
*Corresponding author

Abstract

Oil contaminated marine sediment sample was collected from harbour, Chennai, Tamilnadu and transported to laboratory in sterile polythene bag. The diesel, petrol, kerosene and vegetable oils such as coconut oil, gingelly oil, groundnut oil and sunflower oil used in this study were collected from local petrol bunk and oil shop and stored separately in bottles before being added aseptically to the growth medium. Bushnell Haas (BH) liquid medium was used as the enrichment medium with 1 %(v/v) diesel as the sole carbon source to isol ate 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 7 days. The single colonies were streaked into nutrient agar plates incubated at 30°C overnight and stored at 4°C until further use. The organism was identified morphologically and biochemically as per NCCLS guidelines. Biosurfactant production was carried out in Mineral Salt (MS) medium with diesel, kerosene, petrol and vegetable oil such as coconut oil, gingelly oil, groundnut oil and sunflower oil as carbon source. The extraction of Biosurfactant was done by acid precipitation method. The preliminary characterization of crude surfactant was carried out by TLC and the biosurfacatant was identified as rhamnolipid. The estimation of rhamnose in the Biosurfactant was carried out by Anthrone's method. The antimicrobial activity of biosurfactant against clinical isolates was performed by AWD assay. The Biosurfactant showed effective inhibitory against the clinical isolates.

Keywords: Marine sediment, Biosurfactant organisms, antimicrobial activity.

Introduction

Surfactants are amphiphilic surface active agents possessing both hydrophilic and hydrophobic moieties that reduce surface and interfacial tensions by accumulating at the interface between two immiscible fluids like oil and water. They are of synthetic or biological origin. Due to their interesting properties such as lower toxicity, higher degree of biodegradability, higher foaming capacity and optimal activity at extreme conditions of temperatures, pH levels and salinity, there have been increasingly attracting the attention of the scientific and industrial community (Kosaric, 1992).

Surfactants are key ingredients used in detergents, shampoos, toothpaste, oil additives and a number of other consumer and industrial products. They

constitute an important class of industrial chemicals widely used in almost every sector of modern industry. 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 (Ganesh *et al*., 2009).

Biosurfactants are polymers, totally or partially extra cellular, amphipathic molecules containing polar and non polar moieties which allow them to form micelles that accumulate at interphase between liquids of different polarities such as water and oil thereby reducing surface tension and facilitating hydrocarbon uptake and emulsification.

In order to reduce or eliminate the effect of oil spillage on the environment and living organisms, effort such as applications of chemical dispersant, skimming of the surface oils. Application of biological oil agents and inoculating the spilled area with relevant bacteria are the outcomes of intensive research. The most promising of many research carried out to deal with large scale oil spillage is the use of microorganisms to provide an effective alternative.

Surface-active compounds produced by microorganisms are of two main types, those that reduce surface tension at the air-water interface (biosurfactants) and those that reduce the interfacial tension between immiscible liquids, or at the solid-liquid interface (bioemulsifiers). Biosurfactants usually exhibit emulsifying capacity but bioemulsifiers 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. These amphiphilic compounds have functional properties like surface and interface activity, emulsification, wetting, foaming, detergency, phase dispersing, solubilization and density reduction of heavy hydrophobic compounds and find wide applications in industries (Walter, 2010). 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.

The interest in biosurfactant has been steadily increasing in recent years due to the possibility of their production through fermentation and their potential applications in such areas as the environmental protection (G.S. Kiran, 2010).

Majority of surfactants produced today is of petrochemical origin beside of the renewable resources like fats and oils. Petroleum-related industries have been identified as one of the major source of pollution in all countries. At present, biosurfactants plays an important application in petroleum-related industries which is use in enhanced oil recovery, cleaning oil spills, oil-contaminated tanker cleanup, viscosity control, oil emulsification and removal of crude oil from sludge.

Materials and Methods

Collection of Soil Sample

Oil contaminated marine sediment sample was collected from harbour, Chennai, Tamilnadu and transported to laboratory in sterile polythene bag. The diesel, petrol, kerosene and vegetable oils such as coconut oil, gingelly oil, groundnut oil and sunflower oil used in this study were collected from local petrol bunk and oil shop and stored separately in bottles before being added aseptically to the growth medium.

Isolation and identification of bacterial diesel degraders

Bushnell Haas (BH) liquid medium (Bushnell and Haas, 1941; Atlas and Bartha, 1992) was used as the enrichment medium with 1 %(v/v) diesel as the sole carbon source to isolate diesel degrading bacteria.
1 g of the marine sediment sample was added to 100 ml of the enrichment medium and incubated at 30°C in a rotary shaker at 160rpm. After two weeks, 1 ml of enriched medium was transferred into freshly prepared enrichment media and incubated at the same conditions as described earlier.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.

Characterization of biosurfactant-producing isolates - Morphological Analysis

The selected biosurfactant-producing bacteria were characterized morphologically and biochemically according to NCCLS standards.

Screening of biosurfactant producing microorganism

Biosurfactants production is detected by various techniques such as follows and performed in triplicates.

1. Drop Collapsing technique
2. Hemolytic activity
3. Bacterial adhesion to hydrocarbons (BATH) assay

4. Oil displacement test
5. Emulsification stability test
6. CTAB Agar Plate method
7. Penetration assay
8. Microtitre plate method

Drop collapsing technique

The isolates were grown in BH medium with diesel as carbon source, incubated with shaking for 48 hours at 37ºC 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. 1 µl 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.

Bacterial adhesion to hydrocarbons (BATH) assay

The hydrophobicity of the cells can be measured by BATH assay (Rosenberg *et al.,* 1980). Bacterial cells were washed twice with phosphate buffer salt solution (K_2HPO_4 16.9g/l, KH_2PO_4 7.3g/l) with pH 7 and were suspended in it to give an optical density of ~ 0.5 at 600nm. 100µl of crude oil (petrol) was added to 2ml of cell suspension and was vortex shaken for 3 min in test tubes. After shaking, crude oil and aqueous phase were allowed to separate for 1hour. OD of the aqueous phase was then measured at 600nm in a spectrophotometer. Hydrophobicity is expressed as the percentage of cell adherence to crude oil and was calculated as follows:

$$100 * (1 - OD\ of\ the\ aqueous\ phase / OD\ of\ the\ initial\ cell\ suspension).$$

Three independent determinations were made and the mean values were calculated.

Oil displacement assay

The oil displacement or spreading assay was developed by Morikawa *et al*.2000) For this assay, 2 µl of diesel is added to the surface of 15 ml of distilled water in a petridish to form a thin oil layer. Then, 10 µl of culture or culture supernatant are gently placed on the centre of the oil layer. If biosurfactant is present in the supernatant, the oil is displaced and a clear zone is formed. The diameter of this clearing zone on the oil surface correlates to surfactant activity, also called oil spreading activity.

Emulsification ability assay

For measuring the emulsification index, diesel is added to an equal amount of culture. 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* is calculated as the ratio of the height of the emulsion layer and the total height of liquid. The same is done for petrol, kerosene and vegetable oil.

$$E = \frac{\text{Height of the emulsion}}{\text{Total height of the liquid}} \times 100$$

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.

Penetration Assay

Maczek *et al* developed another assay suitable for high throughput screening, the penetration assay. This assay relies on the contacting of two unsoluble phases which leads to a color change.For this assay, the cavities of a 96 well microplate are filled with 150 µl of a hydrophobic paste consisting of oil and silica gel. The paste is covered with 10 µl of oil. Then, the supernatant of the culture is colored by adding 10 µl of a red staining solution to 90 µl of the supernatant. The colored supernatant is placed on the surface of the paste. If biosurfactant is present, the hydrophilic liquid will break through the oil film barrier into the paste. The silica is entering the hydrophilic phase and the upper phase will change from clear red to cloudy white within 15 minutes. The described effect relies on the phenomenon that silica gel is entering the hydrophilic phase from the hydrophobic paste much more quickly if biosurfactants are present. Biosurfactant free supernatant will turn cloudy but stay red.

Microplate Assay

The surface activity of individual strains can be determined qualitatively with the microplate assay developed and patented by Vaux and Cottingham. This assay is based on the change in optical distortion that is caused by surfactants in an aqueous solution. Pure water in a hydrophobic well has a flat surface. The presence of surfactants causes some wetting at the edge of the well and the fluid surface becomes concave and takes the shape of a diverging lens. For this assay, a 100 µl sample of the supernatant of each production medium is taken and put into a microwell of a 96-mircowell plate. The plate is viewed using a baking sheet of paper with a grid. If biosurfactant is present, the concave surface distorts the image of the grid below. The optical distortion of the grid provides a qualitative assay for the presence of surfactants**.**

Optimization of Growth

Bacterial growth was optimized using different parameters such as pH, temperature and nitrogen source.

Effect of pH

The growth of biosurfactant producing *Pseudomonas aeruginosa* strain at different pH ranging from 6,6.5,7,7.5,8 and 8.5 was analysed.

Effect of temperature

The growth o f biosurfactant producing *Pseudomonas aeruginosa* strain at different temperature ranging from 35,36,37,38 and 39 was analyzed.

Effect of nitrogen source

The growth of biosurfactant producing *Pseudomonas aeruginosa* strain on different nitrogen sources such as ammonium chloride, sodium nitrate, ammonium nitrate and ammonium sulphate was analyzed.

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

The *Pseudomonas aeruginosa* isolate was inoculated in Mineral Salt liquid medium for Biosurfactant production. The pH was adjusted to 7 before autoclaving at 121ºC and for 15 minutes. After sterilization, 2 % of the different carbon sources such as diesel, petrol, kerosene and vegetable oil such as coconut oil, gingly oil, groundnut oil and sunflower oil were added. Then bacteria 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

The culture broth was centrifuged (10000 rpm, 15 min) to remove the cells and there after sterilized with membrane filter. The clear sterile supernatant served as the source of the crude biosurfactant. The biosurfactant was 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. White precipitate was formed is used for TLC and AWD assay against selected human pathogens.

Thin Layer Chromatography

Preliminary characterization of the biosurfactant was done by TLC method. A portion of the crude biosurfactant was separated on a silica gel plate using chloroform: methanol: water (70:10:0.5, v/v/v) developing solvent system. Anthrone reagent (1 g

anthrone in 5 mL sulfuric acid mixed with 95mL ethanol) was used to detect the presence of rhamnolipid as yellow spot.

Structural characterization

Rhamnose test

The presence of carbohydrate groups in the biosurfactant molecule was assayed by rhamnose test. A volume of 0.5 ml of cell supernatant was mixed with 0.5 ml of 5%. Phenol solution and 2.5 ml of sulfuric acid, and incubated for 15 min before measuring absorbance at 490 nm.

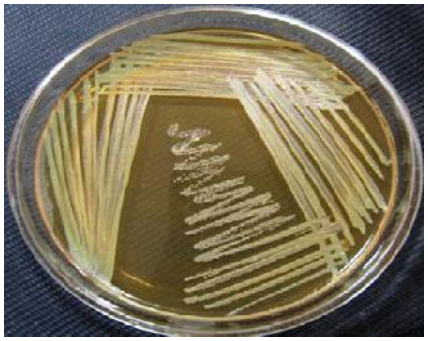
Antimicrobial susceptibility test by AWD assay

The clinical isolate of pathogenic microorganisms obtained from diabetic foot ulcer were subjected for their susceptibility against the biosurfactant obtained by Agar Well Diffusion (AWD) assay on Muller-Hinton agar plates.

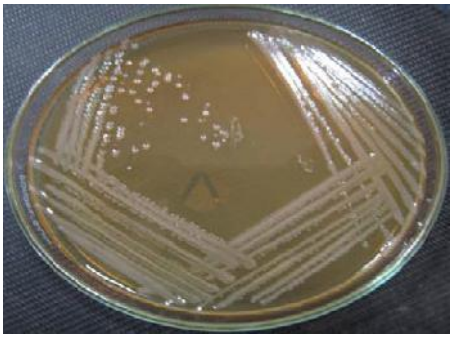
Results

Isolation of bacterial colonies

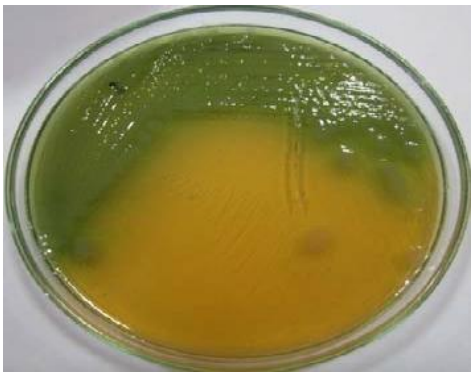
Four strains observed with different colony morphology were streaked on Nutrient Agar plates to obtain pure cultures such as MS1,MS2,MS3 and MS4. Figure – 1.



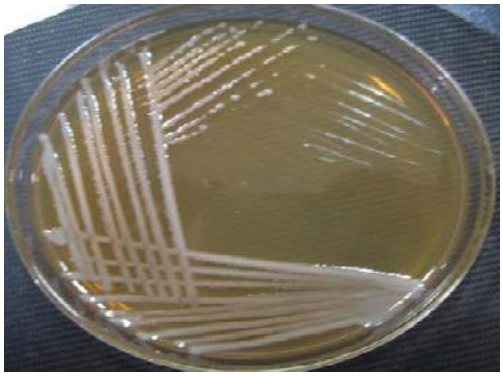
MS1



MS2



MS3



MS4

Screening of biosurfactant

All the four isolated strains were named as MS1, MS2, MS3 and MS4 respectively. All the strains were screened for the biosurfactant production to identify the strain with shows good results were selected for further study.

Drop collapse test

This assay relies on the destabilization of liquid droplets by surfactants. 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 was reduced. All the strains were tested for drop collapse among which MS3 isolate is positive.

Hemolysis test

All the strains were streaked on blood agar plates. All the six strains showed positive results for haemolytic activity i.e., formation of a clear zone around the colonies.

BATH assay

In this assay a turbid, aqueous suspension of washed microbial cells was mixed with a distinct volume of a hydrocarbon, petrol. After mixing for 2 minutes, the two phases were allowed to separate. Hydrophobic cells were bound to hydrocarbon droplets and had risen with the hydrocarbon. They were removed from the aqueous phase. The turbidity of the aqueous phase was measured. The decrease in the turbidity of the aqueous phase correlates to the hydrophobicity of the cells. The percentage of cells bound to the hydrophobic phase (H) is calculated by:

$$H = (1-A/A_0) *100$$

Where, A_0 is the absorbance of the bacterial suspension without hydrophobic phase added and A the absorbance after mixing with hydrophobic phase (Table 1). Among the four strains the MS3 showed high adhesion activity.

Table 1: BATH assay readings

Strains	A_0	A	H%
MS1	0.97	0.76	27
MS2	0.89	0.76	17
MS3	1.96	0.98	50
MS4	0.75	1.47	25

Oil displacement assay

The supernatant of the four strains were added to the plates containing oil. The strain MS3 displaced the oil showing a zone of displacement. The organisms which produce biosurfactant can only displace the oil. The results obtained were noted down (Table 2).

Table 2: Oil spreading technique

STRAIN	DIAMETER (in cm)	INTERPRETATION
MS1	0.7	NEGATIVE
MS2	1.7	NEGATIVE
MS3	5.2	POSITIVE
MS4	0	NEGATIVE

Emulsification ability assay

The emulsification index *E* is calculated as the ratio of the height of the emulsion layer and the total height of liquid. The results for emulsification index of biosurfactant producing strain MS3 for diesel, petrol, kerosene and vegetable oil such as coconut oil, gingly oil, groundnut was given in Table – 3. The maximum emulsification index is obtained for diesel.

Table – 3 Emulsification index

S.No	Emulsification ability test (E24 index) for MS3 strain	Height of emulsion in mm	% of emulsification index
1.	Diesel	**18 mm**	**52%**
2.	Petrol	15mm	44%
3.	Kerosene	17mm	**50**%
4.	Coconut oil	16mm	47%
5.	Giggly oil	15mm	44%
6.	Grounut oil	15mm	44%
7	Sunflower oil	17mm	**50**%

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. Productive colonies surrounded by dark blue halos was observed in the MS3 isolate.

Penetration Assay

Maczek *et al* developed another assay suitable for high throughput screening, the penetration assay. This assay relies on the contacting of two unsoluble phases which leads to a color change showed by MS3 strain.

Microplate Assay

The surface activity of individual strains can be determined qualitatively with the microplate assay developed and patented by Vaux and Cottingham. This assay is based on the change in optical distortion that is caused by surfactants in an aqueous solution. Pure water in a hydrophobic well has a flat surface. The presence of surfactants causes some wetting at the edge of the well and the fluid surface becomes concave and takes the shape of a diverging lens which shows positive for MS3 strain.

Table – 3 Screening of Biosurfactant producing MS3 strain

S.No	Screening technique	Result
1.	Drop Collapsing test	+ (Droplet collapse with the hydrocarbon)
2.	Hemolysis activity	+ (α – hemolysis was observed)
3.	BATH assay	+
4.	Oil Spreading technique	+
5.	Emulsification ability test	+
6.	CTAB Agar Plate method	+
7.	Penetration assay	+
8.	Microplate assay	+

Biochemical characterization of the MS3 strain The results for the biochemical characterization of the isolate is given in Table – 4.

Table – 4 Biochemical characteristics

S.NO	BIOCHEMICAL TEST	RESULT
1.	Gram Staining	Gram Negative rod shaped organism
2.	Motility test	Motile rods
3.	Catalase test	Positive
4.	Oxidase test	Positive
5.	Indole	Negative
6.	Methyl red	Negative
7.	Voges proskauer	Negative
8.	Citrate utilization	Positive
9.	Triple sugar iron test	Acid butt, alkaline slant, H_2S^{-ve}
10.	Urease	Negative

Based on the cell morphology, Gram's staining reaction,biochemical characteristics and green pigment producing colony morphology the MS3 strain was identified as *Pseudomonas aeruginosa.*

Growth optimization studies of MS3 strain

The optimum pH, Temperature and Nitrogen source that showed effective biomass of the Biosurfactant producing MS3 strain was given in Table – 5.

S.No	Parameter	Optimum values	Biomass U/ml
1.	pH	7.5	0.601
2.	Temperature	37^0C	0.561
3.	Nitrogen source	$NaNO_3$	0.524

Biosurfactant recovery

The biosurfactant was recovered from the cell free culture supernatant by acid precipitation method and is identified for the presence of Rhamnolipid by TLC.

Thin Layer Chromatography

The results for the TLC biosurfactants obtained acid precipitation were given in the Table -6.

Table -6 Thin Layer Chromatography

Hydrocarbon	Rf Value
Diesel	0.72
Petrol	0.67
Kerosene	0.61
Sunflower oil	0.62

Rhamnose test

The results for the presence of carbohydrate groups in biosurfactant molecule was assayed by rhamnose test was given in Table – 7 .

Table – 7 Optimization of Rhamnose

Reagents	Blank	S1	S2	S3	S4	S5	Test
Rhamnose (Diesel)	_	0.2	0.4	0.6	0.8	1	0.5
Distilled Water	1	0.8	0.6	0.4	0.2	_	0.5
Phenol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sulfuric acid	2.5	2.5	2.5	2.5	2.5	2.5	2.5
OD at 490nm	0	0.76	1.02	1.10	1.38	1.70	0.80

Antimicrobial susceptibility test by AWD assay

The results for the antimicrobial activity of the biosurfactant by growing *Pseudomonas aeruginosa* in MSM was shown in Table – 8.

Table – 8 Antimicrobial susceptibility test by AWD assay

S.No	Biosurfactant source	Zone of inhibition in mm				
		Staphylococcus aureus	***Escherischia coli***	***Klebsiella sp.***	***Salmonella typhi***	***Vibrio sp.***
1.	Diesel	**7**	12	18	12	**20**
2.	Petrol	15	**15**	**15**	**10**	20
3.	Kerosene	10	10	10	10	10
4.	Coconut oil	10	10	10	0	10
5.	Gingelly oil	18	10	12	0	15
6	Grounut oil	2	10	8	0	10
7.	Sunflower oil	14	10	12	5	5
8.	Standard antibiotic	10	18	22	28	22

Discussion

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 (Ganesh *et al*., 2009).

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.

Pseudomonas is a genus of Gram negative aerobic γ-proteobacteria, belonging to the family *Pseudomonadaceae* containing 191 validly described species. The members of the genus demonstrate a great deal of metabolic diversity, and consequently are able to colonize a wide range of niches. Their ease of culture in vitro and availability of an increasing number of *Pseudomonas* strain genome sequences has made the genus an demonstrate a great deal of metabolic diversity, and consequently are able to colonize a wide range of niches. Their ease of culture in vitro and availability of an increasing number of *Pseudomonas* strain genome sequences has made the genus an excellent focus for scientific research; the best studied

species include *Pseudomonas aeruginosa*. Some members of the genus *Pseudomonas* are able to metabolise chemical pollutants in the environment, and as a result can be used for bioremediation.

The present study focused on studying the production of biosurfactant by bacteria isolated from marine sediment sample selectively *Pseudomonas aeruginosa*, which is assumed to be potent biosurfactant producer.

The screening of biosurfactant producing *Pseudomonas aeruginosa* by was investigated by hemolytic assay, drop collapse test, emulsification index **,** oil displacement test showed the results similar to the studies reported by Saravanan.V, 2012. The optimization of growth that shows maximum yield of biomass obtained was pH 7.5 (OD - 0.601), temperature37°C (OD-0.561) and the nitrogen source NaNO3 (OD-0.524) which is nearly as similar as S.Dhail, 2013. The results for TLC analysis of the biosurfactant was reported as done by Priya .T and G.Usharani, 2009 which shows the yellow colour development. The results for antimicrobial activity of the clinical isolates from diabetic foot ulcer pathogens were performed and are reported by Andrea *et al*., 2007.

References

Adeline., Ting S. Y., Carol., Tan H.C Tan and AW,C.S.2009.Hydrocarbon-degradation by isolate *Pseudomonas lundensis* UTAR FPE 2.*Malaysian Journal of microbiology*. 5(2):104-108.

Adria A. Bodour., Claudia Guerrero- Barajas., Beth V.Jiorle., Mark E. Malcomson., Amanada K.Paull., Arpod Somogyi., Long N.Trinhs., Robert B.Bates and Raina M.Maier.2004.Structure and characterization of flavolipids novel class of Biosurfactants produced by *Flavobacterium* Species strain MTN11. *Appl Environ Microbiol*. 70(1): 114–120.

Ainon hamzah, Noramiza sabturani and Shahidan Radiman.2013. Screening and optimization of biosurfactant production by the hydrocarbon degrading bacteria. *Sains malysiana* 42(5):615-623.

Ali Diab and Shereen Gamal El Din. 2013. Production and characterization of biosurfactants produced by *Bacillus* spp and *Pseudomonas* spp isolated from the Rhizosphere soil of an Egyptian salt marsh plant. *Nature and Science*. 11(5).

Aliahmadi A., Raghanian,R., Emitiazi,G and Ghassempour,A. 2011. A simple method for primary screening of antibacterial pepties in plant seeds. *Iranian Journal of microbiology*. 3 (2):104-108.

Anandi C., Alaguraja,D., Natarajan,V., Ramanathan, M., Subramaniam, C.S., Thulasiram, M, Sumithra, S. 2004.Bacteriology of Diabetic Foot Lesions. *Indian Journal of Medical Microbiology*; 22 (3) : 175 – 178.

Anantharaj,B and Thivakaran,P.2010. Isolation and production of biosurfactant producing organism from oil spilled soil.*J Biosci Tech*, 1 (3):120-126

Andrea Giuliani Pirri, Silvia Fabiole Nicolette.2007.Antimicribial peptides an overview of a promising class of therapeutics. *Central European Journal of biology* 5(2): 21-22

Anuradha,S Nerurkar, Krushi,S Hingurao and Harish G Suthar.2009. Bioemulsifiers from marine microorganisms. *Journal of scientific & Industrial Research.* 68. 273-277.

Armstrong,DG., Liswood,PJ and Todd W.F., 1995. William J Stickel Bronze Award. Prevalence of mixed infections in the diabetic pedal wound. A retrospective review of 112 infections. *J Am Podiatr Med Assoc.* 85(10):533-7.

Arora,S.K., Jitesh Sony, Anayata Sharma and Mridul Taneja. 2015. Production and Characterization of biosurfactant from *Pseudomonas* spp. *Int.J.Curr.Microbiol.App.Sci*. 4 (1): 245-253

Asfar SK, al-Arouj M, al-Nakhi A, Baraka A, Juma T, Johny M. 1993. Foot infections in diabetics: the antibiotic choice. *Can J Surg*.36(2): 170-172.

Atul Pratap Singh., Singh, R. B., Mishra, Sanjay.2012. Microbial and Biochemical Aspects of Antibiotic producing Microorganisms from soil samples of *certain Industrial area of India*. J *of general microbiology*. 5:105-112.

Berheimer,A. W and Luis S.Avigad.1970. Nature and properties of a cytolytic agent produced by *Bacillus subtilis*. *Journal of general microbiology*.6:361-369.

Bidnenko,SI., Mikhnevych,OE., Sapa,SA,Dan'kevych, VP. 2001. Bacteriological investigation and treatment strategies of patients with diabetic foot complicated by purulent necrosis. *Klin Khir*.3: 38 - 40.

Boutoille,D., Leautez,S., Manlaz,D., Krempf M. RaffiF. 2000. Skin and Osteoarticular infections. *Presse Med*. 29 (7): 393 - 395

Cooper,D.G., Macdonald,C.R., Duff S. J. B and Kusart N.C.1981. Enhanced production of surfactinfrom *Bacillus subtilis* by continuous product removed and metal contain additient. *Applied and environmental microbiology*.42(3):408-412.

Dang CN, Prasad YD, Boulton AJ, Jude EB. 2003.Methicillin – resistant *Staphylococcus aureus* in the diabetic foot clinic : a worsening problem. *Diabetmed* 20 (2):159-161.

David G.Cooper and Beena G.Coldberg. 1987. Suface active agent from two *bacillus* species. *Applied and environmental microbiology*.224-229.

Dhail, S.2013.Biosurfactant-producing bacteria and characteristics of selected biosurfactant from marine sample. ICOEST *Cappadocia*, turkey, 18-21.

Dhail,S and N.K. Jasuja. 2012. Isolation of biosurfactant producing marine bacteria. *African journal of environmental science technology* 6(6):263-266.

Erhrat Gharaei-Fathabed, 2010. Biosurfactant in pharmaceutical Industry. *American J.of Drug Discovery and Development*. 1: 58-69.

Fabiala costa., Isabel,F Carvalho.,Ronaid,C.Montelaro., Gomes,P and Christina,M.Martins.2011.Covalent immobilization of antimicrobial peptides(AMPS) onto biomaterial surfaces. *Actabiomaterialia*.7(4):1431-1440.

Fatima Menezes Bento., Flavio A de Oliveira Camargo., Benedict, C Okeke and William T.Franken bege. 2005.Diversity of Biosurfactant producing micro organisms isolated from soils contaminated with diesel oil. *Microbiological research*.160(3):249-255.

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.

Gokhan Coral and Serap Karagoz. 2005. Isolation and characterization of phenanthrene. degrading bacteria from a petroleum refinery soil. *Annals of Microbiology*,55(4):255-259.

Wheat LJ, Allen SD, Henry M, Kernek CB, Siders JA, Kuebler T, Fineberg N, Norton J. 1986. Diabetic foot infections. Bacteriologic analysis. *Arch Intern Med.* 146(10):1935-40.

Jyothi K., Surendra Babu.K., Nancy Clara,K and Amita Kashyap.2012.Identification and Isolation of hydrocarbon Degrading bacteria by Molecular Characterization. *Helix* 2:105-111.

Karanth,N.G.K.,Deo, P.G. and N.K.Veenanadig.,1999.Microbial production of biosurfactants& their importance.*Curr.sci*.,77;116-123.

Kengne,A.P., Chouxem,,S.P., Dehayem,Y.M., Simo,N.L., Fezeu,L.L., Mbanya JC.2006. Diabetic foot ulcers in Cameroon : Can microflora prevalence inform probabilistic antibiotic treatment. *J Wound Care*.15 (8): 363 -366.

Layne O Gentry. 1993. Diagnosis and management of the diabetic foot ulcer. *Journal of Antimicrobial Chemotherapy*. 32:77- 89.

Luis Yuste, Maria Eugenia Corbella,Maria Jesus Turiegano, Ulrich karlson, Antonio Pryet, Fernando Rojo.2000.*FEMS Microbiology Ecology*.32:69-75.

MacFarlane I A, Brown R M, Smyth RW, Burdon DW and Fitzgerald MG. 1986. Bacteraemia in diabetics. *Journal of Infections*.12(3):213-219.

Mahesh N., Murugesh S and Mohana Srinivasan V. 2006. Determination of the presence of Biosurfactant produced by the Bacteria present in the soil samples. *Research Journal of Microbiology*. 1(4):339-345.

Marvaso A, Esposito S, Noviello S, Ianniello F, Leone S, Maiello A, Petronella P.2002. Outpatient parental antibiotic therapy (OPAT) of diabetic foot infections with piperacilllin / tazobactam. *Infez Med.*10(4) : 230-235.

Morikawa M, Daido H, Takao T, Murata S, Shimonishi Y, Imanaka T.1993.A new lipopeptide biosurfactant produced by *Arthobacter species* strain MIS38. *Journal of bacteriology*.175:6459-6466.

Mathiyazhagan N, Danashekar K and Natarajan D,2011.Amplification of biosurfactant producing gene (rhlb) from *Pseudomonas aeruginosa* isolated from oil contaminated soil. *International Journalof pharma and bioscience* 2(1):497-504

McLigeyo, Otieno LS. 1991.Diabetic Ulcers – a clinical and bacteriological study. *East Aftr Med J*.68 (3):204-210

McLigeyo, Oumah S, Otieno, Saaul L. 1990. Diabetic ulcers - a clinical and bacteriological study. *West Aft J Med*. 9(2): 135-138.

Michael Rossini JR. 1992. Bateriology of 100 consecutive Diabetic foot infections and invitro susceptibility to Ampicillin / Sulbactum versus cefoxitin. *Angiology*. Vol 43, No. 4; 357 – 361.

Mohammad Reza Samaci., Syed Bagher Mortazavi., Bita Bathri and Ahmed Jonidi Jafari.2012.Isolation and characterization of bacteria degrading n-Hexadecane from soil. *International conference on biological and life science*. 40

Mukeshkumar D J., Amutha Magesh B., Devika S., Balakumaran M D and kalaichelvan P T.2012. Biodegradation of polycyclic Aromatic Hydrocarbons by *Pseudomonas* sp, Pss6 Isolated from Municipal wastes sediment. *Der chemical sinica*. 3(3):543-547.

Nadem,H. Hayder., Sura Alaa and Hutaf Abdulmalik.2014. Optimized Conditions for Bioemulsifier production by Local *Streptomyces* sp. SS 20 isolated from hydrocarbon contaminated soil. *Romanian Biotechnological Letters*. 19 (1).

Nikolopoulou,M., Eickenbusch.P and PasadakisN.D.2011.Evaluation of autochthonous bioaugmentation to combat marine oil-spills. *Environmental Engineering and Management Journal*.11(3).

Okolieghe,I.N and Agary.O.O,2012. Application Microbial Surfactant. *Journals of Biotech*. 1(1):15-23.

Orathai pornsunhorntavee, Wongpanit and Ratana Rujiravanit. 2010. Rhamnolipid biosurfactant production and their potential in environmental biotechnology. *Adv Exp Med Biol*. 672:211-221.

Padmapriya B., Suganthi S and Anishya R. S.2013. Screening, Optimization and Production of Biosurfactants by *Candida* species Isolated from oil polluted Soil. *American Eurasian J. Agric & Environ. Sci*. 3(2):227-233.

Palashpriya das., 2008.Genetic Regulation of the Bio synthesis of Microbial Surfactants. *Biotech.and Genetic engineering Review* 25: 165-186.

Prabhakar P, Rao AB, Hira J., 1981.Bacteriological study of diabetic foot ulcers. *Trop Geogr Med*.33 (3): 249-252

Priya, T and G.Usharani.2009.Comparative study for biosurfactant production by using *Bacillus subtilis* and *Pseudomonas aeruginosa*. *Botany Research international*. 2(4):284-287

Raza Zulfiqar Ali., Muhammad saleem Khan.,Zafar M.Khalid and Aama Rehman.2006.Production of Biosurfactant using Different Hydrocarbons by *Pseudomonas aeruginosa* EBN-8 Mutant. *Z Naturforsch C*. 61(1-2):87-94.

Rodrigues Ligia R., Jose A. Teixeira., Henny C.Vander Mei and Rosario Oliveira.2006.Physicochemical and functional characterization of a biosurfactant produced *by Lactococcus lactis* 53.*Biointerfaces*.49:78-85.

Saharan,B.S.,R.K. Sahus and D.Sharma. 2012. A review on Biosurfactant Fermentation, Current Developments and Perspectives. *Genetic Engineering and Biotechnology Journal*.29.

Santhini.K and R.Parthasarathi. 2014. Isolation and Screening of biosurfactant producing microorganisms from hydrocarbon contaminated soils from automobile workshop. *Int.J of Pharm and Biol Archives*.5 (2):158-167

Saravanan,V and Vijayakumar.S., 2012.Isolation and Screening of biosurfactant producing microorganism from oil contaminated soil..*Acad.Indus.Res*.1(5).

Satpute,S K.,Bhawsar B.D.,Dhakephalkar P.K and Chopade B.A.2008. Assessment of different screening methods for selective Biosurfactant producing marine bacteria. *Indian Journal of Marine Sciences*. 37(3):243-250.

Shanmugaraju,V., Chidambara Rajan.R and Sivakumar.N.2012.Partial purification and Characterization of anti-MRSA peptide from marine *Pseudomonas aeruginosa*. *International Journal of Current Microbiology and Applied sciences*.1(1):40-49.

Sneha,K.S., Padamapriya,B and Rajeswari,T.2012.Isolation and Screening of Biosurfactants produced by *Pseudomonas aeruginosa* form oil spilled soils. *International journal of Pharmaceutical & Biological Archives*3(2):321-325.

Sridhar,B., Karthik,R., Tamil Venthan,M and Gopiesh Khanna,V.2013. Production,purification and antimicrobial activity of biosurfactants from *Saccharomyces cerevisiae* and *Pseudomonas*

aeruginosa. Int J Advanced Res in Pharmaceutical and biosciences. 3(4):1-7

Suganya,R S.2012. Screening Optimization and Production of Biosurfactants from *Bacillus* and *Pseudomonas* species. *International Journal of current pharmaceutical Research*.5(1):19-23.

Surachai Techaori.2007. Preliminary Screening of Biosurfactant producing microorganisms isolated from hot spring and garages in Northern Thailand. *KMITL Sci.Tech.J*. 7(1).

Surekha,K. 2010. Biosurfactant,bioemulsifiers and Exopolysaccharide from marine microorganisms. *Biotech. adv*.28:436-450.

Svetukhin, AM, Prokudhia, MV, Pavlova, MV, Puchkovva LS and Zemlianoi. 1995. Microflora of purulent necrotic lesions of the foot in patients with Diabetes Mellitus. *AntibiotKhimioter*. 40(8):40-42.

Tayfun kaya., Belma Aslim and Ergin Kariptas. 2014. Production of biosurfactant by Pseudomonas spp isolated from industrial waste in Turkey.*Turkish journal of biology*. 38: 307-317

Tentolouris N, Petrikkos G, Vallianou N, Zachos C,Daikos D.L, Tsapogas P, Markou G and Katsilambros N. 2005.Clinical Microbiology and Infection. *European Society of Clinical Microbiology and Infectious Disease*.12(2):186-189.

Terry J Gentry., Chrissto herrensing and Ian L Peprer.2004. New Approaches for Bioaugmentation as a Remediation Technology. *Critical Reviews in Environmental Science and Technology*. 34:447-494.

Urbancic V Roven and Gubina M. 1997. Infection in Superficial diabetic foot ulcers. *Clinical Infectious diseases*. 25(2):184-185.

Vandana Singh,2012. Biosurfactant-Isolation, production, purification and significance. *International Journal of scientific and Research publication*.2(7).

Vera pingitore,E., Salvucci,E.,Sesma,F and Nadar,M.E. Macias. 2007. Different Strategies for purification of antimicrobial peptides form Lactic acid Bacteria (LAB).*FORMATEX*. *Communicating Current Research and Educational Topics and Trends in Applied Microbiology*.

Vijaya,B.,Jayalakshmi N.R and Manjunath.K., 2014. Isolation and partial characterization of a biosurfactant produced by *Pseudomonas aeruginosa PAVIJ* from contaminated soil. *Research Journal of Pharmaceutical,Biological and Chemical Sciences*. 5 (2): 881-887

Whyte,L.G.,Bourbonniere L and Greer C. W.1997.Biodegradation of petroleum hydrocarbons by psychrotrophic *Pseudomonas* strains possessing both alkane(alk) and naphthalene (nah) catabolic pathways. *Applied and environmental microbiology*. 63:3719-3723.

Yakimov Michail M.., Kenneth N.Timmis.,Victor wray and Herbert L.Fredrickson.1995.Characterization of a new lipopeptide surfacnt produced by Thermotolerant and Halotolerant subsurface *Bacillus licheniforms* BAS50. *Applied and environmental microbiology*.61:1706-1713.

Zhang Guo-liang, Yue-ting Wu, Xing-ping Qian and Qin Mend.2005.Biodegradation of crude oil by *Pseudomonas aeruginosa* in the presence of rhamnolipids. *J Zhejiang Univ Sci B*. 6(8):725-730.

Access this Article in Online

Website: www.ijarbs.com

Subject: Biotechnology

Quick Response Code

DOI:10.22192/ijarbs.2016.03.12.029

How to cite this article:

R.Sumathi and N.Yogananth. (2016). Isolation and identification of Biosurfactant producing *Pseudomonas aeruginosa* from marine sediment samples and its Antimicrobial Properties. Int. J. Adv. Res. Biol. Sci. 3(12): 200-212.

DOI: http://dx.doi.org/10.22192/ijarbs.2016.03.12.029