International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 7; Issue 4(F); April 2018; Page No. 11704-11707
DOI: http://dx.doi.org/10.24327/ijcar.2018.11707.2034

Research Article

SCREENING AND ANTIMICROBIAL ACTIVITY OF BIOSURFACTANT PRODUCED BY *Pseudomonas aeruginosa* ARCH1802 ISOLATED FROM HYDROCARBON CONTAMINATED SOIL

Archana Kalita[1]*., Manab Deka[1] and Suresh Deka[2]

[1]Department of Biological Sciences, Institute of Science and Technology, Gauhati University, Assam
[2]Department of Life science, Institute of Advanced Study in Science and Technology, Assam

A R T I C L E I N F O

Article History:

Received 18th January, 2018
Received in revised form 13th February, 2018 Accepted 15th March, 2018
Published online 28th April, 2018

Key words:

Surfactant, lipopeptide, 16 S r RNA, FTIR, zone of inhibition .

A B S T R A C T

In the present research, attempts were made to isolate biosurfactant producing bacteria using mineral salt media with diesel as sole carbon source and to analyze its antimicrobial activity against non- pathogenic strains. Among 16 different strains, the most efficient strain namely Strain 2_1 has remarkably reduced surface tension up to 28.7 mN/m and emulsification index 74.04%. Biosurfactant was extracted using chloroform methanol in 2:1 ratio and purified using column chromatography. The composition of biosurfactant was confirmed as lipopeptide by biochemical and FTIR analysis. Based on the biochemical and molecular characterization, the strain was identified as *Pseudomonas aeruginosa* ARCH1802. Antimicrobial effect of biosurfactant synthesized by *Pseudomonas aeruginosa* ARCH1802 was confirmed by measuring their zone of inhibition against four non-pathogenic strains.

INTRODUCTION

Surfactants are surface active compounds that reduce the interfacial tension between two liquids or that between a liquid and a solid. In this context, bio-surfactants are extracellular amphiphilic compounds produced by bacteria, yeast and fungi grown on hydrophobic substrates (Mata-Sandoval *et al*., 1999, 2000; Chen *et al*., 2007). Microorganisms utilize a variety of organic compounds as source of carbon and energy. When the carbon source is in insoluble form, like hydrocarbon, microorganisms facilitate their diffusion into the cell by producing bio-surfactants. Bio-surfactants are diverse in chemical composition and its nature and amount depend on the type of microbes produced in a particular media. They are basically classified as glycolipids, lipopeptides, phospholipids, neutral lipids or fatty acids and polymeric biosurfactants (Cooper and Zajic 1980, Kosaric 1993). In recent times, interest in bio-surfactant has increased manifold due to its diversity, flexibility and ecofriendly nature (Thavasi R. *et al* 2011).

Bacteria are the main group of biosurfactant-producing microorganisms, although they are also produced by some yeasts and filamentous fungi (Desai *et al* 1997). Recent studies evidenced that among the many classes of biosurfactants, lipopeptides surfactant has remarkable biological activities, such as antimicrobial, antitumor, antiviral and antiadhesive activities (Banat *et al*.1995, Peypoux *et al*. 1999).

Hence, in this investigation, indigenous bacteria which produce biosurfactant was isolated from petroleum contaminated site and screened for its antimicrobial activity. Biosurfactant was purified and characterized by biochemical and spectral analysis. The bacteria was further characterized by biochemical and molecular techniques.

Corresponding author:* **Archana Kalita
Department of Biological Sciences, Institute of Science and Technology, Gauhati University, Assam

MATERIALS AND METHODS

Collection of soil sample

Petroleum contaminated soil samples and sludge were collected from three different sites (namely S1, S2, S3) of refinery area (IOCL, Guwahati), Assam. The samples were collected from surface and sub –surface (5-30 mm depth) and passed through 2mm sieve to remove the surface litter. Then the soil samples were stored at 4°c for further laboratory use.

Culture media strain isolation

1 g of each soil sample was inoculated in 100ml of Mineral Salt Medium (composition : NH_4No_3 4g,KCl 0.1g, KH_2PO_4 0.5g, K_2HPO_4 1g, $CaCl_2$ 0.1g, $MgSO_4.7H_2O$ 0.1g, $FeSO_4.7H_2O$ 0.1g, Yeast extract 0.1g, Trace element 10 ml; Trace element composition: H_3BO_3 0.26g, $CuSO_4.5H_2O$ 0.50g, $MnSO_4.H_2O$ 0.50g, $(NH_4)_6Mo_7O_{24}$ 0.06g, $ZnSO_4.7H_2O$ 0.70 g) with 2% diesel as the sole carbon source and incubated at 37°C in an orbital shaker at 145 rpm for 72 hours. 1ml from each flask was transferred to fresh mineral salt media and incubated at the same condition (145 rpm and 37°C) for 72

hours (Patwory *et al*. 2016). The culture from each flask were serially diluted up to 10 -6 dilution and inoculated on mineral salt agar (Carbon source 2% diesel) plate and incubated at 37° C, for 48 hours. Pure and representative colonies were transferred to slants for preservation.

Screening of biosurfactant

For screening, a loop full of each strain was inoculated in 5 ml nutrient broth and incubated in an orbital shaker incubator at 145 rpm for 24 hours at 37°c. Each culture was transferred to 100 ml mineral salt media containing 2% glucose as carbon source and were kept in same condition for 72 hours and then screened for biosurfactant production.

Drop-Collapse technique

A drop of 15 µl crude oil was placed on the glass slide and 10 µl aliquot of cell suspension was then applied onto the slide. The result was considered to be positive when the diameter of the drop was increased by 1mm from the diameter that was produced by distilled water (Youssef *et al*., 2004).

Parafilm M test

The 25 µl of bacterial supernatants was added to the hydrophobic surface of parafilm M. The shape of the drop on the surface was inspected after 1 min. The diameters of droplets were measured and compared with water which was used as negative control (Hewald S. *et al*. 2005; Morita T. *et al*. 2007).

Surface tension measurement

Surface tension of crude biosurfactant was determined by a tensiometer with respect to distilled water. If the microbes produce biosurfactant in the culture media then surface tension of the culture media will be lower than the initial value. The reduction in surface tension was measured at every 24 hrs. for 3 days in the K11 tensiometer (KRUSS, Hamburg, Germany). Based on maximum surface tension reduction, the most efficient bacterial were isolated.

Emulsification index

The isolated bacteria were checked for their abilities to emulsify crude oil or hydrocarbons. 2ml of kerosene were added to 2ml of culture supernatant and vortexed at high speed for 2 minutes and allowed for 24 hours. E24 was calculated by dividing the height of the emulsion layer by the mixture total height and then multiplying by 100 (Bodour 2004).
E24 =Height of emulsion/total height of solution X 100

Identification of potential bacteria

Biosurfactant producing bacteria were further taken for biochemical and molecular characterization. For morphological and biochemical characterization Grams staining, MR, VP, Indole, citrate utilization test was performed.

Molecular characterization was performed by 16s RNA technique. The genomic DNA was isolated as described by Sambrook *et al* (Sambrook *et al* 2001). Part of each extracted DNA sample was used for 16S rDNA amplification. The 16S rDNA was amplified using primer set 1492R (5'-CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG 3') and 27F(5'CCCGGGATCCAAGCTTACGGCTACCTT GTTACGACTT-3)'. The polymerase chain reaction (PCR) programmed was set at 30 cycles and the amplification was carried out as follows: 92 °C for 2 min, 42 °C for 30 s, 72 °C for 4 min and 4 °C incubation at the end of the last cycle. The obtained sequences were analyzed by using online databases viz. NCBI-BLAST to find the closest match of the contiguous sequence.

Extraction and purification of biosurfactant

For extraction 48 hours old culture broth was centrifuged at 9,000 rpm for 20 min at 4 ° C and cell free supernatant was acidified to pH 2.0 with 6N HCl and kept at 4 °C for overnight (Nitschke and Pastore 2006). Acidification was followed by extraction of crude biosurfactant by chloroform-methanol (2:1) for three times. The organic phase was separated, concentrated and was further purified on normal phase silica gel column chromatography using stepwise elution with methanol and chloroform ranging from 1:20 to 1:1 (v/v). Biosurfactant containing fractions were combined and dried in a rotary evaporator at 40 °C.

Characterization of biosurfactant

The extracted biosurfactant was characterized to determine its composition and nature. Both biochemical and spectral analyses were carried out to characterize the biosurfactant.

Biochemical analysis

The presence of carbohydrate groups in the biosurfactant molecule was assayed by Molisch's test (Vanavil B *et al*. 2013). 3 ml cell free supernatant was mixed with 1 ml of 10% α-naphthol and was followed by addition of 1 ml of concentrated H_2SO_4 without disturbing the sample. This is a sensitive test for the presence of carbohydrates, based on the dehydration of the carbohydrate by sulfuric acid to produce an aldehyde, which condenses with two molecules of phenol (usually α-naphthol) to give a red- or purple- colored compound.

Qualitative analysis of lipid was carried out by emulsion test. 0.2 g purified biosurfactant was dissolved in 10 ml ethanol in a test tube. The mixture was shaken properly to dissolve maximum portion of the biosurfactant added to a beaker containing distilled H_2O. Lipids do not dissolve in water and thus it falls out of solution to form an emulsion.

Presence of peptide bond was confirmed biuret test. In the presence of peptides, a copper(II) ion forms violet-colored coordination complexes in an alkaline solution.

Biosurfactant was treated with an equal volume of 1% strong base (sodium or potassium hydroxide) followed by a few drops of aqueous copper(II) sulphate (Kalyani A 2014).

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FT-IR) analysis is used to identifying types of chemical bonds (functional groups). Thus, it can be used to elucidate the components of an unknown mixture. For FTIR analysis, purified biosurfactant was sent to CDRI, Lucknow.

Antimicrobial activity against non -pathogenic strain

The partially purified biosurfactant was tested for its antimicrobial activity against four non -pathogenic microbial strains by well diffusion method. The antimicrobial activity of the biosurfactants was tested against four standard bacteria species namely *Staphylococcus aureus, Escherichia coli*,

Enterococcus feacalis and *Bacillus cerus* strain. An overnight nutrient broth culture of each of the four strains was prepared and made a lawn culture on nutrient agar plates. Two wells at each plate was prepared using a sterile borer. Partially purified biosurfactant dissolved in distilled water (200 µg/ml) used as sample and ampicillin (25 µg/ml) used as positive control. The wells were inoculated with 20 µl of biosurfactant and ampicillin and incubated for 24-48 h and observed for clear zone formation which indicates the antibacterial activity of the biosurfactants.

RESULT

It was observed that 16 bacteria strains were able to grow in MSM agar plates using diesel as sole carbon source. Among them five strains showed positive result in drop collapse and parafilm M test. In the screening process, strain 2_1 reduced surface tension from 72 to 28.7 mN/m after 48 hours. The emulsification index of strain 2_1 was74.04%. The strain was identified morphologically as a Gram negative, rod shaped bacteria (Fig 1) which showed negative result to indole, methyl red, VP test but positive to citrate utilization test (Fig 2). The strain was further characterized through 16S molecular technique. After sequencing of the 16S rRNA, gene data were submitted to the genetic sequence database at the National Center for Biotechnology Information (NCBI). The GenBank ID (http://www.ncbi.nlm.nih.gov) of these strain is LC377945 which was molecularly identified as *Pseudomonas aeruginosa* ARCH1802.

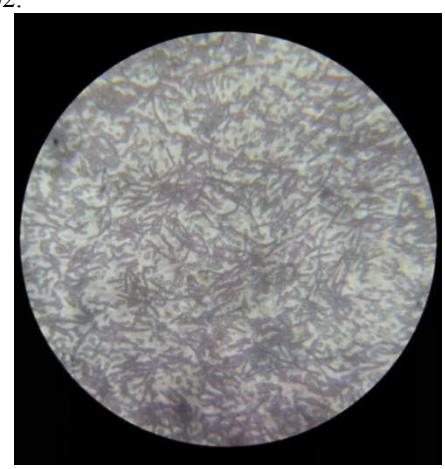

Fig1 Rod shaped Gram negative bacteria

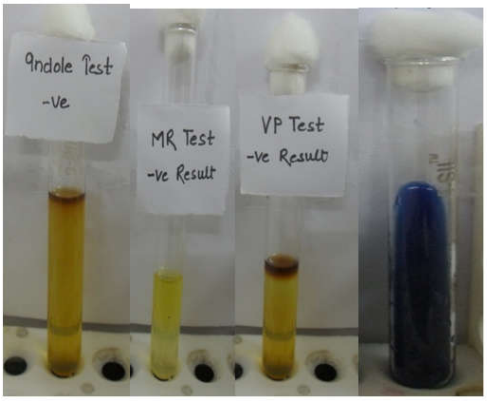

Fig 2 Biochemical test showing indole, MR, VP negative and citrate utilization test positive

The biosurfactant extracted from Pseudomonas aeruginosa ARCH1802 confirms the presence of lipid and protein in emulsion test and Biuret test. FTIR analysis of the biosurfactant showed that, wave numbers 3,452 and 3,000 cm−1(Fig 3) for N–H bonds and amide salt indicated the presence of amine groups. C–H bonds of the CH3, CH2 and CH groups observed at wave numbers 2933cm−1, confirmed the presence of alkanes. The appearance of a weak absorbance signal at 2421-2450 cm−1 may be due to C ▬ N stretch. The wave number 1815 and 1726 cm−1 may be due to c=o stretching. The wave number 1,520 and 1486cm−1 (amide I bond) indicated the presence of peptide groups. The wave number 1,227 and 1163 cm−1 indicated the presence of C–O bonds. High intensity peak in the region of 1000–1100 cm−1 was assigned to O–C–O which extended vibrations of carboxylic acids, aldehydes and ketones. The following vibrations observed at 800–500 cm−1 may be indicative of methylene scissoring vibrations. The above information from the respective wave numbers confirmed the lipopeptide nature of the biosurfactant (Ahmed *et al* 2015,Thavsi R *et al* 2011).

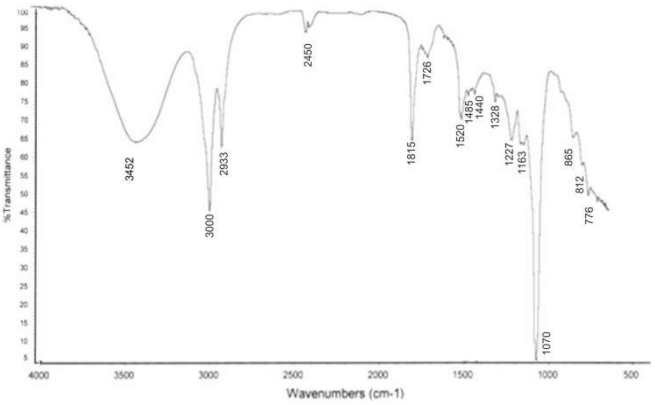

Fig 3 FTIR profile of biosurfactant

Among tested non-pathogenic bacteria, biosurfactant showed antimicrobial activity against all four strains. The maximum zone of inhibition values recorded were *Staphylococcus aureus* (1.5cm) followed by *Escherichia coli* (2.0 cm,), *Enterococcus feacalis* (1.8 cm), *Bacillus cerus* (1.8 cm (Table 1).

Table 1 Zone of inhibition produced by biosurfactant and ampicillin against non- pathogenic strain

Strain	Biosurfactant(cm)	Ampicillin(cm)
Staphylococcus aureus	1.5	1.5
Escherichia coli	2.0	1.2
Enterococcus feacalis	1.8	1.2
Bacillus cerus	1.8	1.2

DISCUSSION

This study revealed the *Pseudomonas aeruginosa* ARCH1802 produces biosurfactant and it has potentiality to use as antimicrobial agent. It is reported that in order to give a positive drop collapse test, a surface tension lower than 45 mN/m is necessary (Viramontes-Ramos *et al* 2010). The strain 2_1 reduced surface tension to 28.7 mN/m and collapsed drop of oil and thus considered to be a good biosurfactant producer. It is also reported that emulsification index is another criterion to screen potential biosurfactant producers because more than one screening method should be included (Satpute *et al*. 2008). The FT-IR profile of the biosurfactant showed similarity to surfactin, a lipopeptide biosurfactant and other lipopeptide biosurfactants like arthrofactin (Morikawa *et al*. 1993, Ahmed *et al* 2015) and thus confirming the lipopeptide nature of a biosurfactant. The lipopeptide nature of

biosurfactant produced by *P. aeruginosa* was also reported (Thavsi R *et al* 2011) and their results correlate with peaks observed the present study. Based on antimicrobial studies, it was observed that the isolated lipopeptide biosurfactant has wide antimicrobial activities.

CONCLUSION

From the above investigation, it can be concluded that the lipopeptide bisurfactant of indigenous bacterial isolate *Pseudomonas aeruginosa*ARCH 1802 used in the study has potential antimicrobial activity.

References

Ahmed M. Elazzazy, T.S. Abdelmoneim, O.A. Almaghrabi, 2015.Isolation and characterization of biosurfactant production under extreme environmental conditions by alkali-halo-thermophilic bacteria from Saudi Arabia. *Saudi Journal of Biological Sciences*, 22(4): 466-475.

Banat IM,1995. Characterization of biosurfactants and their use in pollution removal-state of the art. *Acta Biotechnol* 15: 251-267.

Bodour A.A., Gerrero-Barajas, C. and Maier M. 2004. Structure and characterization of flavolipids, a novel class of biosurfactants produced by Flavolipid sp. strain MTN11. *Appl. Environ. Microbiol*. 10(6): 1114-1120.

Chen S.Y, Wei Y.H. and Chang J.S., 2007. Repeated pH-stat fed-batch fermentation for rhamnolipid production with indigenous Pseudomonas aeruginosa S2. *Appl. Microbial & Biotechnol*. 76, 67-74.

Cooper DG, Zajic JE ,1980. Surface-active compounds from microorganism. *Adv Appl Microbiol* 26:229–256.

Desai J. D. and Banat I. M., 1997.Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews,*61(1): 47–64.

Hewald, S., Josephs K. and Bolker M. 2005. Genetic Analysis of Biosurfactant Production in *Ustilago maydis*. *Applied and Environmental Microbiology*, 71(6), 3033-3040.

Kalyani A.L. T, Naga Sireesha G, Girija Sankar G, Prabhakar T, 2014.Isolation, Identification and Antimicrobial activity of Bio-surfactant from Streptomyces matensis (PLS-1). I*nt. J. Pharm. Sci. Rev. Res.*, 25(1): 165-170

Kosaric N (1993) Biosurfactants. Production, property, application, surfactant sciences series, 48. Dekker, New York

Mata-Sandoval J.C., Karns, J. and Torrents, A., 1999. High-Performance Liquid Chromatography method for the characterization of rhamnolipid mixtures produced by Pseudomonas aeruginosa UG2 on corn oil. *J. Chromat. A.* 864, 211-220.

Mata-Sandoval J.C., Karns, J. and Torrents, A., 2000. Effect of nutritional and environmental conditions on the production and composition of rhamnolipids by Pseudomonas aeruginosa UG2. *Microbiol. Res*. 155, 1-8.

Morita T., Konishi M., Fukuoka T., Imura, T. and KitamotoD., 2007. Physiological Differences in the Formation of the Glycolipid Biosurfacants, Mannosylerythritol Lipids, between *Pseudozyma antartica* and *Pseudozyma aphidis*. *Applied of Microbiology and Biotechnology*, 74, 307-315.

Morikawa M., Daido H., Takao T., Murata S., Shimonishi Y, Imanaka T.,1993. A new lipopeptide biosurfactant produced by Arthrobacter sp. *strain MIS38. J. Bacteriol.*, 175: 6459-6466

Nitschke M, Pastore GM., 2006. Production and properties of a surfactant obtained from *Bacillus subtilis* grown on cassava wastewater. *Bioresour Technol.*97(2):336-41.

Patowary K, Patowary R, Kalita MC, Deka S., 2016. Development of an Efficient bacterial consortium for the potential remediation of hydrocarbons from contaminated sites. *Front Microbiol*.14; 7:1092.

Peypoux F, Bonmatin JM, Wallach J., 1999. Recent trends in the biochemistry of surfactin. *Appl Microbiol Biotechnol.* 51: 553-563.

Sambrook J., Maccallum P. and Russell D., 2001. Molecular Cloning: A Laboratory Manual. 3rd. Cold Spring Harbor Press, NY.

Satpute S.K., Bhawsar B. D, Dhakephalkar, P.K., Chopade B.A., 2008. Assessment of different screening methods for selecting biosurfactant producing marine bacteria. *Ind. J. Mar. Sci*., 37: 243-250

Thavasi R, Subramanyam Nambaru VR, Jayalakshmi S, Balasubramanian T, Banat IM., 2011. Biosurfactant Production by *Pseudomonas aeruginosa* from Renewable Resources. *Indian J Microbiol*. 51(1):30-6.

Thavasi R, Jayalakshmi S, and Banat IM, 2011.Application of biosurfactant produced from peanut oil cake by *Lactobacillus delbrueckii* in biodegradation of crude oil. *Bio resource Technology*, vol. 102(3):3366–3372.

Vanavil B, Perumalsamy M, Rao AS., 2013. Biosurfactant production from novel air isolate NITT6L: screening, characterization and optimization of media. *J Microbiol Biotechnol.* 23(9):1229-43.

Viramontes-Ramos S, Cristina Portillo-Ruiz M, Ballinas-Casarrubias Mde L, Torres-Muñoz JV, Rivera-Chavira BE, Nevárez-Moorillón GV, 2010. Selection of biosurfactan/bioemulsifier-producing bacteria from hydrocarbon-contaminated soil. *Braz J Microbiol*. 41(3):668-75.

Youssef, N.H., Dunacn, K.E., Nagle, D.P., Savage, K.N., Knapp, R.M. and McInerney. M.J. ,2004. Comparison of methods to detect biosurfactant production by diverse microorganism. *J. Microbiol. Meth*, 56: 339-347.

How to cite this article:

Archana Kalita *et al* (2018) 'Screening And Antimicrobial Activity of Biosurfactant Produced By Pseudomonas aeruginosa ARCH 1802 Isolated From Hydrocarbon Contaminated Soil', *International Journal of Current Advanced Research,* 07(4), pp. 11704-11707. DOI: http://dx.doi.org/10.24327/ijcar.2018.11707.2034

****