Available online at http://www.ijcrls.com

International Journal of Current Research in Life Sciences
Vol. 07, No. 04, pp.1800-1803, April, 2018

ISSN: 2319-9490

RESEARCH ARTICLE

BIOSURFACTANT PRODUCTION FROM ISOLATED BACTERIUM AND ITS EMULSIFICATION ACTIVITY

[1]Dnyaneshwar Sonune, [1]Shravan Ligade, [1]Vishal Chavan,[1]Sumit Waghmare, [1]Swapnil Warade, and *[2]Neha Chavan

[1,2]Department of Biotechnology, Shivchhatrapati College, Aurangabad, MS, India

Received 19th February, 2018; Accepted 27th March, 2018; Published 30th April, 2018

ABSTRACT

Biosurfactants are extracellular surface active, amphiphilic compounds produced by bacteria, fungi, and yeast which reduce surface and interfacial tension. The present study aimed at the isolation of biosurfactant producing bacteria and production of biosurfactant. The one isolated bacterium which is screened for highest production of biosurfactant was identified as *Pseudomonas spp.* based on biochemical and cultural characteristics. *Pseudomonas spp.* was used for biosurfactant production in Bushnell-Hass medium. The emulsification activity of extracted biosurfactant was tested against different vegetable oils and hydrocarbons and compared with chemical surfactant Triton X100 as a standard. The highest emulsification index [EI_{24}] of biosurfactant was found against peanut oil and safflower oil i.e. 56.06 %.The extracted biosurfactant was characterized by FTIR analysis.The carbohydrate content was estimated at 46 µg/ml by using Anthrone method.

Key words: Biosurfactant, Screening, Biochemical characterization, Emulsification activity, FTIR analysis.

Citation: Dnyaneshwar Sonune, Shravan Ligade,Vishal Chavan, Sumit Waghmare, Swapnil Warade, and Neha Chavan, 2018. "Biosurfactant production from isolated bacterium and its emulsification activity" *International Journal of Current Research in Life Sciences*, 7, (04), 1800-1803.

INTRODUCTION

Surfactant is a structurally diverse group of a surface- active molecule. They are amphiphillic molecules having both hydrophilic and hydrophobic moieties that reduce surface and interfacial tension between two liquid or between a liquid and a solid. Biosurfactant are synthesized by microorganisms like bacteria, fungi and yeast. In the past few decades, Biosurfactant had gained attention because they exhibit some advantage, such as biodegradability, low toxicity environmental compatibility, high selectivity, and specific activity at extreme temperatures, pH, and salinity, diversity ecological acceptability and their ability to produce from cheaper substrate (Rosenberg and Ron, 1999). The first microbiological biosurfactant on the market was sophorolipid. Of all currently known biosurfactants, rhamnolipids have the highest potential for becoming the next generation of biosurfactants introduced in the market (Mu¨ llera *et al.,* 2012). Microorganism have been reported to produce several classes of biosurfactant such as glycolipid, lipopeptide, phospholipid, neutral lipid or fatty acid and polymeric biosurfactant. (Cooper, 1986 and Kosaric, 1993). Biosurfactant can be intracellular (remain attached to the cellwell) and can be excreted to the media.

Corresponding author: **Neha Chavan,**
Department of Biotechnology, Shivchhatrapati College, Aurangabad, MS, India.

When the Biosurfactant are intracellular, their structure includes membrane lipids and promote the transport of insoluble substrates through the membrane; when they are extracellular, the Biosurfactant help on the surface of lipids, proteins and carbohydrates.The main difference in the chemical nature of the different Biosurfactant molecules is in hydrophilic head, allowing for wide range of variation in their physical and biological properties (Peter and Singh, 2014). Biosurfactant producing microorganisms were naturally present in oil contaminated soil and aquatic environment. The most prevalent bacterial hydrocarbon degraders and biosurfactant producers, belong to the genera are *Pseudomonas, Achromobacter, Flavobacterium, Micrococcus, Bacillus, Arthrobacter, Klebsiella, Acinetobacter, Aeromonas, Alkaligenes, Streptococcus* sp., *Corynebacterium* sp., *Moraxella* sp., and *Proteobacteria* (Mishra *et al.,* 2001). Among the different classes of biosurfactants rhamnolipid and surfactin are best studied. Rhamnolipid is mostly produced by *Pseudomonas aeruginosa* (Peypoux *et al.,* 1999). Most Biosurfactant are either anionic or neutral and the hydrophobic portion can be carbohydrate, amino acid, phosphate or cyclic peptide (Nitschke and Costa, 2007).Biosurfactant have been shown to have a variety of application, including enhancing crude oil recovery from oil reservoirs, mobilizing heavy crude oil transport in pipelines and cleaning oil sludge from oil storage facilities.

They are also used in soil or sand bioremediation, remediation of organics and metals, and as emulsifier in agriculture and medicine in biological control (Desai and Banat, 1997 and Lang and Wullbrandt, 1999). Pollution of the sea, especially by oil, which is caused by standing of tankers, is one of the serious environmental problems over the world. The use of Biosurfactant can play an important role by emulsifying the polluted oil prior to biodegradation. From this point view, present study aimed to isolate highly productive Biosurfactant producing bacterium and its emulsification activity against different oils.

MATERIALS AND METHODS

Collection of water sample

Water sample was collected in presterilized bottle from Salim Ali Lake in Aurangabad city and transported to laboratory for further analysis.

Isolation of Biosurfactant producing bacteria

Enrichment of water sample was done in Mineral salt medium [MSM] and the enriched sample was spread on agar media for isolation of bacteria.Isolated colonies were purified on Nutrient agar medium and screened for the production of Biosurfactant.

Screening for Biosurfactant production

The selected bacterial cultures were grown in mineral salt medium [MSM] in shaking condition with 150 rpm at 37^0c for 24-48 hrs. After incubation the supernatant of each bacterial isolates was collected by centrifugation at 10,000 rpm for 20 min. The Biosurfactant property of bacterial culture supernatant was analyzed by using standard methods such as Drop collapse test (Mahalingam and Sampath, 2014), Oil spreading test, Emulsification activity [Youssef *et al.,* 2004). Based on screening test results, the bacterial isolates showing highest Biosurfactant activity were selected and used for production of Biosurfactant.

Identification of bacteria

Identification of hightest biosurfactant producing bacterium was done on the basis of Bergey's Manual of Determinative bacteriology.

Production of biosurfactant

The hightest producing bacterial culture was inoculated in nutrient broth and incubated at 37^0c for 24 hrs on rotary shaker [150 rpm]. This inoculum was transferred to 100 ml of Bushnell-Hass mineral salt medium [BHMS] in 250 ml Erlenmeyer flask and incubated on shaker with 150 rpm at 30^0 c for 90 hrs (El-sheshtwy and Doheim, 2014).

Extraction of biosurfactant

The culture supernatant was obtained by centrifugation at 10,000 rpm for 20 min. The biosurfactant was recovered from the cell free supernatant by precipitation with two volumes of chilled acetone and allowed to stand overnight at 4^0C.The precipitate was collected by centrifugation and stored in refrigerator. The resulting product was considered as the crude Biosurfactant (Mahalingam and Sampath, 2014).

Characterization of Biosurfactant by FTIR Analysis

Fourier transform infrared spectroscopy is technique for the characterization and identification of the functional groups in the sample. The extracted biosurfactant was subjected to FTIR analysis. The spectral region used was 4000-500cm^{-1}.

Estimation of carbohydrate content extracted

Carbohydrate content of Biosurfactant was analyzed by Anthrone method using Rhamnose as standard (Nigam and Ayyagari, 2007).

Emulsification activity of crude Biosurfactant

Biosurfactant solution (2ml) was added to different test tube containing 2ml of different oils and hydrocarbons. The contents of tubes were vortexed for 2 min and stand for 24 hrs. The volume of oil separated after 24 hrs of standing was measured.This showed the ability of biosurfactant to form stable emulsion (Jyotsna and Dhirendra, 2014).

The emulsification activity [E_{24} index] was calculated by,

$$E_{24}\ \text{index} = \frac{\text{Height of emulsion layer}}{\text{Total height}} \times 100$$

RESULTS AND DISCUSSION

Collection of water sample

Water sample was collected in pre-sterilized bottle from Salim Lake, Aurangabad and stored in laboratory.

Isolation of Biosurfactant producing bacteria

Seven morphologically distinct bacterial colonies were isolated on nutrient agar plates and colony characteristics were studied.

Screening for Biosurfactant production

All the isolates were screened for Biosurfactant activity by oil spreading technique, drop collapse technique and emulsification activity. All isolates exhibited varied results for screening test. However, the extent of oil displacement, drop collapse and emulsification activity differed considerably among isolates suggesting direct correlation to Biosurfactant present in supernatant. Similar observations were made by Rodrigues *et al.* (2006). Among 7 isolates, only isolate 4 showed good Biosurfactant property and hence further selected for identification and production.

Identification of isolated bacterium

Among seven isolate, isolate 4 was able to show highest biosurfactant activity and hence selected for identification. Identification of highest Biosurfactant producing isolate was carried out on the basis of morphological and biochemical characterization according to Bergey's Manual of Systematic bacterialogy (Table.2). It was also confirmed further by their growth on Pseudomonas isolation agar. Bhat *et al.* (2015) were also identified isolated bacteria for biosurfactant production on the basis of similar characterization Isolate 4 was identified as *Pseudomonas spp.*

Table 1. Result of Biochemical characterization

Test	Result
Gram stain	-
Oxidase	+
Catalase	+
Indole	-
Methyl red	-
VP test	-
Citrate	+
Glucose fermentation	+
Growth on *Pseudomonas* isolation agar	+

Production of Biosurfactant

Production of Biosurfactant was carried out in Bushnell-Hass mineral salt medium using isolated *Pseudomonas spp.* After production and extraction, 4.2 g/liter of Biosurfactant yield was obtained, which is less than the yield reported by R.Thavasi *et al.* But the value is more than the yield reported by Youseff *et al.* (90mg/lit) and H.S. El-sheshtwy etal (1g/lit).

Extraction of Biosurfactant

Extraction of biosurfactant was done by adding two volume of chilled acetone in cell free supernatant. Precipitate of Biosurfactant was collected by centrifugation. Yield of extracted biosurfactant was 4.2 g/ litr.(Fig.12)

FTIR Analysis

Characterization of recovered Biosurfactant was done by FTIR analysis as shown in Fig.13. FTIR spectra of the Biosurfactant showed characteristic absorption bands corresponding to functional groups typically forming part of Rhamnolipids. The strong absorbance at 1638.33cm$^{-1,}$ which is considered to be the characteristic peak of Biosurfactant by many researchers (Li .Q.X. *et al.* 2002, Patil S. et al.2014),must be assigned to C-H stretching vibrations of the hydrocarbon chain positions. The spectral analysis showed strong absorption band at 3335.89 cm^{-1}, this is nearly similar to the value (3370 cm^{-1}) reported by Rashmi Saikia *et al.,* 2012. This was due to stretching vibration of the –OH group. It was also reported by Bordoloi & Konwar, 2009 and Rath K. *et al.,*2016. The Symmetric stretch of CH_2 and CH_3 groups of aliphatic chains could be observed around at 2854.41 – 2924.26 cm^{-1}. Nearly similar stretching pattern observed by Rath K. *et al.* (2016), at 2856.55 – 2928.66 cm^{-1}. Absorption around 1740.20 cm^{-1} and 1638.33 cm^{-1} represents ester (C=O) group and carbonyl group (COO-) respectively. The presence of the Carboxylic acid functional group was also confirmed by the bands in the region of 1456.56 -1368.74 cm^{-1}for bending of hydroxyl (O-H) group (Moussa. T.A.A. *et al.,* 2014).This pattern of absorption bands observed for *Pseudomonas spp.* was reported previously for rhamnolipids by Bordoloi et al.in 2009. The absorption at 1157.68 cm^{-1} was due to stretching vibration of ether linkages of Rha C8-C10 and Rha- C10-C8 molecules (Saikia R. 2012). According to this data obtained in FTIR spectrum, Biosurfactant composed of carbohydrates and lipids in combination. Therefore, it confirms the presence of Glycolipid type of biosurfactant corresponding to Rhamnolipid.

Estimation of carbohydrate content in extracted biosurfactant

Carbohydrate content in extracted Biosurfactant was estimated as 46 µg/ml by Anthrone method. This confirmed the presence of sugar in the extracted Biosurfactant and hence the presence of Glycolipid type of Biosurfactant.

Table 2. Emulsification activity of extracted Biosurfactant and Triton X100

Sr. No.	Oils / Hydrocarbon	Emulsification activity	
		Extracted Biosurfactant	Triton X100
1)	Soyabean oil	52.17 %	73.91 %
2)	Peanut oil	56.06 %	69.56 %
3)	Sunflower oil	54.54 %	69.56 %
4)	Safflower oil	56.06 %	69.56 %
5)	Benzene	45.83 %	47.82 %
6)	Xylene	52.17	69.56

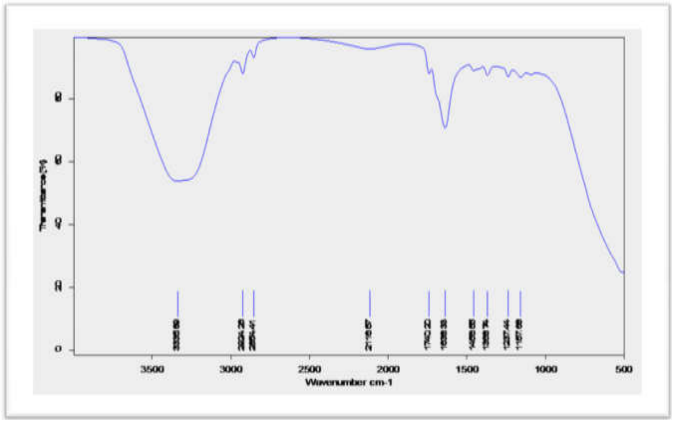

Fig. 1. Result of FTIR Analysis

Fig. 2. Emulsification activity of Triton X100

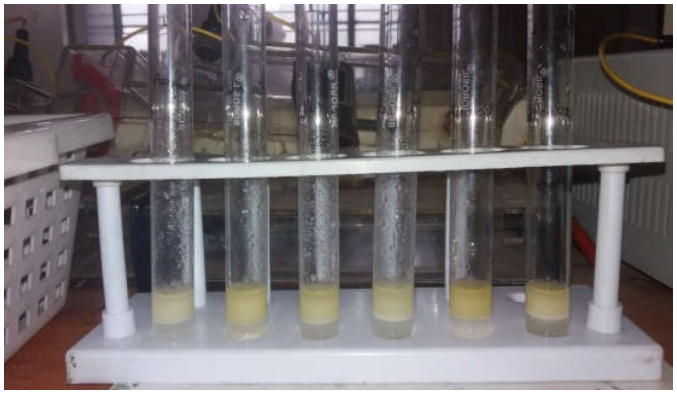

Fig. 3 Emulsification activity of extracted Biosurfactant

Emulsification activity of extracted biosurfactant

Emulsification activity [E24 index] of extracted Biosurfactant was checked against different oils and hydrocarbons, and compared with TritonX100 as standard surfactant (Fig.No.2 &

3) Highest emulsification activity was found in peanut oil and safflower oil which is 56.06 %. (Table 2) Biosurfactant produced in this study showed good Emulsficatiom activity against six different oils and Hydrocarbon source tested. Rahman *et al.* (2002) showed *P. fluorescence* has higher emulsification activity in Soyabean oil. The reason behind emulsification of oils and hydrocarbon by *Pseudomonas species* is the nature of Biosurfactant produced by them. Emulsification result showed that Biosurfactant produced from isolated bacterium can emulsify different range of hydrocarbons and oils, which confirmed its applicability against different hydrocarbon pollution and in oil spills and for hard surface cleanser.

REFERENCES

Arora, S.K. *et al.* 2006. production and stability studies of the bioemulsifier obtained from strain of Candida glabrata UCP1002 *J. of biotech.,* 9(4); 400-401.

Bhatia, V. and Saharan, B. S. 2015. Isolation and characterization of glycolipid produced by *Saccharomyces cerevisiae* LF14 isolated from leather factory,IOSI *J. Micro.Biotech.Food.Sci*.,1(2),22-27.

Cooper D.G. and Zajic, J.E. 1983. *Adv. Appl. Microbiol.***,** 26, 229–253,

Cooper, D.G. 1986. *Microbiol. Sci.***,** 3, 145–149

Desai, J.D. and Banat, I.M. 1997. Microbial production of surfactants and their commercial potential. *Microbiol. Mol. Biol.,* 61, 47-64.

El-sheshtwy, H.S. and Doheim, M.M. 2014. Selection of pseudomonas aeruginosa for biosurfactant production and studies of its antimicrobial activity; *Egyptian journal of petroleum*, 23; 1-6.

Jazeh, G. Forghani F. *et al.* 2012. biosurfactant production by Bacillus spp. Isolated from petroleum contaminated soils of sirri Island; *American J. of App sci.,* 9(1);1-6.

Jyotsna, K.P. and Dhirendra P.S. 2014. characterization of emulcification activity of partially purified Rhamnolipids from Pseudomonas fluorecences; *Int. J. of innovation and sci. research.,* 3(1); 88-100

Kaskatape, B. *et al.* 2015. Biosurfactant production by Pseudomonas aeruginosa in kefir and fishmeal; *Brazilian J. of microbiology,* 46(3), 855-859.

Kosaric, N., 1993. Biosurfactants Production–Properties–Applications, Marcel Dekker, New York.

Lang, S. and Wullbrandt, D. 1999. Rhamnose lipids-biosynthesis, microbial production and application potential. *Applied Microbiol. Bioethanol.,* 51: 22-32.

Li, Q.X. *et al.,* 2002. Several method for the screening of biosurfactant producingmicroorganisms, *the Chinese academ.medi.mag. org.*, 2; 13-52**.**

Mahalingam, P.U. and Sampath, N. 2014. Isolation characterization and identification of bacterial biosurfactant; *European J. of Exp. Biology*, 4(6), 59-64;

Mishra, S.J., Jyot, R.C. and Kuhad, B. 2001. Evaluation of inoculum addition to stimulate in situ bioremediation of oily sludge contaminated soil. Appl. Env. *Micro*., 67(04),1675-1681.

Moussa. T.A.A. *et al.,* 2014. Production and characterization of Di-Rhamnolipid produced by *Pseudomonas aeruginosa* TMN, *Brazilian J.Chem.Engi*.,31(4)'867-880**.**

Mu¨ llera, M.M., Ku¨ glera, J.H., Henkela, M., Gerlitzki, M., Hormanna, B., Pohnleina, M., Syldatka, C. and Hausmannb, R. 2012. *J.Biotechnol.,* 162,366–380.

Nigam, A. and Ayyagari, A. Lab manuals in Biochemistry, Immunology and Biotechnology,Tata McGraw-Hill publishing company limited,New Delhi.

Nitschke, M. and Costa, S.G. 2007. Biosurfactants in food industry. *Trends Food Sci. Technol.,* 18,252-259.

Patil, S. *et al.* 2014. Studies on optimization of biosurfactant production by Pseudomonas aeruginosa F23 isolated from oil contaminated soil sample, *Iner.J.Curr.Biotech*., 2(4),20-30**.**

Peter, J. K. and Singh, D.P**.** 2014**.** Characterization of emulsification activity of partially purified Rhamnolipids from *Pseudomonas fluorescens, Inter. J. Innov. Sci. Research,* 3(1), 88-100.

Peypoux, F., Bonmatin, J. M. and Wallach, J. 1999. Recent trends in Biochemistry of Surfactin. *Applied Microbiol. Biotechnol.,*51: 553-563.

Rahman, K. S. *et al*. 2002. Rahmnolipid biosurfactant production by strains of pseudomonas aeriguinosa using low cost raw materials, *Biotechnology progress,* 18(6), 1277-1281**.**

Rosenberg, E. and Ron, E. 1999. *Appl. Microbiol. Biotechnol.,* 52, 154–162

Santhini, K. and Parthasarrathi, R. 2014. Isolation and screening of biosurfactant producing microorganisms from Hydrocarbon contaminated soils from automobile workshop; *Int. J. pharma. Biological archies.,* 5(2); 158-167

Saravanan, V. and Vijaykumar S. 2012. Isolation and screening of biosurfactant producing micro-organisms from oil contaminated soil; *J Acad. Indus. Ress.,* 1(5); 264-268.

Shaikh, R.A. *et al*. 2013. Screening and characterization of biosurfactant producing micro-organisms from natural environment; *J. of natural sci. research.,* 3(13); 53-58**.**

Youssef, N.H. *et al.* 2004. comparison of methods to detect biosurfactant production by diverse mocro-organisms; *J. microbial. Methods.*, 56; 339-347**.**

****