www.ioirp.com
International Journal of Innovative Research in Technology, Science & Engineering (IJIRTSE)
ISSN: 2395-5619, Volume – 1, Issue – 10. December 2015

Isolation and Evaluation of Screening Methods for Isolation of Biosurfactant producing Microorganism from Soil

Madhura Chandgude, Roshni Nair and Sasirekha B
Department of Microbiology & Biotechnology, Acharya Bangalore B School, Andrahalli Main road, Magadi road, Bangalore, Karnataka, India. E-mail: sasirekha.b@acharyabbs.ac.in

Abstract - Biosurfactants are amphiphilic compounds produced by microorganisms as secondary metabolite. The unique properties of biosurfactants make them possible to replace or to be added to synthetic surfactants which are mainly used in food, cosmetics and pharmaceutical industries and in environmental applications. The biosurfactants have several advantages over chemical surfactants including lower toxicity and higher biodegradability, and effectiveness at extreme temperatures or pH Values. Furthermore possibility of their production on large scale, selectivity, performance under intense conditions and their future applications in environmental fortification also these have been increasingly attracting the attention of the scientific and industrial community. Therefore the present study is aimed to screen and identify biosurfactant producers from environmental samples. A total of 22 soil samples and 6 water samples were collected from different places of Bangalore were plated onto minimal media containing 1% petrol as sole source of carbon and isolated colonies were screened for their biosurfactant potential. A total of 40 bacterial isolates were isolated, of which BS15 showed high emulsification index and activity of 54.30 % and 122.54 EU/ ml respectively. BS15 was negative for hemolytic assay and positive for oil spread assay. It showed positivity for blue agar plate indicating the biosurfactant is of rhamnopilid in nature. Isolate BS15 exhibiting maximum biosurfactant potential was gram negative rods by gram's staining.

Keywords: Biosurfactants, surface tension, emulsification index, rhamnopilid.

INTRODUCTION

Surfactants are basically chemical compounds which lower the surface tension of a liquid, the interfacial tension between two liquids, or that between a liquid and a solid. These surfactants are produced by a variety of microorganisms such as yeast, bacteria and filamentous fungi and thus are called Biosurfactants [1]. Biosurfactants have different properties such as they act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. These are usually organic compounds that are amphiphilic in nature, which contains both the hydrophobic and hydrophilic component. The hydrophobic (non-polar) part of the biosurfactant is insoluble in water and may have a long-chain of fatty acids, hydroxyl fatty acids or α-alkyl-β-hydroxy fatty acids.

The hydrophilic (polar) end can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol [2]. Surface active compounds produced by microorganisms are divided into two main types. These that reduce surface tension at air water interface (biosurfactants) and those that reduce the interficial tension between immiscible liquids or at the solid-liquid interfaces (bioemulsifires). Biosurfactants are amphipathic compounds excreted by microorganisms that exhibit surface activity [3]. There are many complex molecules included in biosurfactants, e.g. glycolipids, lipopeptides, fatty acids, polysaccharide protein complexes, peptides, phospholipids and neutral lipids. Biosurfactant have special advantage over their commercially manufactured counterparts because of their lower toxicity, biodegradable nature, and effectiveness at extreme temperature, pH, salinity and ease of synthesis. They are potential candidate for much commercial application in the pharmaceutical and food processing and oil recovery industries. Biosurfactant reduce surface tension, critical micelle concentration (CMC) and interfacial tension in both aqueous and hydrocarbon mixture [4]. Although biosurfactants are exhibiting advantages, they have not yet been employed extensively in industry because of high production costs [5]. There have been very few studies so far that evaluated the presence of indigenous bio-surfactant producing microbes. Therefore the present study is focused on the isolation, screening, and characterization of biosurfactant producing bacteria.

International Journal of Innovative Research in Technology, Science & Engineering (IJIRTSE)
ISSN: 2395-5619, Volume – 1, Issue – 10. December 2015

MATERIALS AND METHODS

A total of 22 soil samples and 6 water samples were collected from different places of Bangalore and stored at *4˚C* until *further processing*.

Isolation of biosurfacant producing microorganisms

Soil and water samples were serially diluted and 100 µl of 10^{-3} each dilution was plated onto Minimal Salt agar medium containing 1% petrol as sole source of carbon. Plates were incubated at 37± 2˚ C. Bacterial colonies showing different morphological appearance were selected, sub- cultured and stored at 4˚C.

Screening for bio-surfactant production

Isolated colonies were screened for their bio-surfactant production potential by the following methods. All the screening experiments were performed in triplicates and the mean values were calculated

Haemolytic assay

Hemolytic assay was performed in 5% sheep blood agar plates. 50µl of bacterial culture grown in mineral salt medium was spot inoculated on to blood agar plates and incubated for 48 h at 37˚C. The plates were visually inspected for clear zone (hemolysis) around the colony [6].

Oil spreading assay

Twenty millilitre of distilled water was added to a plastic petridish followed by addition of 20 µl of crude oil to the surface of the water. 10 µl of cell free culture broth was then added to the oil surface. If bio-surfactant is present in the cell free culture broth, the oil will be displaced with an oil free clearing zone and diameter of this clearing zone indicates the surfactant activity, also called oil displacement activity [7].

Emulsification test (E_{24})

Hydrocarbon (2 ml) was taken in a test tube to which 1ml of cell free supernatant obtained after centrifugation (10,000 rpm for 10 min) of the culture was added and vortexed for 2 minutes to ensure homogenous mixing of both the liquids. The emulsification index was observed after 24 h and it was calculated by using the formula Cooper and Goldenberg (1987).

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

Bacterial Adhesion to Hydrocarbons (BATH assay)

Bacterial isolates were inoculated into Erlenmeyer flasks containing 50 ml of minimal salt media supplemented with petrol (1%). Flasks were incubated at 37˚C for 48 h in a rotary shaker (120 rpm) and centrifuged for 30 min at 7,500 x g. The cell pellets were washed twice and suspended in a buffer salt solution (g/l) 16.9 K_2 HPO_4 and 7.3 KH_2PO_4; pH 7) and diluted using the same buffer solution to an optical density (OD) of ~ 0.5 at 610 nm. Hundred µl of crude oil was added to 2ml of the cell suspension and vortex-shaken for 3 min. After shaking, crude oil and aqueous phases were allowed to separate for 1h. OD of the aqueous phase was then measured at 610 nm using spectrophotometer. The decrease in the turbidity of the aqueous phase correlates to the hydrophobicity of the cells [8]. The percentage of cells bound to the hydrophobic phase is calculated by:

$$\text{Bacterial cell adherance } (\%) = 1 - \frac{\text{OD of the aqueous phase}}{\text{OD of initial cell suspension}} \text{ X } 100$$

Blue agar plate (Bap) method

Mineral salt agar medium supplemented with glucose as carbon source (2%) and cetyltrimethylammonium bromide (CTAB: 0.5 mg/mL) and methylene blue (MB: 0.2 mg/mL) were used for the detection of anionic bio-surfactant [9)]. Thirty microlitre of cell free supernatant was loaded into the each well prepared in methylene blue agar plate using cork borer (4 mm). The plate was then incubated at 37°C for 48-72 h. A dark blue halo zone around the culture was considered positive for anionic bio-surfactant production.

Emulsification assay

Culture inoculums were prepared by growing the isolates in erlenmeyer flasks containing 50 ml of minimal salt media supplemented with petrol (1%). Flasks were incubated at 37˚ C for 48 h in a rotary shaker (120 rpm) and centrifuged for 30 min at 7,500 x g. Three ml of supernatant was mixed with 0.5 ml of petrol, vortexed vigorously for 2 min and incubated at 37°C for 1 h. Uninoculated broth was taken as control. Absorbance of the aqueous phase was measured by using spectrophotometer at 400 nm. Emulsification activity per ml (EU/ml) was calculated by using the formula, 1 emulsification unit= 0.01 × dilution factor [10].

Characterization of bacterial isolate BS15

Isolate BS15 exhibiting maximum biosurfactant potential was subjected to cultural, morphological and biochemical test as described in Bergey's manual of systematic Bacteriology [11].

RESULTS AND DISCUSSION

A total of 40 isolates were isolated from 28 samples. Pure cultures of bacterial isolates were maintained on mineral salts medium with petrol as sole source of carbon. The culture supernatants of all 40 isolates were screened for their biosurfactant activity by haemolytic assay; oil spread method and by Emulsification tests (E_{24}). Results of screening process are listed in Table 1.

Table 1 Screening result of isolate BS15 for biosurfactant production

Screening test	Result
Haemolytic assay	-
Oil spread assay	+
Emulsification Index (%)	54.30± 0.06
BATH assay (%)	54.3
Emulsification assay (EU/ml)	122.54
Blue agar plate	+

Haemolysis assay

Of the 40 bacterial strains tested, 7 isolates were positive for hemolysis. Haemolytic assay in this study as a criterion for biosurfactant production as it was widely used method to screen biosurfactant production and in some reports it is the sole method used to screen biosurfactant production [12]. Selvi and Nithya in his studies endorsed that the haemolytic activity could be considered as one of the major criteria for biosurfactant production. Our study is in line with that of [13] used blood haemolysis test for screening biosurfactant producing organisms.

Oil spread method

Among the 40 isolates screened, 8 isolates were positive for oil spread method. One nonhaemolytic culture (BS15) showed biosurfactant production by oil spread method. The positive drop collapse assay also revealed the extracellular production of the biosurfactant and its surface active nature. Shoeb *et al.* (14) showed drop collapse activity in 84% of isolates. Urum *et al.* [15] in his study selected C3 strain based on oil displacement method.

Emulsification index

Out of 40 isolates, BS15 showed the higher emulsification activity of 55 ± 0.03 %. Techaoei (2011) in his study reported 60 % emulsification index with *Pseudomonas aeruginosa*. The *Bacillus cereus* culture isolated during the present study showed 53% emulsification [16]. Vijaya and Raashi reported 70-80% emulsification index with *Bacillus licheniformis. Pseudomonas* sp. showed the highest emulsification test (70.5 ± 0.55) towards n-hexadecane [17].

BATH assay

Out of 40 isolates, 14 isolates were positive for BATH assay, maximum cell adherence of 54.3 % was found with BS15. Thavasi *et al.* [18] in his study reported maximum cell adherence of 95.15 % by *Pseudomonas aeruginosa* followed by *L. delbrueckii* (92.6%). Selvi and Nithya [19] reported 76 % adherence by BATH assay for *Pseudomonas aeruginosa* SBS1001.

Blue agar plate

Of the 40 isolates, presence of dark blue halo zone in the methylene blue agar plate supplemented with CTAB confirmed the presence of anionic bio-surfactant in BS15. Other isolates grew weakly on blue agar plates and produced no halos. Aparna *et al.* [20] detected rhamnolipid type of biosurfactant from *Pseudomonas* sp. 2B using the CTAB-Methylene blue agar medium based method. Priya and Usharani, [4] also reported rhamnolipid from *Peudomonas aeruginosa* and surfactin from *Bacillus subtilis* by TLC plate.

Emulsification assay

In the emulsification assay BS15 showed highest activity of 122.54 EU/ ml. Our results are in agreement with that of Satpute *et al.* [9] who reported maximum biosurfactant production of 213.8 (EU/ml) and 187.5 EU/ml for petrol and xylene. Tulevaa *et al.* [21] in his study reported emulsifying activity of 69%. Out of the 14 isolates, isolate BS15 showed maximum emulsification activity based on drop collapse test, oil spreading technique and by hemolytic activity.

Morphological, biochemical and molecular characterization of BS15 isolate

The isolate produced smooth irregular creamy colonies on nutrient agar. Isolate was found to be Gram positive rod, positive for catalase and oxidase, liquefied gelatine, reduced nitrate and Voges-Proskauer reaction. Arginine dihydrolase, ornithine and lysine carboxylase, indole test were negative. However, it utilized an array of carbon sources like glucose, mannitol, glycerol, glycogen, fructose, galactose, sucrose, sorbitol and xylose (acid production). Based on Bergey's Manual of Systematic Bacteriology, the isolate was identified as genus of *Bacillus* sp.

CONCLUSIONS

In the present study, isolate BS15 isolated from soil showed significant biosurfactant activity. We

recommend oil spread method, emulsification index and surface tension more suitable for primary screening. The importance of this bio-surfactant for industrial use is shown by their physical properties like high emulsification index and reduced surface tension. The functional characterization of isolated biosurfactant indicated that the bio-surfactant produced was rhamnolipid type.

ACKNOWLEDGMENT

We would like to express our sincere gratitude to our institution Acharya Bangalore B School for allowing us to do our project in the college.

REFERENCE

[1] A. Ebrahimi and N. Tashi N, " Isolation of biosurfactant producing bacteria from poultry breast skin". Jundishapur Journal of Natural Pharmaceutical Products, 2012,7, 93- 96.
[2] R.C. Jaysre, S. Basu, P. Singh,T. Ghosal, P. Patra, *et al.*" Isolation of biosurfactant producing bacteria from environmental sample", Pharmacology online, 2011,3, 1427- 1433.
[3] S. Lang S. "Biological amphiphiles (microbial biosurfactants)". Curr. Opin. Colloid Inter. Sci., 2002,7, 12–20.
[4] T. Priya and G. Usharani, "Comparative study for biosurfactant production by using *Bacillus subtilis* and *Pseudomonas aeruginosa*," Bot. Res. Inter., 2009, 2, 284-287.
[5] C. Sivapathasekaran, S. Mukherjee, A. Ray, A. Gupta, R. Sen, "Artificial neural network modeling and genetic algorithm based medium optimization for the improved production of marine biosurfactant", Bioresoure Technology, 2010, 101, 2884- 2887
[6] B. Anandaraj and P. Thivakaran, "Isolation and production of biosurfactant producing organism from oil spilled soil", Journal of Bioscience and Technology, 2010, 1, 120 – 126.
[7] M. Morikawa, Y. Hirata, T. Imanaka, "A study on the structure function relationship of lipopeptide biosurfactants", Biochimica Biophysica Acta, 2000, 1488, 211- 218.
[8] M. Rosenberg, D. Gutnick and E. Rosenberg, E " Adherence of bacteria to hydrocarbons a simple method for measuring cell-surface hydrophobicity", FEMS Microbiology Letter, 1980, 9, 29- 33.
[9] S.K. Satpute, B.D. Bhawsar, P.K. Dhakephalkar and B.A. Chopade BA, "Assessment of different screening methods for selecting biosurfactant producing marine bacteria", Indian Journal of Marine Science, 2008, 37, 243- 250.
[10] J.R.Patil and B.A. Chopade, BA, "Studies on bioemulsifier production by *Acinetobacter* strains isolated from human skin" Journal of Applied Microbiology,2001, 91, 290- 298.
[11] G.M. Garrity, J.A. Bell and T.G. Lilburn, Bergey's Manual of Systematic Bacteriology, Springer-Verlag, New York, USA, 2004.
[12] A. Samanta, P. Pal, A. Mandal, C. Sinha, A. Lalee *et al*., "Estimation of biosurfactant activity of an alkaline protease producing bacteria isolated from municipal solid waste", Central European Journal of Experimental Biology, 2012, 1, 26-35.
[13] P.G. Carrillo, C. Mardaraz, S. J. Pitta-Alvarez and A.M. Giulietti, "Isolation and selection of biosurfactant-producting bacteria", J. World Microbiol.Biotechnol., 1996, 12, 82-84.
[14] E. Shoeb, N. Ahmed, J. Akhtar, U. Badar, K. Siddiqui *et al.* "Screening and characterization of biosurfactant-producing bacteria isolated from the Arabian Sea coast of Karachi. Turk", J. Biol., 2015,39,210-216.
[15] K. Urum and T. Pekdemir, "Evaluation of biosurfactants for crude oil contaminated soil washing," Chemosphere, 2004, 57, 1139- 1150.
[16] Arpita Gupte and Sheetal Sonawdekar, "Study of Oil Degrading Bacteria Isolated From Oil Contaminated Sites" International Journal for Research in Applied Science & Engineering Technology, 2015, 3(2), 345-349.
[17] S. Dhail and N.D. Jasuja, "Isolation of biosurfactant-producing marine bacteria", African Journal of Environmental Science and Technology, 2012, 6, 263 - 266.
[18] Thavasi R, Sharma S, Jayalakshmi S, "Evaluation of screening methods for the isolation of biosurfactant producing marine bacteria", Journal of Petroleum and Environmental Biotechnology, 2011, doi:10.4172/2157-7463.S1-001
[19] K.V. Selvi and N. Nithya, "Production and biodegradation potentials of biosurfactant from *Pseudomonas aeruginosa* isolated from oil polluted water samples",Int. J. Adv. Res. Biol. Sci. 2014, 1(1):149-161.
[20] A. Aparna, G. Srinikethan and H. Smitha, "Isolation, screening and production of biosurfactant by *Bacillus clausii* 5B", Research in Biotechnology, 2012, 3, 49- 56.
[21] B.K.Tuleva, G.R. Ivanov and N.E. Christova, "Biosurfactant production by a new *Pseudomonas putida* strain", Zeitschrift Naturforschung C, 2002, 57: 356- 360.