View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by ePrints@Bangalore University

INT J CURR SCI 2013, 5: 86-94

RESEARCH ARTICLE

ISSN 2250-1770

Enumeration of biosurfactant producing microorganisms from oil contaminated soil in and around Bangalore (India)

Vijaya B*, N.R. Jayalakshmi and K. Manjunath

Department of Microbiology and Biotechnology, Jnana Bharathi Campus, Bangalore University

Bangalore-560 056, Karnataka, India

**Corresponding author: E-mail: vsd_bhuvana@rediffmail.com; Phone: +91- 9980836183*

Abstract

Enhanced production of biosurfactants was carried out with six soil samples, SS-A to SS-F collected from petrochemical contaminated sites in and around Bangalore city. The enumeration of microorganisms was done by determining their colony forming units (CFU's). Among 42 isolates, six were selected with higher CFU's (*Pseudomonas, Bacillus, Streptococcus, Micrococcus, Rhizobium and Lactobacillus*) for induction of biosurfactant production with four different carbon sources (palm oil, castor oil, coconut oil and honge oil) at different concentrations of 0.5-2.0%. To confirm the ability of isolates in biosurfactant production, different screening methods including blood hemolysis, emulsification, bacterial adherence test for hydrocarbon (BATH) assay, determination of surface tension, drop-collapse, cetyl tri ammonium bromide (CTAB) and methylene blue reduction assay were assessed. The ethyl acetate extracted was detected for rhamnolipids, biosurfactant by orcinol method with rhamnose as standard and expressed as rhamnose equivalents suggesting the two species, *Pseudomonas* and *Bacillus* has high potential for biosurfactant production with palm oil and castrol oil treatment.

Keywords: emulsification, screening assays, interfacial surface tension, CFU's

Received: 14th October; Revised: 24th October; Accepted: 26th November; © IJCS New Liberty Group 2013

Introduction

Surfactants are amphiphilic molecules with both hydrophilic and hydrophobic regions attributing towards decrease in surface tension by the formation of aggregates at interfaces between fluids of different polarities. This unique surface characteristic have been applied in various areas like detergency, emulsification, adhesion, coatings, wetting, foaming, soil and water remediation, paints, chromatographic separation, medicine, agriculture, cosmetics etc. (Feichter, 1992; Georgiou et al., 1993; Kosaric, 1993; Rouse et al., 1994; Shafi and Khanna, 1995; Lin, 1996; Volkering et al., 1998; Karanth et al., 1999).

Almost all surfactants currently in use are chemically derived from petroleum and are synthetic in nature exhibiting low rate of biodegradation and high potential to aquatic toxicity. For these reasons, biosurfactants are seen to be a promising alternative with better industrial application. Microorganisms like bacteria, fungi and yeast produce large number of surface metabolites with varied chemical structures and properties (Feichter, 1992; Desai and Banat, 1997; Rosenberg and Ron, 1999). The biosurfactants produced by the microorganism exhibits emulsifying activity to utilize

hydrocarbons as a sole carbon source and to convert them to harmless products (Deleu and Paquot, 2004).

The organisms carry out biosurfactant production when grown either on insoluble substrates (such as hydrocarbons, oils and waxes) or on soluble ones (carbohydrates). Hence the isolation of microbial strains capable of biosurfactant production by using these substrates is of interest in commercial production. Kerosene, petrol, diesel, oils are examples of alternative substrates to produce various biosurfactants like glycolipids- rhamnolipids, lipopeptides and surfactins by different species of *Pseudomonas, Bacillus, Serratia, Candida* etc. (Desai and Banat, 1997; Lang and Wullbrandt, 1999; Nishanthi et al., 2010). Hence, a study was undertaken to isolate, screen and enhance biosurfactant producing microorganisms from different oil contaminated soil in and around Bangalore city with supplementation of different cheap carbon sources.

Materials and Methods

Sampling area

Soil samples were collected from six petrochemical contaminated sites in and around Bangalore city and the sites were designated as SS-A to SS-F. The samples were collected in sterile air tight polytene bags and further tested for their texture, color, consistency, pH and biosurfactant producing microflora.

Isolation and enumeration of bacterial isolates from the sample

1 gm of the oil spilled soil sample was subjected to serial dilution. The organisms were enumerated by spreading on nutrient agar plates and colony characterization was performed with enumeration of bacterial population by determining CFU's.

Production and extraction of biosurfactants from microorganisms using various carbon sources

The six dominant organisms were transferred to 250 ml Erlenmeyer flask containing 50 ml of mineral salt medium (MSM) containing different concentrations of various carbon sources like palm oil, castor oil, coconut oil and honge oil in the range of 0.5 to 2%. The culture flasks were incubated at 30°C for five days in a shaking incubator at 150 rpm. The cells were removed from the culture broth by centrifugation at 12,000 rpm for 15 min at 4°C. The supernatant was filtered with 0.45 µm Millipore filter paper and acidified to pH 3 with 2 N HCl and kept in freezer at 4°C overnight. The biosurfactants were extracted by ethyl acetate on mild shaking at RT overnight. The solvent was evaporated and the oily residue was dissolved in 1 ml of methanol and used for further screening.

Preliminary detection of biosurfactants by methylene blue complexation method

The presence of biosurfactant was recorded by the appearance of sky blue color in the bottom of the tube. The method relies on measuring the absorbance (at 638 nm) of the biosurfactant-methylene blue complex that partitions into the chloroform phase (Pinzon and Ju, 2009).

Estimation of biosurfactants by orcinol method

The positive samples for methylene blue complexation were analysed for rhamnose concentration by the orcinol method according to (Koch et al., 1991). A standard curve was drawn up from L-rhamnose (100 µg/ml)

with a suitable blank at 421 nm and the concentrations of biosurfactants were expressed as rhamnose equivalents.

Purification of biosurfactants by Silica G-60 column

The silica G-60 column was regenerated with three volumes of 100% methanol for standardization. The crude ethyl acetate extract from the four organisms treated with four different carbon sources was dissolved in 1 ml of 100% chloroform. The chloroform was run through the column and allowed to equilibrate for five minutes. Equal volumes of chloroform: methanol in the ratio 9:1 was added to the column to remove the impurities. The gradient of methanol was made from 60% to 100% to completely remove the impurities and finally the biosurfactant was eluted with ethyl acetate and methanol in the ratio 7:3.

Screening methods

The purified extracts were used for further screening to confirm for their biosurfactant production by performing different tests like, surface tension, E_{24} (emulsification method), BATH assay, drop collapse, CTAB reduction and blood haemolysis (Rosenberg et al., 1980; Cooper and Goldenberg, 1987; Robert et al., 1989; Bodour and Maier, 1998; Siegmund and Wagner, 1991; Mulligan, 2004). The results were recorded and their mean values were tabulated.

Statistical analysis

Statistical analysis was performed by using SPSS 11.5 Windows software. The difference in mean biosurfactant production between different concentrations from different carbon sources and different bacteria were analyzed by applying two way ANOVA. The variations between different carbon sources in different organisms for all the five screening tests were analyzed statistically with multiple ANOVA. The result presented are averaged over the independent experiments with ten quantifications within each sample, mean values are expressed as ± S.D at 0.001% level of significance.

Results

Forty two different colonies were isolated from six oil contaminated soil samples. The enumeration of microbial count was done for all the six soil samples by determining their CFUs at 10^{-4} dilution and tabulated (Table 1). After morphological and microscopic observations six organisms were selected by considering their dominance in occurrence in all the plates. Based on the colony characteristics and biochemical tests the six organisms were identified respectively as, *Pseudomonas, Bacillus, Streptococcus, Micrococcus, Rhizobium* and *Lactobacillus* (Table 2). Initially, all the six organisms were tested for biosurfactant production and only four *(Pseudomonas, Bacillus, Streptococcus, Micrococcus)* were found to be positive by the formation of sky blue color when tested with methylene blue complexation method. The confirmed organisms were selected for enhanced production of biosurfactants by treating with four different concentrations from 0.5 - 2% with various carbon sources (palm oil, castor oil, coconut oil and honge oil).

Quantification of the biosurfactant was done after column purification of the extract using orcinol reagent detection method. The variation in biosurfactant production amongst four organisms was statistically analyzed by

Table 1. Soil analysis and enumeration of soil microbial counts

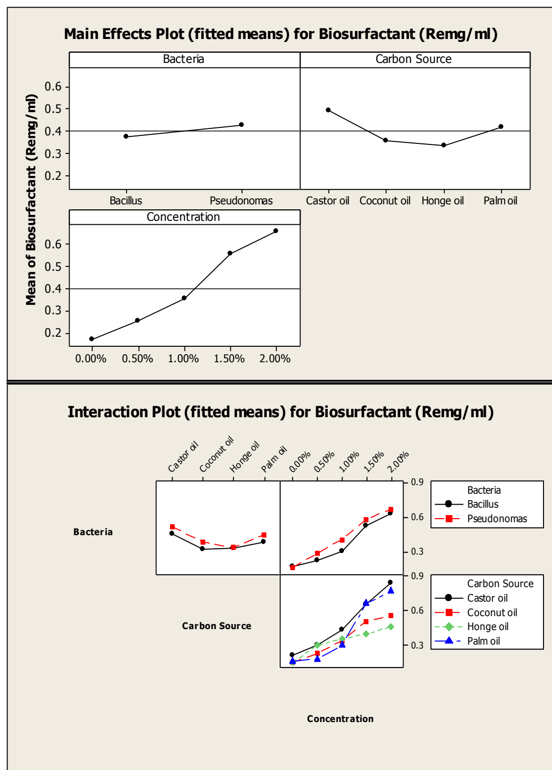
Soil sample	Contamination	Texture	SPC CFU/ml of soil	Total No. of colonies	Colonies selected for screening	Biosurfactant producing isolates
SS-A	Petrol and diesel	Loamy	$(9.9 \pm 03.8) \times 10^5$	56	9	1
SS-B	Motor oil and petrol	Sandy	$(7.1 \pm 57.7) \times 10^6$	54	10	1
SS-C	Petrol and diesel	Loamy	$(3.4 \pm 38.0) \times 10^7$	43	6	1
SS-D	Petrol and diesel	Sandy loam	$(5.3 \pm 34.2) \times 10^6$	42	4	1
SS-E	Petrol and diesel	Loamy	$(2.9 \pm 11.4) \times 10^6$	33	7	1
SS-F	Petrol and diesel	Sandy loam	$(1.2 \pm 17.4) \times 10^6$	18	5	1

Table 2. Morphological and biochemical tests of the six organisms selected from soil samples

Organisms	Gram stain	Cell shape	Spores	Motility	Oxidase activity	Catalase	Gelatinase	Starch hydrolysis	Urease
Pseudomonas sp.	-ve	Rod	-	motile	+	+	+	+	-
Bacillus sp.	+ve	Rod	+	motile	-	+	-	+	+
Micrococcus sp.	+ve	Cocci	-	Non motile	+	+	-	-	+
Rhizobium sp.	-ve	Rods	-	Non motile	+	+	-	-	-
Streptococcus sp.	+ve	Cocci	-	Non motile	-	+	-	-	-
Lactobacillus sp.	+ve	Rods	-	motile	-	+	-	+	-

applying factorial ANOVA and their mean values are graphically represented (Fig. 1).

Fig. 1. Mean biosurfactants recorded in combination of bacteria, carbon source and concentrations (0.001% level of significance)



Among the different concentrations of carbon sources used, 2% addition enhanced more of biosurfactant production in all the four organisms for all the oils used. Palm oil and castor oil treatment produced very high amount of biosurfactants when compared to coconut and honge oil treatment. Among the four organisms tested *Pseudomonas* and *Bacillus* showed good quantification when compared to *Streptococcus* sp. and *Micrococcus* sp. The difference in mean biosurfactants production by using two way ANOVA was found to be statistically significant at the p-values of $P<0.001$ (at 0.001% level of significance).

The four organisms were further screened to confirm for their biosurfactant production by five different screening methods - E_{24} (emulsification method), BATH assay, drop collapse, CTAB reduction and blood haemolysis. Among the four organisms selected two organisms proved best by showing positive results for all the screening methods they are, *Pseudomonas* sp and *Bacillus* sp. The other two organisms *Micrococcus* sp. and

Table 3. Mean values in combination of bacteria and (C) carbon sources (1-Palm Oil; 2-Castor Oil; 3-Coconut Oil; 4-Honge Oil; 5-Untreated)

Bacteria	C	Mean ±S.D. ±S.E				
		Drop collapse test	Bath assay	Surface tension	Emulsification index	Blood hemolysis
Pseudomonas	1	0.73 ± 0.02± 0.01	120.70 ±1.06 ± 0.33	30.26 ± 0.53 ± 0.17	46.37 ± 0.59 ± 0.19	4.10 ± 0.18 ± 0.06
	2	0.51 ± 0.01 ± 0.00	144. 91 ±2.35 ± 0.74	34.12 ± 0.01 ± 0.00	42.10 ± 0.04 ± 0.01	3.77 ± 0.04 ± 0.01
	3	0.53 ± 0.02 ± 0.01	50.80 ± 1.03 ± 0.33	49.77 ± 0.28 ± 0.09	41.70 ± 0.31 ± 0.10	3.73 ± 0.29 ± 0.09
	4	0.41 ± 0.01 ± 0.00	73.70 ± 1.77 ± 0.56	52.43 ± 0.10 ± 0.03	35.92 ± 0.02 ± 0.01	2.41 ± 0.17 ± 0.05
	5	0.32 ± 0.02 ± 0.01	47.30 ± 2.06 ± 0.65	62.49 ± 0.15 ± 0.05	22.18 ± 0.07 ± 0.02	0.00 ± 0.00 ± 0.00
Bacillus	1	0.64 ± 0.01 ± 0.00	142.80 ± 1.48 ± 0.47	28.39 ± 0.03 ± 0.01	45.30 ± 0.04 ± 0.01	4.32 ± 0.07 ± 0.02
	2	0.41± 0.01 ± 0.00	141.80 ± 1.55 ± 0.49	31.02 ± 0.01 ± 0.00	48.42 ± 0.04 ± 0.01	3.77 ± 0.02 ± 0.01
	3	0.53 ± 0.02 ± 0.01	132.40 ± 1.54 ± 0.34	47.61 ± 0.01 ± 0.00	44.12 ± 0.03 ± 0.01	3.54 ± 0.06 ± 0.02
	4	0.45 ±0.02 ± 0.01	75.80 ±1.87 ±0.59	56.59 ± 0.04 ± 0.01	34.10 ± 0.04 ± 0.01	2.33 ± 0.12 ±0.04
	5	0.33 ± 0.02 ± 0.00	61.30 ± 0.82 ± 0.26	71.46 ± 0.33 ± 0.10	9.12 ± 0.06 ± 0.02	0.00 ± 0.00 ±0.00
Micrococcus	1	0.44 ± 0.01 ± 0.00	46.60 ± 1.17 ± 0.37	50.09 ± 0.05 ± 0.02	20.41 ± 0.16 ± 0.05	1.04 ± 0.09 ± 0.03
	2	0.43 ± 0.01 ± 0.00	49.40 ± 0.84 ± 0.27	50.61 ± 0.13 ± 0.04	18.25 ± 0.54 ± 0.17	0.96 ± 0.05 ± 0.02
	3	0.35 ± 0.01 ± 0.00	41.30 ± 0.82 ± 0.26	64.35 ± 0.12 ± 0.04	20.42 ± 0.15 ± 0.05	0.00 ± 0.00 ± 0.00
	4	0.34 ± 0.02 ± 0.01	39.30 ± 0.82 ± 0.26	62.20 ± 0.14 ± 0.04	25.16 ± 0.09 ± 0.03	0.00 ± 0.00 ± 0.00
	5	0.34 ± 0.01 ± 0.00	39.90 ± 1.29 ± 0.41	72.46 ± 0.04 ± 0.01	20.12 ± 0.08 ± 0.02	0.00 ± 0.00 ± 0.00
Streptococcus	1	0.54 ± 0.01 ± 0.00	48.50 ± 2.07 ± 0.65	52.12 ± 0.03 ± 0.01	22.37 ± 0.13 ± 0.04	1.13 ± 0.09 ± 0.03
	2	0.39 ± 0.01 ± 0.00	51.00 ± 0.94 ± 0.30	50.37 ± 0.19 ± 0.06	20.09 ± 0.05 ± 0.02	1.14 ± 0.06 ± 0.02
	3	0.31 ± 0.01 ± 0.00	42.50 ± 1.18 ± 0.37	65.13 ± 0.02 ± 0.01	21.08 ± 0.07 ± 0.02	0.00 ± 0.00 ± 0.00
	4	0.34 ± 0.01 ± 0.00	41.40 ± 0.84 ± 0.27	54.25 ± 0.19 ± 0.06	30.20 ± 0.05 ± 0.02	0.00 ± 0.00 ± 0.00
	5	0.28 ± 0.06 ± 0.02	39.40 ± 0.84 ± 0.27	68.24 ± 0.12 ± 0.04	18.43 ± 0.23 ± 0.07	0.00 ± 0.00 ± 0.00

Streptococcus sp. showed positive results but it was very less and delayed response for all or most of the tests. The variations between palm oil, castor oil, coconut oil and honge oil in different organisms for all the five screening tests were analyzed statistically with multiple ANOVA (Table 3). The statistical hypothesis was accepted for the differences in the screening methods showing variations at 0.001% level of significance.

Discussion

The biosurfactant producing microorganisms are basically isolated from oil spilled soil samples (Thavasi et al., 2008; Lai et al., 2009). These hydrocarbon-contaminated sites house all the typical genera of microorganisms that are hydrocarbon degraders (Naitali et al., 1999; Rahman et al., 2003). Hence, in this study, six different oil contaminated soil samples were used for the isolation of various biosurfactant producing microorganisms and the selection of various soil samples from oil spilled sites in and around Bangalore city provided good number of about forty two isolated colonies. The selection of the cheapest and viable carbon source is essential to obtain commercially viable product with minimum production cost (Mukherjee et al., 2006). The best solution is to minimize the substrate costs by using renewable sources from different varieties of waste oils like olive oil (Robert et al., 1989), palm oil (Deshpande and Daniels, 1995), sunflower oil (Fiebig et al., 1997; Haba et al., 2000; Raza et al., 2007). The advantage of these substrates is that they are low priced, high in purity and as hydrophobic substrates they can possibly enhance the production of biosurfactants (Van Hamme et al., 2006). Hence, palm oil, castor oil, coconut oil and honge oil were

selected in the study to induce biosurfactants production from the soil isolated microorganisms.

The strains of *Pseudomonas* sp. and *Bacillus* sp. selected for enhanced production by palm oil and castor oil treatments produced mainly rhamnolipid and surfactin biosurfactants. Basically, biosurfactants are frequently classified according to the organism which produces them. Rhamnolipids are extracted from *Pseudomonas* sp, the lipopeptides, surfactin and iturin from *Bacillus,* glycoside and rhamnolipids from *Streptococcus* sp. (Vasileva-Tonkova et al., 2008) and *Micrococcus* sp. (Yilmaz et al., 2009). *Rhizobium* sp. is also found to produce rhamnolipid type of biosurfactant (Van Bogaert et al., 2011). It indicates in few of the organisms the three *lactobacillus acidophilus* strains inhibit the integrity of biofilm formation. Initial detection of biosurfactants was done by methylene blue complexation method. This is a rapid, simple and new method for biosurfactant detection. This method is a non tedious and non laborious one which systematically investigates the complexation of biosurfactants and methylene blue (Pinzon and Ju, 2009).

Biosurfactant have a wide variety of structures which means that no standard method can be applied to determine the concentration of all biosurfactants directly. In this study, orcinol assay-a colorometric method was used for direct biosurfactant quantification (Jenny et al., 1991). Quantification of biosurfactants was done by using standard graph obtained from L- rhamnose (0-100 µg/ml) and the amount of biosurfactants was expressed as rhamnose equivalents (RE mg/ml) (Heyd et al., 2008). Apart from this method even anthrone method can be used as an efficient method for quantifying biosurfactants (Chandrana and Dasa, 2012). Qualitatively different tests were performed for the four organisms to confirm for biosurfactant production, surface tension, drop collapse, emulsification assay, BATH assay, CTAB reduction test and hemolytic activity were used. In all these tests the two strains, *Pseudomonas* sp and *Bacillus* sp exhibited good activity and this is encompassed by their rhamnolipid and surfactin production in higher concentration (Cooper and Goldenberg, 1987; Fiebig et al., 1997; Desai and Banat, 1997; Rosenberg and Ron, 1999; Youssef et al., 2004; Pinzon and Ju, 2009).

Conclusion

The result from the study reports that even from the cheapest carbon sources like palm and castor oil at a very lesser concentration of 2% a good concentration of biosurfactants can be produced. This has opened up a practically significant and commercially viable biotechnological approach to produce varieties of biosurfactants having huge industrial application.

Acknowledgement

I thank AIRD (Adarsh Institute of Research and Development, Karnataka, India) for providing funds and I extend by sincere thanks to Mr. V.M. Tejasvi for his assistance in applying statistical tools.

References

Bodour AA, Maier MM (1998). Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant producing microorganisms. J. Microbiological Methods, 32: 273-280.

Chandrana P, Dasa N (2012). Role of sophorolipid biosurfactants in degradation of diesel oil by *Candida tropicalis*. Bioremediation, Sssa Spec Publ., 16: 19-30.

Cooper G, Goldenberg GB (1987). Surface active agents from two *Bacillus* species. Appl. Environ Microbiol., 53: 224-229.

Deleu M, Paquot M (2004). From renewable vegetables resources to microorganisms: new trends in surfactants. Comptes Rendus Chimie, 7: 641-646.

Desai JD, Banat IM (1997). Microbial production of surfactants and their commercial potential. Microbiol. Mol. Biol. Rev., 61: 47–64.

Deshpande M, Daniels L (1995). Evaluation of sophorolipid biosurfactant production by *Candida bombicola* using animal fat. Bioresour Technol., 54: 143-150.

Feichter A (1992). Biosurfactants: moving towards industrial application. Trends in Biotechnology, 10: 286-293.

Fiebig RD, Schulze J, Chung C, Lee ST (1997). Biodegradation of polychlorinated biphenyls (PCBs) in the presence of a bio-emulsifier produced on sunflower oil, Earth and Environmental Science. Biodegradation, 8: 67-75.

Georgiou GS, Lin C, Sharma MM (1993). Surface activecompounds from microorganisms. Biotechnology, 10: 60-65.

Haba E, Espuny MJ, Busquets M, Manresa A (2000). Screening and production of rhamnolipids by *Pseudomonas aeruginosa* 47T2 NCIB 40044 from waste frying oils. J. Appl. Microbiol., 88: 379-387.

Heyd M, Kohnert A, Tan T.H, Nusser M, Kirschhöfer F, Brenner-Weiss G, Franzreb M, Berensmeier S (2008). Development and trends of biosurfactant analysis and purification using rhamnolipids as an example. Analaytical and Bioanalystical Chemistry. 391: 1579-1590.

Jenny K, Kappeli O, Fiechter A (1991). Biosurfactants from *Bacillus licheniformis*: structural analysis and characterization. Appl Microbiol Biot., 36: 5-13.

Karanth NGK, Deo PG, Veenanadig NK (1999). Microbial production of biosurfactants and their importance. Current Science, 77: 116-126.

Koch AK, Kappeli O, Fiechter A, Reiser J (1991). Hydrocarbon assimilation and biosurfactant production in *Pseudomonas aeruginosa* mutants. J.of Bacteriol., 173: 4212.

Kosaric N (1993). Biosurfactants: production, properties, applications, NewYork, USA, Marcel Dekker.

Lai CC, Huang YC, Wei YH (2009). Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil. Journal of Hazardous Materials, 167: 609-614.

Lang S, Wullbrandt (1999). Rhamnose lipids-biosynthesis, microbial production and application potential. Appl. Microbiol. Biot., 51: 22-32.

Lin S (1996). Biosurfactants: Recent Reviews. J. Chem. Tech. Biotechnol., 66: 109-120.

Mukherjee S, Das P, Sen (2006). Towards commercial production of microbial surfactants in Biotechnology, 24: 509-515.

Mulligan NC (2004). Environmental applications for Biosurfactants. Environmental Pollution., 133: 183-198.

Naitali MB, Rakatozafy MH, Marchal R, Leveau JY, Vandecasteele JP (1999). Diversity of bacterial strains degrading hexadecane in relation to the mode of substrate uptake. J Appl Microbiol., 86: 421-428.

Nishanthi R, Kumaran S, Palani P, Chellaram C, Premanand T, Kannan V (2010). Screening of Biosurfactants from Hydrocarbon degrading bacteria. J. Ecobiotechnology, 2/5: 47-53.

Pinzon NM, Ju LK (2009). Analysis of rhamnolipid Biosurfactants by methylene blue complexation. Appl Microbiology and Biotechnology, 82: 975-981.

Rahman KSM, Rahman TJ, Kourkoutas Y, Petsas I, Marchant R, Banat (2003). Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. Bioresource Technology, 90: 159-168.

Raza ZA, Rehman A, Khan MS, Khalid ZM (2006). Improved production of biosurfactant by a *Pseudomonas aeruginosa* mutant using vegetable oil refinery wastes. Earth and Environment Science. Biodegradation., 18: 15-121.

Robert M, Mercade ME, Bosch MP, Parra JL, Espuny MJ, Manresa MA and Guinea J (1989). Effect of the carbon source on biosurfactant production by *Pseudomonas aeruginosa* 44T1. Biotechnology Letters, 11: 871-874.

Rosenberg M, Gutnic, D and Rosenberg E (1980). Adherence of bacteria to hydrocarbons: A simple method for measuring cell-surface hydrophobicity. FEMS Microbiology Letters, 9: 29-33.

Ron EZ and Rosenberg E (1999). High and low molecular mass microbial surfactants. Appl. Microbiol. Biotechnol., 52: 154-162.

Rouse JD, Sabatini DA, Suflita JM and Harwell JH (1994). Influence of surfactants on microbial degradation of organic compounds. Critical Reviews in Environmental Science and Technology, 24: 325-370.

Shafi R and Khanna S (1995). Biosurfactants-A Review. Indian J. Microbiol., 35: 165-184.

Siegmund I and Wagner F (1991). New method for detecting rhamnolipids excreted by Pseudomonas species during growthon mineral agar. 5: 265-268.

Thavasi R, Jayalakshmi S, Balasubramanian T and Banat IM (2008). Production and characterization of a glycolipid biosurfactant from *Bacillus megaterium* using economically cheaper sources.W.J.of Microbiology and Biotechnology. 24: 917-925.

Van Bogaert INA, Zhang JX and Soetaert W (2011). Microbial synthesis of sophorolipids. Process Biochem., 46: 821-833.

Van Hamme JD, Singh A, Ward OP (2006). Physiological aspects Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotechnol Adv., 24: 604-620.

Vasileva-Tonkova ED, Galabova E, Stoimenova, Lalchev Z (2008). Characterization of bacterial isolates from industrial wastewater according to probable modes of hexadecane uptake. Microbiol Res., 163: 481-486.

Volkering F, Breure AM, Rulkens WH (1998). Microbiological aspects of surfactant use for biological soil remediation. 8: 401-417.

Walencka E, Rozalska S, Sadowska B, Rozalska B (2008).The influence of *Lactobacillus acidophilus*-derived surfactants on staphylococcal adhesion and biofilm formation. Folia Microbiol. (Praha). 53: 61-6.

Yilmaz F, Ergene A, Yalçin E, Tan S (2009). Production and characterization of biosurfactants produced by microorganisms isolated from milk factory waste waters. Environ Technol., 30: 1397-404.

Youssef NH, Duncan KE, Nagle DD, Savage KH, Knapp RM, McInerney MJ (2004). Comparison of methods to detect biosurfactant production by diverse microorganisms. J. Microbiological Methods, 56: 339-347.