



International Journal of ChemTech Research

CODEN (USA): IJCRGG ISSN: 0974-4290 Vol.8, No.7, pp 260-270, **2015**

Isolation and identification of oil utilizing microorganisms from different environmental sources

Jaysree, R.C., Rajam C.*, and Rajendran, N.

School of Bio Sciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India

Abstract: Microorganisms such as bacteria, fungi, yeast etc. present in a wide range of environments have been capable of producing biosurfactants. Thus different biosurfactants are classified according to the presence of sugar, amine or lipid group and they are rhamnolipid, lipopeptide, polymeric biosurfactants etc. Their multiple uses as antimicrobial agent, bioremediation of environmental pollutants, pesticides etc. have increased its significance for its large production. Total 27 different environmental samples were collected from VIT University, Vellore and Ottappalam, Palakkad District of India. 27 isolates from soil, 12 from water and 11 from salt pan samples were isolated and biosurfactant activity of these isolates was measured by oil displacement. 54%, 30% and 16% of the isolates were categorized into low, moderate and high biosurfactant producing isolates, respectively. 14 isolates were further selected for 16S rRNA sequencing according to their activities. Using these results, phylogenetic tree analysis was performed with the strains submitted in NCBI which have been reported to have biosurfactant activity. From those 14 strains, six strain sequences were submitted in the GenBank and acquired accession numbers. Biochemical characterization was also used for the identification of strains. Since these isolated strains were found to be capable of producing biosurfactants, they can be potential candidates for environmental application.

Keywords: Biosurfactant, Environment, Oil displacement, 16S rRNA, Phylogenetic tree.

Introduction

Microorganisms present in a wide range of environment have shown capability in utilising the organic and inorganic chemicals present in environment and transform it into a non harmful or non-toxic by-product and thus cleaning up the polluted environment which is useful for the man to live in (1). The environments that serve as reservoirs of such ecofriendly microorganisms can be a potential area for identifying the novel enzymes and new compounds that would help to clean up the environment. One of the useful compounds from microorganisms is biosurfactants. Microorganisms such as bacteria, yeast, fungi etc. that have been collected from different environmental conditions are capable of producing biosurfactants. Biosurfactants have already been reported to have antimicrobial activity (2), good adhesive agents and also help in the biodegradation of heavy metals and oil recovery (3, 4). They are also known to solubilize or split-up hydrocarbon that would make them bioavailable to the microorganisms and can be utilized as carbon energy.

These biosurfactants occur in different names such as rhamnolipid, lipopeptide, surfactin, polymeric biosurfactants etc. according to the sugar, amine or lipid group present with the compound (5). They are stable and effective at extreme and harsh temperatures, pH, salinity, and are specific in its action and causing less

toxicity to the environment led to their popularity. Their application in many industries such as detergent, cosmetics, food industry, clinics, chemical industry etc. and remediation of oil spills led to their vast significance in the large production of the biosurfactant (6, 7). One of the main objectives of the present study was to isolate and identify oil utilizing microorganisms from different environmental sources.

Materials and methods

Sampling

For isolation of bacteria, environmental samples were collected from two different areas of India.

- (a) VIT University, Vellore, Tamil Nadu.
- (b) Ottappalam, Palaghat, Kerala.

Soil samples were collected from a petrol station in Vellore, and the campus of VIT University, and coconut plantations and garden in Ottappalam, Kerala. Water samples were collected from an effluent treatment plant, VIT University, Vellore. The numbers of samples collected from VIT University, Vellore and Ottappalam, Kerala were presented in Table 1.

Sr. No.	Location	Type of sample	Sample numbers (16)
1.	VIT University, Vellore,	Soil	6
	Tamil Nadu	Water	3
2.	Ottappalam, Kerala	Soil	4
		Salt pan	3

Table	1 Number	of samples	and the	type of san	ple collected	from different	locations
		01 000000000		.,			10 000010 110

The soil and salt pan samples were collected in sterile polythene bags and were transported to Molecular and Microbiology Research Laboratory, VIT University, Vellore, India and stored at 4°C until further analysis. Water samples were also collected in a sterile centrifuge tubes of 50ml and stored at 4°C until further analysis.

Media preparation

To isolate bacteria from the soil sample, nutrient agar medium was used. The composition of nutrient agar medium was as follows (g per 1000 ml of distilled water); peptone (10); meat extract (10); agar (15). The composition of nutrient broth medium was as follows (g per 1000 ml of distilled water); peptone (10); meat extract (10). Medium pH was 7.2 ± 0.2 and the medium was autoclaved at $121 \,^{0}$ C for 20 min at 15 lbs pressure. The composition of the medium used for the research work was added with 2% of sodium chloride, in addition to the sodium chloride present in the medium for salt pan sample.

Isolation of microorganisms

One gram of soil sample was serially diluted up to 10^{-5} and 10^{-6} , then plated by spread plate method on nutrient agar plates and incubated under aerobic conditions at 37°C for 24 hours. The colonies were randomly selected for further study. The strains isolated were maintained in agar slants at 4°C for future use.

To select only the biosurfactant producing microorganisms, nutrient broth that was supplemented with 1% water soluble fraction (WSF) of diesel as carbon source was used for the enrichment of the bacteria.

Screening for biosurfactant production

Qualitative oil displacement test was performed as explained by Morikawa (8). 25 ml of distilled water was taken in a petri plate and 20 μ l of hydrocarbon source (kerosene) was added making a thin layer of oil on the surface of water. Then, 10 μ l aliquot of supernatant of the particular bacteria was delivered onto the oil. Distilled water was used for negative control and displacement of oil was considered as positive and that was measured in cm.

Identification of bacterial isolates

The different methods for identification of the bacterial isolates were followed as described below:

I. Morphological and biochemical characterization

Bacterial strains isolated from the medium were subjected to Gram staining (9). Spore staining was done using Schaeffer-Fulton method and capsule staining was done using Indian ink. Biochemical tests were also carried out by using Himedia kit method. The strain identification was done by using Bergey's manual of Determinative Bacteriology (10).

II. 16S rRNA sequencing

The bacterial strains were identified using 16S rRNA sequencing. The different steps involved in 16S rRNA sequencing are:

- 1. Extraction of DNA.
- 2. The quality of extracted DNA was checked by running agarose gel electrophoresis.
- 3. Extracted DNA was amplified using polymerase chain reaction (PCR).
- 4. Analysis of the PCR product was done by running gel electrophoresis.
- 5. The PCR products were purified and sequenced for 16S rRNA.
- 6. Results were analyzed and deposited to GenBank.

Extraction of DNA was done using cetyl trimethyl ammonium bromide (CTAB) and phenol:chloroform:isoamylalcohol method using Sambrook and Russell (11). The extracted DNA was checked by running agarose gel electrophoresis, and then DNA was amplified by following polymerase chain reaction using universal primers listed in the Table 2. The universal 16S rRNA primers 518F and 800R and 27F and 1492R were used for 16S rRNA sequencing.

Universal primers	Sequences (5'- 3')
518F	5'-CCAGCAGCCGCGGTAATACG-3'
800R	5'-TACCAGGGTATCTAATCC-3'
27F	5'-AGAGTTTGATCMTGGCTCAG-3'
1492R	5'-TACGGYTACCTTGTTACGACTT-3'

Table 2: Universal primer sequences used for the PCR.

After PCR amplification, agarose gel electrophoresis was carried out using 1.2% agarose gel.

BLASTN and phylogenetic tree construct

The 16S rRNA gene sequences were compared with other bacterial sequences having the same biosurfactant producing capability by using NCBI BLASTN (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for their pairwise identities. Multiple alignments of these sequences were carried out by Clustal Omega (<u>http://www.ebi.ac.uk/Tools/msa/clustalo/</u>). Phylogenetic tree was constructed in Jalview software using neighbour joining with distance calculation.

Results and discussion

Sampling

16 environmental samples were collected from Ottappalam, Palakkad, Kerala and VIT University, Vellore, Tamil Nadu. There were three different types of environmental samples which were soil, water and salt pan samples. Salt pan samples were also soil samples in which salt was added to the soil around the roots of coconut trees to defend them against insects. The number of samples collected from different environments was shown in Table 3.

Environmental source	Number of samples (16)
Soil	10
Water	3
Salt pan	3

Table 3: Number of different samples collected from various environmental sources.

Isolation of biosurfactant producing bacterial strains

Isolation of microorganisms was carried out by using two methods i.e. serial dilution and enrichment method. From 16 different environmental samples, 50 strains were isolated. The number of isolates from each environmental source was shown in Table 4. Table 5 shows the list of microorganisms collected from different sources.

Table 4: Number of isolates from environmental sources.

Environmental source	Number of isolates (50)
Soil	27
Water	12
Salt pan	11

Table 5 shows the number of strains isolated from soil, water and sludge samples from different contaminated or uncontaminated sources. According to the collected strains, 50% were *Pseudomonas* sp. which was found in contaminated soil, sludge and water samples as observed in many investigations (12, 13, 14, 15, 16, 17, 18, 19, 20, 21). The least number of isolates were found to be *Rhodococcus erythropolis, Kocuria marina* and *Virgibacillus salarius* which were collected from alkaline soil and solar salt respectively. *Rhodococcus* sp. *Kocuria* sp. and *Virgibacillus sp.* reported to produce trehalose lipids, nonanoic acid and Lipopeptide (22, 23, 24). In the collected samples of the present study, 35% were *Bacillus* sp. mainly from uncontaminated and contaminated soil and water. Different biosurfactants produced by the bacterial isolates from environmental samples have been characterized as rhamnolipid, lipopeptides, polymeric and glycolipid (Table 5).

Sr. No.	Microorganisms	Area of sample collection	Biosurfactant	References
1.	Pseudomonas aeruginosa	Petroleum contaminated soil	Rhamnolipid	17
2.	Rhodococcus erythropolis	Alkaline soil	Trehalose lipids	22
3.	Pseudomonas fluorescens	Soil near roots of Wheat	Lipopeptides (Iturin	14
		plant	A)	
4.	Pseudomonas syringae	Soil	Lipopeptides	12
5.	Pseudomonas fluorescens	Soil	Lipopeptides	16
6.	Bacillus subtilis	Soil	Surfactin	25
7.	Pseudomonas aeruginosa	Hydrocarbon contaminated	HAAs Rhamnolipid	15
		soil		
8.	Bacillus sp.	Soil contaminated by diesel	Lipopeptide	26
		oil		
9.	Pseudomonas stutzeri	Petrol contaminated soil	Polymeric	21
10.	Bacillus clausii	Soil contaminated from	Lipopeptide	28
		hydrocarbon		
11.	Virgibacillus salaries	Oil contaminated soil	Lipopeptide	24
12.	Bacillus subtilis	Oil field from brine area	Surfactin	27
13.	Kocuria marina	Solar salt	Nonanoic acid	23
14.	Pseudomonas aeruginosa	Sludge contaminated with	Rhamnolipid	20
		oil		

Table 5. List of microorganisms isolated from different environmental sources.

15.	Pseudomonas aeruginosa	Petroleum sludge	Rhamnolipid	18
16.	Pseudomonas aeruginosa	Water from hydrocarbon contaminated area	Rhamnolipid	13
17.	Pseudomonas sp.	Oil sea water sediments	Glycolipid	19
18.	Bacillus subtilis	Water from oil field	Lipopeptide	32
19.	Bacillus sp.	Oil well	Glycolipid	34
20.	Bacillus subtilis	Water from oil field	Glycolipid	33

According to the area of collection, 55% of microorganisms were from soil (22, 12, 25, 14, 15, 17, 16, 26, 27, 28, 21). These results confirm that soil may be a good source for the isolation of biosurfactant producing bacteria. In our study, maximum activity was seen in soil isolates collected from uncontaminated soil. Therefore, soils could be considered as a good source for bacterial isolation when compared to other water sources used for biosurfactant producing bacteria.

Screening for biosurfactant production

Oil displacement is more sensitive method for biosurfactant detection than any other methods used such as microplate and drop collapse method (29). All the 50 bacterial strains were screened by using oil displacement activity for selecting the suitable bacterial strains which could be potential for the production of biosurfactant. The oil displacement activity of 50 isolates from different environmental sources was shown in Fig. 1(a) and 1(b). Based on the high and moderate activities of oil displacement, 14 bacterial strains were further selected for molecular characterization by using 16S rRNA sequencing.





Fig. 1(a): Oil displacement activity of 25 isolated strains from different environmental sources.

Fig. 1(b): Oil displacement activity of 25 isolated strains from different environmental sources.

The different bacterial strains which were further taken for sequencing were JSO1, JSO2, JSO5, JSO6, 2AH, 6AH, 8AH, JCO3, JS, JB, FC1, FC2, FC5, and FC6.The bacterial strains presented in Fig. 1(a) and 1(b) were collected from different environmental sources and they were listed in Table 6.

Figure	Sample code	Collection area	
	JSO1, JSO2, JSO5, JSO6	Salt pan samples, Ottappalam, Kerala.	
	EFTW1, EFTW2, EFTW3, EFTW4,	Effluent treatment plant water, VIT	
	EFTW5, EFTW6, EFTW7, EFTW8,	University, Vellore.	
	EFTW9, EFTW10, EFTW11, EFTW12		
Fig 1(a)	VBS1, VBS2, VBS3, VBS4	Vellore petrol station soil, VIT University,	
		Vellore.	
	BFC	Soil from the campus, VIT University,	
		Vellore.	
	NG1, NG2, NG3, NG4	Soil from garden, Ottappalam, Kerala.	
	NG5	Petrol station soil, Ottappalam, Kerala.	
	1AH, 2AH, 3AH, 4AH, 5AH, 6AH, 7AH,	Garden soil, Ottappalam, Kerala.	
	8AH		
	JS, JB	Nursery soil, VIT University, Vellore.	
Fig 1(b)	JCO1, JCO2, JCO3	Salt pan samples, Ottappalam, Kerala.	
	FC1, FC2, FC3, FC4, FC5, FC6	Soil from food court, VIT University, Vellore.	
	JSY1, JSY2, JSY3, JSY4, JSY5	Salt pan samples, Ottappalam, Kerala.	

Table 6 Isolated bacterial strains from different environmental sources.

The results of oil displacement shown by 50 strains have been categorized according to their activity (Fig. 1a and 1b). The isolated bacterial strains were shown in Table 7 according to their biosurfactant activities. Based on their biosurfactant activity, category I, II and III were grouped into low, moderate and high respectively. 54% of the isolated strains came under category I, in which EFTW3, EFTW8, EFTW10, EFTW11, and EFTW12 showed activity below 1cm and VBS2 and NG3 showed no activity (Fig. 1a).

The rest of the 20 strains had activity above 1 cm (Fig. 1a and b). Moderate activity was shown by 30% of the strains. High activity was exhibited by 16% of the isolated strains. Of which, 3AH, 5AH, FC1 and JSY5 showed values between 3 and 4 cm as shown in Fig. 1(a) and (b). The minimum activity was shown by EFTW3 and EFTW12 both having 0.5 cm and maximum activity was seen in JB with 5 cm and JS with 4.2 cm.

The low and moderate activities exhibited by the isolated strains might be due to the insufficient amount of biosurfactant produced. This could mainly be due to a number of reasons i.e. less carbon to nitrogen ratio, exhaustion of carbon source or nitrogen source, stress conditions, change in the environmental conditions from the collected area to the laboratory conditions, which usually makes them unable to express the genes fully in the organism or give specific responses and signals (30, 31). The distribution of bacterial isolates by their oil displacement activity was shown in Fig. 2. The bacterial isolates were categorized into three by their oil displacement activity as shown in Table 7.

Category	Oil displacement activity (in cm)	Isolates
I	0 - 2	JSO1, JSO2, JSO6, EFTW1, EFTW2, EFTW3, EFTW4, EFTW5, EFTW6, EFTW7, EFTW8, EFTW9, EFTW10, EFTW11, EFTW12, VBS1, VBS2, VBS3, VBS4, BFC, NG1, NG2, NG3, 1AH, 6AH, FC3, JSY3
II	2 – 3	JSO5, NG4, NG5, 2AH, 4AH, 7AH, 8AH, JCO3, FC2, FC5, JSY1, JSY2, JSY4, JCO1, JCO2
III	3 - 5	3AH, 5AH, JS, JB, FC1, FC4, FC6, JSY5

Table: 7. Isolated strains categorized according to their biosurfactant activ	ccording to their biosurfactant activity.	,
---	---	---



Fig. 2: Percentage distribution of bacterial isolates by their oil displacement activity. Note: I = low, II = Moderate, III = High.

Biochemical characterization

These 6 bacterial strains which were selected based on their high and moderate biosurfactant activities were further identified and confirmed by biochemical characterization by using Bergey's manual of Determinative Bacteriology, 1974. Biochemical characterization of the six strains was presented in Table 8.

Identification of isolates by 16S rRNA sequencing

The identified 14 strains by 16S rRNA sequencing were shown in Table 9. The bacterial strains which have been identified were mainly *Pseudomonas* sp., *Micrococcus* sp., *Bacillus* sp. and *Serratia* sp. Other species were *Proteus* sp., *Acinetobacter* sp., *Brevibacterium* sp. and *Bordetella* sp. Of these strains, one species could not be identified based on the sequence data.

Biochemical characterization	B. thuringiensis (JB)	P. vulgaris (2AH)	B. lutescens (JSO5)	Bacillus sp. (JSO6)	M. lylae (JSO1)	M. luteus (JSO2)
Gram staining	Positive	Negative	Positive	Positive	Positive	Positive
Spore staining	Positive	Negative	Positive	Positive	Negative	Negative
Capsule staining	Positive	Negative	Positive	Positive	Negative	Negative
Indole	Negative	Positive	Negative	Negative	Negative	Negative
Methyl red	Negative	Positive	Positive	Positive	Negative	Negative
Voges-Proskauer	Negative	Negative	Negative	Negative	Positive/Negative	Positive/Negative
Citrate	Negative	Positive	Positive	Positive	Positive	Negative
Glucose	Positive	Positive	Negative	Negative	Negative	Positive
Lactose	Negative	Positive	Positive	Positive	Negative	Negative
Sucrose	Positive	Positive	Negative	Negative	Negative	Positive
Rhamnose	Negative	Positive	Positive	Negative	Negative	Negative
Mannitol	Negative	Negative	Positive	Negative	Negative	Positive

Table 8 Biochemical characterization of GenBank submitted bacterial strains.

Phylogenetic tree analysis

The 14 bacterial strains which have been identified by 16S rRNA sequencing were confirmed again using BLASTN for their pairwise identities. Clustal Omega and Jalview software were used for Phylogenetic tree analysis. The phylogenetic tree of those 14 bacterial strains with other bacteria collected from NCBI which were already been reported to have biosurfactant producing capability was shown (Fig. 3). The phylogenetic tree construct was done to know the neighbouring distance between the isolated strains and already reported strains obtained from NCBI as listed in Table 10 with their accession numbers. The blue highlighted ones were the strains isolated in the present study and the other strains collected from NCBI (Fig. 3).

Sr.no.	Isolates	Sequenced
1	JB	Bacillus thuringiensis
2	2AH	Proteus vulgaris
3	JS	Bordetella petrii
4	6AH	Acinetobacter pitti
5	8AH	Pseudomonas stutzeri
6	JCO3	Serratia marcescens
7	FC1	Pseudomonas aeruginosa
8	FC2	Serratia marcescens
9	FC5	Uncultured organism
10	FC6	Pseudomonas stutzeri
11	JSO1	Micrococcus lylae
12	JSO2	Micrococcus luteus
13	JSO5	Brevibacterium lutescens
14	JSO6	Bacillus sp.

Table 9 Identified bacterial strains by 16S rRNA sequencing.

Table: 10 Biosurfactant producing bacterial strains and their accession numbers collected from NCBI.

Bacterial strain	Accession number
Alcaligenes faecalis SOL-B	JX534504
Bacillus pumilus SS-1	JX534508
Bacillus cereus strain SNW3-3	JX534506
Bacillus subtilis SNW3	JX534509
Pseudomonas putida SOL-10	JX534510
Serratia marcescens strain MUD - MaC2	JX534512
Serratia sp. MUD-MaC1	JX534511

GenBank submission

16S rRNA sequences of six bacterial strains have been submitted in GenBank, NCBI and received the accession numbers which were listed in Table 11. The sequences of other strains are being in the process of submission to the GenBank through BankIt.

Table 11 Bacterial strains with their GenBank accession numbers.

Isolate	Accession number
Bacillus thuringiensis (JB)	KJ372208
Proteus vulgaris (2AH)	KP289283
Micrococcus lylae (JSO1)	KC351486
Micrococcus luteus (JSO2)	KC351487
Brevibacterium lutescens (JSO5)	KC351488
<i>Bacillus</i> sp. (JSO6)	KC351489



Fig. 3: Phylogenetic tree analysis of the identified strains with the bacterial strains collected from NCBI.

Conclusion

16 different environmental samples were collected from VIT University, Vellore, Tamil Nadu and Ottappalam, Palakkad, Kerala. From these16 samples, 50 strains were isolated by using serial dilution and enrichment method with the addition of 1% of water soluble fraction of diesel oil. Of these 50 strains, 27 isolates were from soil, 12 from water and 11 from salt pan. Oil displacement activity was used as a screening method for selection of bacteria producing biosurfactant. According to the biosurfactant activity, the isolated strains were grouped into three categories in which 54% strains were under category I having low activity, 30% strains were having moderate activity category II and 16% strains were in category III with high activity. The minimum activity was shown by EFTW3 and EFTW12 both having 0.5cm and maximum activity was noticed in JB with 5 cm and JS with 4.2 cm.

Based on the results of screening by oil displacement activity, 14 isolates having high and moderate activities were further selected for molecular characterization by 16S rRNA sequencing. Using the partial 16S rRNA sequences of these 14 isolates, BLASTN was performed to confirm and identify the organisms. Phylogenetic tree analysis was done using CLUSTAL Omega software to know the neighbouring distance between the present 14 isolates and the isolates from NCBI that have already been reported to have the biosurfactant producing capability. Six sequences of the 14 strains were submitted in NCBI GenBank and obtained accession numbers. The remaining rRNA sequences are being in the process of submission.

Biochemical characterization was also used for the identification of these six bacterial strains. These bacterial strains could be tested further to understand their potential for future applications.

Acknowledgement

We wish to thank the management and staff of VIT University, Vellore, India for providing financial support and facilities to carry out the research work.

References

- 1. Trudinger P.A. and Bubela P., Microorganisms and the natural environment. Miner. Deposita, 1967, 2, 147 157.
- 2. Singh, P. and Cameotra S. S., Potential applications of microbial surfactants in biomedical sciences. Trends Biotechnol., 2004, 22, 142–146.
- 3. Soberón Chávez, G., Lépine F. and Déziel E., Production of rhamnolipid by *Pseudomonas aeruginosa*. Applied Microbiol. Biotech., 2005, 68, 718-725.
- 4. Van-Hamme, J. D., Singh A. and Ward O. P., Surfactants in microbiology and biotechnology . Biotech . Advances., 2006, 24, 604-620.
- 5. Desai J. D. and Banat I. M., Microbial production of surfactants and their commercial potential. Microbiol Mol Biol., 1997, 61, 47-64.
- 6. Fiedler H.P. and Umbach W. Cosmetic and toiletries. In Surfactants in Consumer Products: Theory, Technology and Applications (ed.) Falbe, J. Heidelberg: Springer-Verlag 1987 350–398.
- 7. Desai J.D. and Desai A. J., Biosurfactants: production, property, application. In Surfactant Science Series ed. Kosaric, N. 1993, 48. New York: Marcel Dekker.
- 8. Morikawa, M., Hirata Y. and Imanaka T., A study on the structure-function relationship of lipopeptide biosurfactants. Biochim Biophys Acta., 2000, 1488, 211–218.
- 9. Dussault H. P., An improved technique for staining red halophilic bacteria, J. Bacteriol., 1955, 70, 484–485.
- 10. Buchanan R. E., N. E. Gibbons. Bergey's manual of Determinative Bacteriology, 8th (ed.), 1974, 22, The Williams and Wilkins Company, Baltimore, 1146, USA.
- 11. Sambrook, J. and Russell D.W. Molecular cloning: a laboratory manual , 3rd edn, vol 1. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001, 1.31- 1.12.
- 12. Hutchison M.L. and Gross D.C., Lipopeptide phytotoxins produced by *Pseudomonas syringae* pv. *syringae*: comparison of the biosurfactant and ion channel-forming activities of syringopeptin and syringemycin. Mol. Plant-Microb Interact., 1997, 10, 347-54.
- Pimienta A. L. R., Díaz M. M. P., Carvajal F. G. S. and Grosso V J. L., Production of biosurfactants (Rhamnolipids) by *Pseudomonas aeruginosa* isolated from Colombian sludges CT&F - Ciencia, Tecnología y Futuro 1997, 1, 95-108.
- 14. De Souza J. T., de Boer M., de Waard P., van Beek T.A. and Raaijmakers J.M., Biochemical, genetic, and zoosporocidal properties of cyclic lipopeptide surfactants produced by *Pseudomonas fluorescens*. Appl. Environ. Microbiol., 2003, 185, 1027-1036.
- 15. Déziel E., Lépine F., Milot S. and Villemur R. rhlA is required for the production of a novel biosurfactant promoting swarming motility in *Pseudomonas aeruginosa*: 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs), the precursors of rhamnolipids. Microbiology. 2003, 149, 2005-13.
- 16. Kuiper I., Lagendijk E. L., Bloemberg G. V. and Lugtenberg B. J. Rhizoremediation: a beneficial plant-microbe interaction. Mol Plant Microbe Interact., 2004, 17, 6-15.
- 17. Benincasa M., Abalos A., Oliveira I. and Manresa A. Chemical structure, surface properties and biological activities of the biosurfactant produced by Pseudomonas aeruginosa LB1 from soapstock. Ant. Van Leeuw., 2004, 56, 93-105.
- 18. Bharali P., Das S., Konwar B.K. and Thakur A.J., Crude biosurfactant from thermophilic *Alcaligenes faecalis*: Feasibility in petro-spill. Int. Biodeterior biodegradiation, 2011, 65, 682–690.
- 19. Dhail S., Isolation of potent biosurfactant producing bacteria from oil spilled marine water and marine sediments. Afr. J. Biotechnol., 2012, 11, 16751-16757.

- 20. Saadat A., B. Roozbehani, Jaafarzadeh Haghighi Fard N. Production of biosurfactants from oil sludge using isolated *Pseudomonas*. Journal of Environmentally Friendly Processes, 2013, 1, 22-25.
- 21. Pradnya A. Joshi and Dhiraj B. Shekhawat. Screening and isolation of biosurfactant producing bacteria from petroleum contaminated soil. European J. of Exptl. Biol., 2014, 4, 164-169.
- 22. Uchida Y., Misawa S., Nakahara T. and Tabuchi T. Factors affecting the production of succinnoyl trehalose lipids by *Rhodococcus erythropolis* SD-74 grown on *n*-alkanes. Agric. Biol. Chem., 1989, 53, 765–769.
- 23. Sarafin Yesurethinam., Mariathasan Birdilla Selva Donio, Subramanian Velmurugan, Mariavincent Michaelbabu, Thavasimuthu Citarasu. Kocuria marina BS-15 a biosurfactant producing halophilic bacteria isolated from solar salt works in India. Saudi J Biol. Sci., 2014, 21, 511–519.
- 24. Ahmed M. Elazzazy, T.S. Abdelmoneim, O.A. Almaghrabi. Isolation and characterization of biosurfactant production under extreme environmental conditions by alkali-halo-thermophilic bacteria from Saudi Arabia. Saudi J. Biol. Sci. (2015) in press.
- 25. Carrillo C., Teruel J., Aranda F. and Ortiz A., Molecular mechanism of membrane permeabilization by the peptide antibiotic surfactin. Biochem. Biophys. Acta., 2003, 4, 91–97.
- Bento F. M., De Oliveira Camargo F. A., Okeke B. C. and Frankenberger W. T. Diversity of biosurfactant producing microorganisms isolated from soils contaminated with diesel oil. Microbiol. Res., 2005, 160, 249–255.
- 27. Simpson D.R., Natraj N.R., McInerney M.J. and Duncan K.E., Biosurfactant-producing *Bacillus* are present in produced brines from Oklahoma oil reservoirs with a wide range of salinities. Appl. Microbiol. Biotechnol., 2011, 91, 1083-93.
- 28. Aparna A., Srinikethan G., Smitha Hegde Isolation, screening and production of biosurfactant by *Bacillus clausii* 5B. Res. Biotech., 2012, 3, 49-56.
- 29. Ainon Hamzah, Noramiza Sabturani, Shahidan Radiman. Screening and optimization of biosurfactant production by the hydrocarbon-degrading bacteria. Sains Malaysiana, 2013, 42, 615–623.
- Reis R. S., da Rocha S. L. G., Chapeaurouge D. A., Domont G. B., Santa Anna L. M. M., Freire D. M. G. et al. Effects of carbon and nitrogen sources on the proteome of *Pseudomonas aeruginosa* PA1 during rhamnolipid production. Process Biochem., 2010, 45, 1504-10.
- 31. Reis R. S., Pereira A. G., Neves B. C., Freire D. M. Gene regulation of rhamnolipid production in *Pseudomonas aeruginosa-* A review. Bioresour Technol., 2011, 102, 6377-84.
- 32. Namir Haddad I.A., Ji Wang and Bozhong Mu Identification of a Biosurfactant Producing Strain: *Bacillus subtilis* HOB2. Protein & Peptide Letters, 2009, 16, 7-13.
- 33. Langping Wu, Jun Yao, A.K. Jain, Radhika Chandankere, Xudong Duan, and Hans H. Richnow An efficient thermotolerant and halophilic biosurfactant-producing bacterium isolated from Dagang oil field for MEOR application. Int. J. Curr. Microbiol. App. Sci., 2014, 3, 586-599.
- Tabatabaee A., Mazaheri Assadi M., Noohi A. A., Sajadian V. A. Isolation of Biosurfactant Producing Bacteria from Oil Reservoirs Iranian J. Env. Health Sci. Eng., 2005, 2, 6-12.