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ORIGINAL RESEARCH ARTICLE

Effect of Carbon and Nitrogen Sources on the Production of Biosurfactant by *Pseudomonas fluorescens* isolated from Mangrove Ecosystem

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ABSTRACT

The production of rhamnolipid biosurfactant by *Pseudomonas fluorescens* MFS03 isolated from mangrove ecosystem was investigated. Optimization of carbon, nitrogen and carbon to nitrogen ratio was carried out with an objective to achieve maximum production of biosurfactant by *Pseudomonas fluorescens* MFS03 using mineral salts medium as the growth medium. Production of biosurfactant was estimated in terms of emulsification (E24%) index. The best carbon, nitrogen source were glucose and sodium nitrate shows high rhamnolipid yields of 8.76 g l^{-1} and 7.52 g l^{-1} respectively. The best result of maximum rhamnolipid production of 8.63g l^{-1} was observed when glucose and sodium nitrate with a C:N ratio of 20. The biosurfactant production was an growth associated and it was represented with the biomass and the emulsification index E_{24}% in 48 to 70 hrs. The biosurfactant was extracted with ethyl acetate and the positive result for the rhamnose test indicates the biosurfactant was rhamnolipid-type. FT-IR spectrum revealed that the important adsorption bands at 3466.24 cm^{-1}, 2926.45 cm^{-1}, 1743.47 cm^{-1}, 1407.30 and 1162.26 cm^{-1} confirms the chemical structure of rhamnolipid. It showed stability during exposure to high temperatures upto 120°C for 15 min, high salinity 10% NaCl and a wide range of pH. The characteristics study of rhamnolipid produced by *Pseudomonas fluorescens* MFS03 leads to application in bioremediation activities of oil contaminated sites.

Key words: Biosurfactant, C:N ratio, stability, *Pseudomonas fluorescens* MFS03, Emulsification activity and Antimicrobial activity.

1. INTRODUCTION

Microbial surfactants are surface-active metabolites produced by microorganisms when grown on water miscible or oily substrates. They possess the characteristic property of reducing the surface and interfacial tensions using the same mechanisms as chemical surfactants. Biosurfactant constitute a diverse group of surface - active molecules and are known to occur in a variety of chemical structures, such as glycolipids, lipopeptides and lipoproteins, fatty acids, neutral lipids, phospholipids and polymeric and particulate structures.

The features that make them commercially promising alternatives to chemically synthesized surfactants are their lower toxicity, higher biodegradability and hence, greater environmental compatibility, better foaming properties and stable activity at extreme of pH, salinity and temperature [1].

Thus, interest towards these biomolecules has increased considerably in recent years, as they are potential candidates for several industrial and environmental processes [2, 3, 4], e.g. in the bioremediation of hydrocarbons, organic pollutants and heavy metal-contaminated sites, treatment of oil spills, enhanced oil recovery, petroleum, food and beverages, mining, agriculture, pharmaceutical, textile, leather, cosmetic, health care, building and construction, paints and protective coatings, detergent industry, industrial cleaning, water treatment, and chemical and other industries [1, 5].

In spite of their important biological activities, the main bottlenecks in their commercialization are their low productivities and high production costs. Different strategies including the use of inexpensive substrates have been suggested

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towards making their production economically viable [6].

The genus *Pseudomonas* was found as capable of using glycerol, mannitol, fructose, n-paraffins and vegetable oils as carbon sources to produce rhamnolipid-type biosurfactants, which are among the most effective surfactants known today [7]. Studies have been carried out to verify the effect of the nitrogen source and the C/N ratio in order to increase the production of rhamnolipid [8, 9, 10, 11]. Optimizing factors that affect growth in biosurfactant producing organisms with potential for commercial exploitation is of paramount importance.

The aim of the present study was to evaluate the effect of carbon source, nitrogen source and carbon to nitrogen ratio on the production of biosurfactant by *Pseudomonas fluorescens* MFS03 isolated from mangrove ecosystem and characterization of the biosurfactant.

2. MATERIALS AND METHODS

Isolation and Identification of Microorganisms

Pseudomonas fluorescens MFS03 used in this study was isolated from crude oil enriched Mangrove forest soil, Pitchavaram, Tamil Nadu, India. To isolate the organism's serial dilution of the soil sample were introduced into Nutrient agar medium. The plates were incubated at room temperature 28 ± 2°C for 48 hrs. The isolated colonies were again introduced into King's B medium to identify the strain. The culture were maintained on Nutrient agar slant at 4°C and sub-cultured every week. The inoculums was prepared by transferring a loopful of culture from the slant to 250 ml Erlenmeyer flask containing 50 ml of mineral salt medium and 2 % glucose as carbon source and incubated at 30°C in an orbital shaker for 24 hrs.

Screening for Biosurfactant production

Biosurfactant production was examined with drop collapsing test [12], Oil spreading test [13], CTAB plate assay [14], Haemolytic activity [15] was detected as the occurrence of a define clear zone around the colony. Lipase activity was measured using the Tributyrin agar plates as described according to [16]. Emulsification activity was performed according to [17]. The entire assays were performed in triplicates.

Growth medium

Mineral salt medium (MSM) with the following components (g/l^{-1}) was used for the growth of the strain, KH_2PO_4, 2.0; K_2HPO_4, 5.0; $(NH_4)_2SO_4$, 3.0; $NaNO_3$, 2.0; NaCl, 0.10; $MgSO_4.7H_2O$, 0.2; $FeSO_4.7H_2O$, 0.01; $CaCl_2$, 0.01. The trace elements solution presents in the medium with the following composition (mg l^{-1}) $ZnSO_4.7H_2O$, 5.25; $MnSO_4.4H_2O$, 200; $CuSO_4.5H_2O$, 70.5; $NH_4MoO_4.2H_2O$, 15; $CoCl_2.6H_2O$, 200; H_3BO_3, 15. The pH of the medium was adjusted to 7 using 1 M NaOH and sterilized by autoclaving at 121°C for 15 min. Glucose (2%) was used as the sole source of carbon which was sterilized separately and added to the flask containing MSM aseptically. Laboratory scale biosurfactant production was carried out in 250 ml Erlenmeyer flasks containing 50 ml of the medium inoculated with 3 ml of inoculums and the incubated in an orbital shaker for 5 days at 30 ° C.

Effect of carbon and nitrogen source on biosurfactant production

The MSM medium described above was used for the biosurfactant production. To evaluate the effect of carbon sources (glucose, olive oil, groundnut oil, sucrose and diesel) on the production of biosurfactant, 2% of each of the carbon sources was aseptically added to the MSM. Using the most appropriate carbon source, the nitrogen sources (NH_4Cl, $NaNO_3$, NH_4NO_3, urea and yeast extract) were evaluated for the production of biosurfactant.

Effect of carbon to nitrogen ratio on the biosurfactant production

Using the selected carbon source and a variable concentration of the selected nitrogen source, different C/N ratios were tested; 20, 40, 60, 80 and 100. The C/N ratio (with optimized carbon and nitrogen sources) was varied from 10 to 100 by keeping a constant carbon source concentration 45 g l^{-1}.

Extraction, purification and characterization of biosurfactant

The culture broth was centrifuged at 10,000 rpm for 15 min to separate the cell debris. The biosurfactant was extracted from the cell-free supernatant by acidifying with conc. HCl to attain the pH 2.0 and extracted with an equal amount of ethyl acetate. The resultant aliquot was concentrated to dryness in a rotary vacuum evaporator. The purification of the biosurfactant was performed by liquid column chromatography according to the method of [18]. The orcinal assay was used for the estimation of glycolipid in the sample [19]. To 1 ml of sample 9 ml of a solution

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containing 0.19 % orcinol (in 53% H_2SO_4) was added. After heating for 30 min at 80 ° C the samples were cooled at room temperature and the OD_{421} was measured. The rhamnolipid concentration was calculated from a standard curve prepared with L-rhamnose and expressed as mg ml^{-1}. The purified biosurfactant was subjected to the FT-IR spectra was recorded in a Thermo Niocolet, AVATAR 330 FT-IR system, Madison WI 53711- 4495, in the spectral region of 4000-400 cm^{-1} using potassium bromide (KBr) solid cells. The analysis was done in the Department of Chemistry, Annamalai University, India. The spectra were recorded and analyzed using the standard methods described by the previous authors [20, 21].

Stability characterization

Determination of the effect of temperature, pH and NaCl on the activity of the biosurfactant

The stability of the biosurfactant was determined by dissolving the active fraction in 2-10% of NaCl and the emulsification index was calculated. The stability of the biosurfactant with pH values was performed by dissolving the biosurfactant in 0.1 M sodium acetate buffer (pH 4.0-7.0) and 0.1 M sodium phosphate buffer (pH 8.0-12). After 1 hrs incubation with reciprocal agitation, the emulsification activity was measured as previously described. To determine the stability of the biosurfactant the biosurfactant solution was maintained at different temperature range of 25 – 125 ° C for 15 min, and cooled at room temperature.

Antimicrobial activity of biosurfactant

The extracted compound as well as the culture supernatant was tested for antimicrobial activity using well diffusion method and area of the halo was calculated [22]. The extracted active compounds were tested against human pathogens such as *Candida albicans, E. coli, Proteus mirabilis, Klebsiella pneumonia,* Haemolytic *Streptococcus.* Muller Hilton agar plates were prepared and swabbed with pathogens well was made with a steel cork borer (1 cm in dm) and 50 µl of each of extracted compound was added in wells, incubated at 30°C for 24 hrs after incubation, the clear halo was measured and the area of inhibition in mm^2 was calculated.

3. RESULTS AND DISCUSSION

Isolation and identification of bacteria

Totally five strains were isolated from the crude oil enriched mangrove forest soil. Among these strain *Pseudomonas fluorescens* MFS03 shows higher activity, for the further studies *Pseudomonas fluorescens* MFS03 was selected and studied in detailed for the biosurfactant production. The cultural, morphological and biochemical characters of the isolate was studied and the species level is identified by following Bergey's Manual of Determinative Bacteriology [23].

Screening of biosurfactant production

Haemolysis of Blood agar was included in this study since it is widely used to screen biosurfactant production and in some cases, it is sole method used [24]. The results obtained from *Pseudomonas fluorescens* MFS03 haemolyzed Blood agar with wide zone of clearance as the diameter of the zone of clearance is a qualitative method used as an indicator of biosurfactant production [24]. In the CTAB plate it forms halos around the well, which detect the production of extracellular anionic glycolipids by *Pseudomonas* spp. [25]. The biosurfactant synthesized by *Pseudomonas fluorescens* MFS03used in this study are most probably a mixture of rhamnolipids, the amphiphilic surface-active glycolipids usually secreted by *P. aeruginosa* [26]. The drop collapsing test, oil spreading method and lipase activity was also performed as a part of screening. In drop collapsing test a flat drop was observed and in oil spreading method, a clear diameter of 6mm was observed (**Table 1**). The lipase acts on water – oil surfaces and therefore it was suggested that the lipase production is one of the characteristic of biosurfactant/bioemulsifier producers [27]. The reduction in surface tension in the liquid- liquid interface leads to complete spreading of liquid drop over the surface of oil [12]. So the drop collapsing and oil displacement tests are the easiest and effective method for screening biosurfactant production. Time course study on the growth of *Pseudomonas fluorescens* MFS03 on MSM medium showed a maximum biomass yield of 4.78 g after 72 hrs. It indicates the end of the exponential phase and the beginning of stationary phase of the growth. The cell free supernatant of the *Pseudomonas fluorescens* MFS03 shows high emulsification activity (86%) (**Fig 1**).

Table 1: Screening results for the isolate *Pseudomonas fluorescens* MFS03

Screening methods	Results
Haemolytic activity	9 mm
CTAB assay	+
Drop collapsing test	+
Oil spreading test	12 mm
Lipase activity	78 U/mg
Emulsification activity	86%

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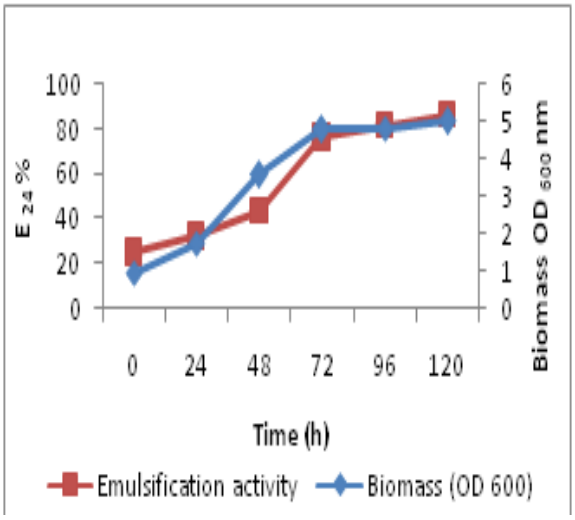

Fig 1: Time course study on the growth of *Pseudomonas fluorescens* MFS03 and the emulsification activity

Effect of carbon source on biosurfactant production

Among the carbon source tested, glucose shows the high biosurfactant yield of 8.76 g l^{-1} while olive oil shows the least yield (2.78 g l^{-1}). The biosurfactant production with different carbon sources were as follows: glucose, groundnut oil, diesel, sucrose and olive oil (**Fig 2**). The lower rhamnolipid yield by *Pseudomonas aeruginosa* from olive oil and soybean oil than that from glucose and glycerol [28]. Vegetable oils have been frequently used as the carbon source for the rhamnolipid production by *Pseudomonas aeruginosa* [29]. The reports by earlier studies support the finding of the present study which shows that glucose is preferred as the carbon source for the rhamnolipid production by *Pseudomonas fluorescens* MFS03.

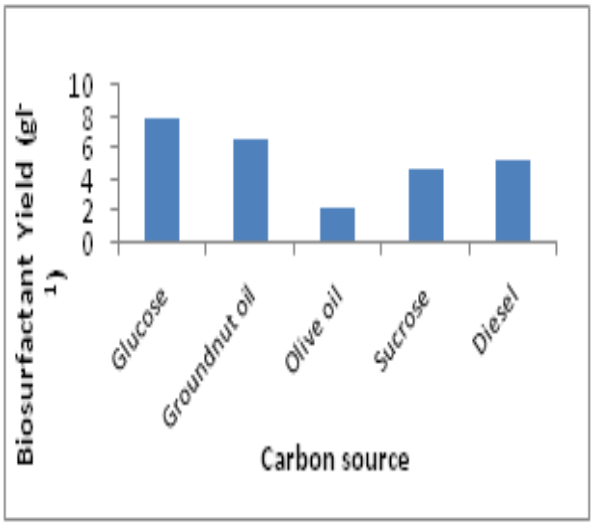

Fig 2: Effect of different carbon source on the production of rhamnolipid biosurfactant by *Pseudomonas fluorescens* MFS03

Effect of nitrogen source on biosurfactant production

Nitrogen source also plays an important role in the biosurfactant production. The limitation of nitrogen source increased the biosurfactant production [1]. In the present study, sodium nitrate shows high yield of rhamnolipid 7.52 g l^{-1} which was followed by yeast extract (Fig-3). The least amount of biosurfactant yield was observed in Urea. Sodium nitrate was a good substrate for the growth with good productivity was reported by [30]. The earlier reports have shown that rhamnolipid production is more efficient under nitrogen-limiting conditions [31, 32].

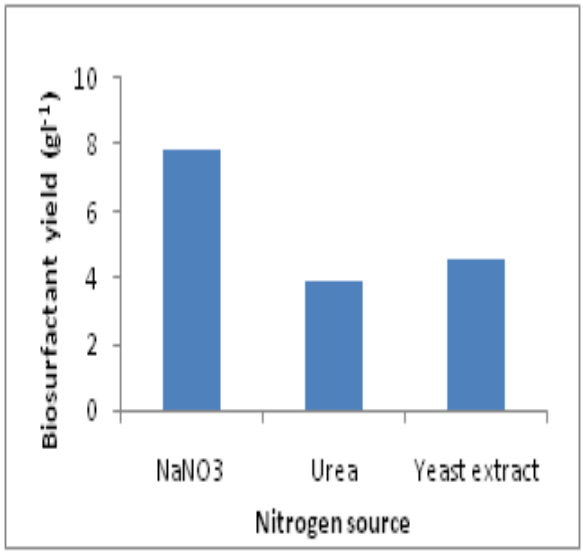

Fig 3: Effect of different Nitrogen source on the production of rhamnolipid biosurfactant by *Pseudomonas fluorescens* MFS03

Effect of carbon to nitrogen ratio on rhamnolipid yield

The carbon-nitrogen ratio is one of the most important factors which induce the biosurfactant production. By using the best carbon source (glucose) and the nitrogen source (sodium nitrate), the effect of carbon to nitrogen ratios on biosurfactant production was studied. The best C/N ratio was 20 which gave a biosurfactant yield of 8.63 g l^{-1} while the least yield was 3.26 g l^{-1} which was recorded for C/N ratio of 60 (**Fig 4**). The concentration of carbon and nitrogen sources affected the yield of rhamnolipid production was reported by [33]. In the present study, the C/N ratio of 20 shows high yield in biosurfactant production, this result correlated with the findings of Wu *et al*. 2008. The present results were supported by [34, 35], the increased rhamnolipid production under the limitation of nitrogen rather than carbon sources.

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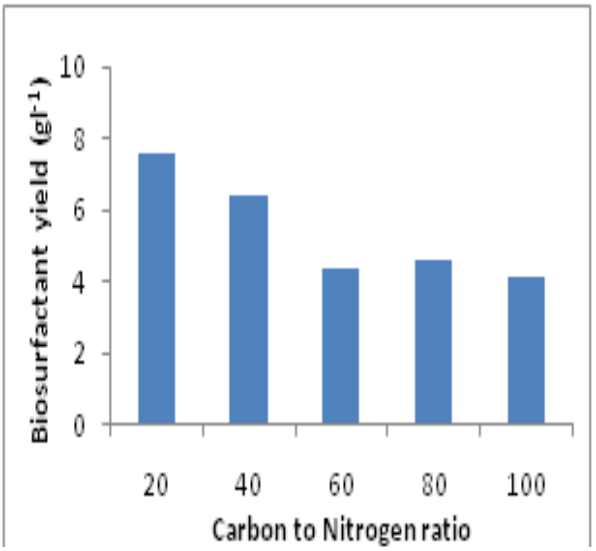

Fig 4: Effect of carbon to nitrogen ration on the production of rhamnolipid biosurfactant by *Pseudomonas fluorescens* MFS03

Characterization of biosurfactant

The rhamnose test was positive which indicates that the separated biosurfactant could be of glycolipid type. Fourier Transform Infrared (FT-IR) spectrum revealed that, the most important adsorption bands located at 3466.24 (OH bond, typical polysaccharides), 2926.45 and 2856.23(CH band: CH_2-CH_3, hydrocarbon chains), 1743.47 and1601.26 cm-1(for C=O, C=O ester bond), 1407.30 cm^{-1}(C-N amide groups). The C-O stretching bands at 1162.26 - 1232.88 cm^{-1} confirm the presence of bonds formed between carbon atoms and hydroxyl groups in the chemical structures of the rhamnose rings and 846.93, 652.05 (for the CH_2 groups) (**Fig 5**). Based on the FT-IR analysis, the biosurfactant produced by *Pseudomonas fluorescens* MSF03 was elucidated as rhamnolipid. FT-IR spectrum of cyclic lipopeptides like surfactin produced by *B. subtilis* and lincheysin produced by *B. licheniformis* as the most effective biosurfactant [36]. In this study, the FT-IR spectral analysis of biosurfactant produced from *Pseudomonas fluorescens* MFS03 was further corroborates with the findings of [37, 38, 39].

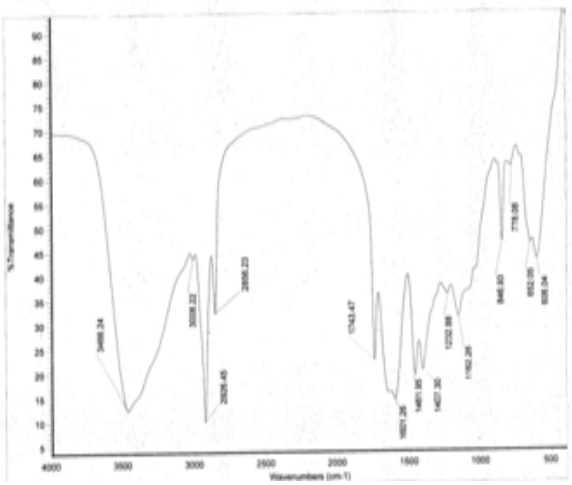

Fig 5: FT-IR analysis of the rhamnolipid biosurfactant produced by *Pseudomonas fluorescens* MFS03

Stability of biosurfactant

The biosurfactant was analyzed under different environmental conditions including pH, temperature and salinity to elucidate the stability of the biosurfactant. The biosurfactant produced by *Pseudomonas fluorescens* MFS03 was shown to be thermostable. Heating of the biosurfactant solution to 100°C caused no significant effect on the biosurfactant performance. The biosurfactant was stable at a broad pH range between 4 and 12. The biosurfactant activity was restored upto 10% sodium chloride concentration. Similar findings were reported for *Pseudomonas aeruginosa* isolate Bs20 which exhibited excellent stabilities at high temperatures (heating at 100°C for 1 hrs and autoclaving at 121°C for 10 min), salinities upto 6% NaCl and pH values up to pH 13 [40].

Antimicrobial activity of the biosurfactant

The biosurfactant from *P. fluorescens* MFS03 showed a wide activity against the pathogenic culture, *E. coli* (11 mm^2) and *Proteus mirabilis* (10 mm^2) showed a higher activity followed by *Candida albicans* (6 mm^2) and *Klebsiella pneumoniae* (5.6 mm^2), Haemolytic *Streptococcus* (4.0 mm^2) (**Fig 6**). This showed strain ability to inhibit various pathogenic microorganisms. Biosurfactants antimicrobial activity has been reported earlier by several authors [41, 42, 43].

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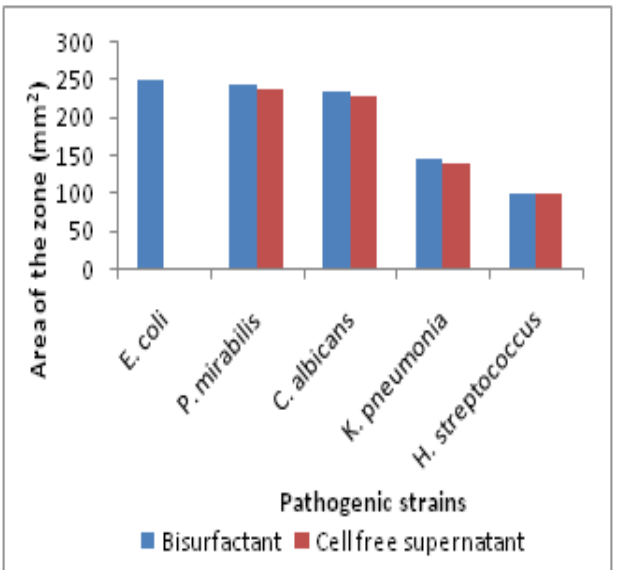

Fig 6: Antimicrobial activity of rhamnolipid produced by *Pseudomonas fluorescens* MFS03 against different clinical pathogens using well-diffusion method

4. CONCLUSION

In conclusion of the present study, shows that natures of carbon source, nitrogen sources and carbon to nitrogen ratio are known to be main factors influencing the performance of biosurfactant production by Pseudomonas fluorescens MFS03 isolated from mangrove ecosystem. In this study, the best carbon source, nitrogen source and C/N (glucose/sodium nitrate) were glucose, sodium nitrate and 20 respectively. Biosurfactant that are stable in environments with high pH, temperature and salinity would find applications in bioremediation. Hence, in the present study the attributes mentioned above makes the biosurfactant as a potential candidate for various bioremediation application processes.

REFERENCES

1. Desai JD and Banat IM. Microbial production of surfactants and their commercial potential. Microbiol. Mol. Biol. Rev, 61: 47–64, (1997).
2. Mulligan CN. Environmental applications of biosurfactants. Env Poll, 133:183–98, (2005).
3. Benincasa M, Abalos A, Oliveira I, Manresa A. Chemical structure, surface properties and biological activities of the biosurfactant produced by *Pseudomonas aeruginosa* LBI from soapstock. Antonie van Leeuwenhoek, 85:1–8, (2004).
4. Lang S, Wullbrandt D. Rhamnose lipids—biosynthesis, microbial production and application potential. Appl Microbiol Biotechnol, 51:22–32, (1999).
5. Banat IM, Makkar RS, Cameotra SS. Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol, 53:495–508, (2000).
6. Mukherjee S, Das P, Sen R. Towards commercial production of microbial surfactants. Trends Biotechnol 24(11):509–515, (2006).
7. Boulton C, Ratledge C. Biosynthesis of lipid precursors to surfactant production. In: Kosaric N, Cairns WL, Gray NCC, editors. Biosurfactants and biotechnology. New York: Marcel Dekker Inc, p. 47–87, (1987).
8. Hamid R, Mahnaz MA, Esmaeil J, Babak B. Optimization of the production of biosurfactant by *Pseudomonas aeruginosa* HR isolated from an Iranian southern oil well. Iran J Chem Eng, 25(1):25–30, (2006).
9. Prieto LM, Michelon M, Burkert JFM, Kalil SJ, Burkert CAV. The production of rhamnolipid by a Pseudomonas aeruginosa strain isolated from a southern coastal zone in Brazil. Chemosphere, 71:1781–1785, (2008).
10. Wu JY, Yeh KL, Lu WB, Lin CL, Chang JS. Rhamnolipid production with indigenous Pseudomonas aeruginosa EM1 isolated from oil-contaminated site. Bioresour Technol, 99:1157–1164, (2008).
11. Abouseoud M, Maachi R, Amrane A, Boudergua S, Nabi A. Evaluation of different carbon and nitrogen sources in production of biosurfactant by Pseudomonas fluorescens. Desalination, 223:143–151, (2008).
12. Youssef NH, Dunacn K.E, Nagle DP, Savage KN, Knapp RM and Mcinerney MJ. Comparison of methods to detect biosurfactant production by diverse microorganism. J. Microbial. meth, 56: 339-347, (2004).
13. Morikawa M, Hirata Y and Imanaka T. A study on the structure-function relationship of the lipopeptide biosurfactants. Biochem. Biophys. Acta, 1488: 211 – 218, (2000).
14. Siegmund I and Wagner F. New method for detecting rhamnolipids exerted by *Pseudomonas* species grown on mineral agar. Biotechnol. Tech, 5: 265- 268, (1991).

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15. Carrillo P, Mardarz C and Pitta-Alvarz S. Isolation and selection of biosurfactant producing bacteria. World. J. Microbiol. Biotechnol, 12: 82- 84, (1996).
16. Seghal Kiran G, Anto Thomas T, Joseph Selvin , Sabarathnam B, Lipton AP. Optimization and characterization of a new lipopeptide biosurfactant produced by marine *Brevibacterium aureum* MSA 13 in solid state culture. Bioresoure Tech, 101: 2389- 2396, (2010).
17. Cooper DG and BG Goldenberg. Surface-active agents from two *Bacillus* species. Appl. Environ. Microbiol, 53: 224-229, (1987).
18. George S, Jayachandran K. Analysis of rhamnolipid biosurfactants produced through submerged fermentation using orange fruit peelings as sole carbon source. Appl. Biochem. Biotechnol, 158: 694 – 705, (2009).
19. Chandrasekaran EV, Bemiller JN. Constituent analyses of glycosaminoglycans. In: Whistler RL (ed) Methods in carbohydrate chemistry. Academic Press, New York, pp 89–96, (1980).
20. Yin H, Qiang J, Jia Y, Ye J, Peng H, Qin, H, Zhang and He B. Characteristics of biosurfactants produced by *Pseudomonas aeruginosa* S6 isolated from oil-containing wastewater. Process, 7: 262-266, (2008).
21. Pornsunthorntawee O, Maksung S, Huayyai O, Rujiravanit R and Chavadej S. Biosurfactant production by *Pseudomonas aeruginosa* SP4 using sequencing batch reactors: Effects of oil loading rate and cycle time. Bioresour. Technol., 100: 812–818, (2009).
22. Cappuccino JG, Sherman N. Microbiology: a laboratory manual. Pearson Education, Singapore, pp. 49, (1999).
23. Buchanan RE, Gibbons NE, Cowan ST, Holt TG, Liston J, Murry RG, Niven CF, Ravin AW, RY Stainer. Bergey's manual of determinative bacteriology. Williams and Wilkins Co.,Baltimore, 1246 p, (1974).
24. Rodrigues L, Moldes A, Teixeira J, Oliveira R. Kinetic study of fermentative biosurfactant production by Lactobacillus strains. J Biochem Eng, 28:109–116, (2006).
25. Siegmund I, Wagner, F. New method for detecting rhamnoli-pids exerted by Pseudomonas species grown on mineral agar. Biotechnol Tech, 5:265–268, (1991).
26. Deziel E, Paquette G, Villemur R, Lepine F, Bisaillon JG. Biosurfactant production by a soil Pseudomonas strain growing on polycyclic aromatic hydrocarbons. Appl Environ Microbiol, 62(6):1908–1912, (1996).
27. Kokare CR, Kadam SS, Mahadik KR, Chopade BA. Studies on bioemulsifier production from marine *Streptomyces* sp. S1. Ind . J Biotechnol, 6: 78-84, (2007).
28. Wu JY, Yeh KL, Lu WB, Lin CL, Chang JS. Rhamnolipid production with indigenous Pseudomonas aeruginosa EM1 isolated from oil-contaminated site. Bioresour Technol, 99:1157–1164, (2008).
29. Mata-Sandoval JC, Karns J, Torrents A. Effect of nutritional and environmental conditions on the production and composition of rhamnolipids byPseudomonas aeruginosa UG2. Microbiol Res, 155:249–256, (2001).
30. Ouled-Hadder H, El-Gilawi MH, Aziz GM, Iraqi. J. Biotechnol. 2:82, (2004).
31. Benincasa M, Contiero J, Manresa MA, Moreaes IO. Rhamnolipid production by Pseudomonas aeruginosaLBI growing on soapstock as the sole carbon source. J Food Eng, 54:283–288, (2002).
32. Kim HS, Jeon JW, Kim BH, Ahn CY, Oh HM, Yoon BD. Extracellular production of a glycolipid biosurfactant, mannosylerythritol lipid, by Candida sp. SY16 using fed-batch fermentation. Appl Microbiol Biotechnol, 70:391–396, (2006).
33. Jeong HS, Lim DJ, Hwang SH, Ha SD, Kong JY. Rhamnolipid production by Pseudomonas aeruginosa immobilized in polyvinyl alcohol beads. Biotechnol Lett, 26:35–39, (2004).
34. Guerra-Santos L, Kappeli O, Fiechter A. Appl. Environ. Microbiol, 48: 301, (1984).
35. Guerra-Santos L, Kappeli O, Fiechter A. Appl. Microbiol. Biotechnol, 24: 443, (1986).
36. Carrera P, Cosmina P, Grandi G, Guido. Method of producing surfactin with the use of mutant of *Bacillus*. United State Patent, 5227294, (1993).
37. Rodrigues L, Teixeira J, Oliveira R and van der Mei HC. Response surface

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optimization of the medium components for the production of biosurfactants by probiotic bacteria. Process Biochem, 41: 1–10, (2006).

38. Pornsunthorntawee O, Arttaweeporn N, Paisanjit S, Somboonthanate P, Abe M, Rujiravanit R and Chavadej S. Isolation and comparison of biosurfactants produced by *Bacillus subtilis* PT2 and *Pseudomonas aeruginosa* SP4 for microbial surfactant enhanced oil recovery. J Biochem. Eng. J, 42: 172–179, (2008).
39. Olivera ND, Nievas ML, Lozada M, del Prado G, Dionisi HM and Sineriz F. Isolation and characterization of biosurfactant-producing *Alcanivorax* strains: hydrocarbon accession strategies and alkane hydroxylase gene analysis, Res. Microbiol, 160: 19-26, (2009a).
40. Abdel-Mawgoud AM, Aboulwafa MM, Hassouna NA. Characterization of rhamnolipid produced by *Pseudomonas aeruginosa* isolate Bs20. Appl. Biochem. Biotechnol, 157: 329 – 345, (2009).
41. Peypoux F, Bonmatin J, Mand Wallach J. Recent trends in the biochemistry of surfactin. Appl. Microbiol. Biotechnol, 51: 553-563, (1999).
42. Richter M, Willey M, Suessmuth R, Jung G and Fielder HP. Streptofactin a novel biosurfactant with aerial mycelia inducing from *streptomyces tendar*. Tue 901/8c. FEMS Microbiol. Lett, 163: 165-171, (1998).
43. Krauss EM and Chan SI. Complexation and Phase transfer of nucleotides of gramicidin. S. Biochemistry, 181: 133-140, (1983).

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