IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

ISOLATION, SCREENING AND IDENTIFICATION OF BIOSURFACTANT PRODUCING BACTERIAL STRAINS FROM OIL CONTAMINATED PLACES OF TRICHY

[1]S. Chithra, [2]Dr. N. Hemashenpagam

[1]HOD & Professor , [2]Professor

[1]Department of Microbiology, Sri Sarada Niketan College of Science for Women, Karur, Tamil Nadu, India.,

[2]PG and Research Department of Microbiology, Hindustan College of Arts and Science, Tamil Nadu, India.

Abstract

Bio-surfactant production from bacteria are still an interested research at industrial biotechnology, microbial cell factories and applied microbiology. Mineral Salt Medium (MSM) was used for the bio-surfactant production. Totally 40 bacterial isolates were isolated for the bio-surfactant production. Drop collapse assay, oil spreading assay, emulsification assay and Hydrocarbon Overlay Agar Assay was carried out for screening the potent bio-surfactant efficient strain. Results from 40 *Pseudomonas species* isolates and the followed method drop collapse method showed 22 isolates, oil spread assay showed 20 isolates and emulsification assay showed 34 isolates exhibited good bio-surfactant activities. Screening method reveals that many isolates showed good response for bio-surfactant production. Therefore further secondary screening methods are required to identify the high efficient strain, culture media optimization, molecular identification and analytical studies are required.

Keywords

Bio-surfactant production, Mineral Salt Medium (MSM), 40 bacterial isolates, *Pseudomonas species*.

1. Introduction

Surfactants are molecules with hydrophobic (non-polar) and hydrophilic (polar) moieties that reduce surface and interfacial tension between liquid. According to their nature, they can be distinguished as chemical surfactants or bio-surfactants (obtained from microbial sources) [1,2]. The common classes of biosurfactants such as glycolipids, lipopeptides, phospholipids, fatty acids, lipoproteins and polymeric and their derivatives compounds [3,4].

Biosurfactants are organic compounds produced by a class of bacteria, yeast and fungi with a nature of reducing the surface tension [5].

Biosurfactants have specific features, such as low toxicity, short time biodegradability, and flexibility in nature [6-9]. A wide range of microbes can produce biosurfactants to utilize their substrates such as oils, alkanes, sugars, and agro-industrial wastes to consume their food sources for cellular metabolic activity. A versatile examples such as agro-wastes [10]. They are amphipathic molecules in that the polar portion is ionic (cationic or anionic), non-ionic or amphoteric, and the non-polar portion is hydrocarbon chain [11]. Such properties exihibited as broad spectrum of industrial applications involving emulsifying, detergent preparation, lubricating, wetting, foaming, frothing and solubilization of different phases [12]. Compounds of a microbial source that exhibit surfactant characters were mainly based on metabolic by-products of their cellular pathways [13].

Thus, the main aim of this current study was to screen bio-surfactant producing bacterial isolates from the respective sources.

2. Experimental Procedures

2.1 Samples Collection

Oil Contaminated soils were collected from Trichy and used as source of isolation of bio-surfactant producing bacterial strains. The collected samples were stored in a fresh zip-lock covers and stored until further studies.

2.2 Isolation of bacterial strains

3 g of collected samples were diluted using 1g/L peptone for serial dilutions. From the serially diluted samples, 50 µl of each dilutions was aseptically spread on Crude Oil containing Nutrient Agar plates. The inoculated plates were incubated at 37 ℃ for 24 - 48 hours. After incubation, the isolated colonies were picked up and sub-cultured at regular interval time period for maintaining a pure cultures for further uses. Totally 120 bacterial isolates were isolated. The isolated strains were examined to study their cellular pattern.

2.3 Production of bio-surfactant producing bacterial strains

The bacterial isolates were inoculated on Mineral Salt Medium (MSM). The isolated pure cultures were inoculated in 100 mL of autoclaved MSM medium containing (g/L - W/V) 1.0 K_2HPO_4, 0.2 $MgSO_4.7H_2O$, 0.05$FeSO_4.7H_2O$, 0.1 $CaCl_2.2H_2O$, 0.001 $Na_2MoO_4.2H_2O$, 30 NaCl and Castor Oil (1.0 % w/v). The MSM medium containing conical flasks (250 mL) and incubated in an orbital shaker (200 - 250 rpm) for 7 days at 37℃. After incubation, the culture broths from each conical flasks were subjected to centrifuge at 7000 rpm using Micro-cooling centrifuge for 20

minutes. After centrifugation, the upper layer supernatant was further filtered using 0.2 µm pore size filter paper. Then the cell free supernatant was labeled and stored in -20℃ for further use.

2.4 Screening of bio-surfactant producing bacteria

For screening efficient bio-surfactant producing bacteria, the cell free supernatant was used to study drop collapse assay; oil spreading assay, emulsification assay and Hydrocarbon Overlay Agar Assay to screen and identify bio-surfactant producing bacteria

2.4.1 Drop Collapse Assay

1 ml of castor oil was applied on the clean glass slides and equilibrated to obtain even application on the glass slides. The cell free supernatant was transferred to the oil applied glass slides and the formed drop sizes were observed using magnifying viewing glass. The flat drop shape was considered as the positive indication for bio-surfactant production by the bacterial isolates.

2.4.2 Oil Spreading Assay

10 ml of distilled water and 10 µl of castor oil was added in a Petri Plates to water surface. Then 5 µl of cell free supernatant was added to the above Petri Plates. The oil displacement activity was measured by displacement of oil with oil free clear zone by measuring the diameter of the clear zone. A negative control (Distilled water only) and positive control (Triton X-100) was used for the comparison and references.

2.4.3 Emulsification Assay

The bacterial isolates from pure cultures was suspended in 3 mL of MSM Medium with addition of 1mL of petrol and incubate for 24 - 48 hours at 37℃. Then the reaction mixtures were mixed vigorously for 2 minutes and left undisturbed for an overnight. 1% SDS and distilled water (W/V) was taken as positive and negative control respectively. Finally the emulsion index were calculated using the formula

$$\text{Emulsification Index} = [\text{ Height of Emulsion Layer / Total Height }] \times 100$$

2.4.4 Critical Micelle Concentration

The CMC method was followed to identify at which point the formation of micelles are initiated. The different concentrations of phosphate buffered saline (PBS) serially diluted bio-surfactant samples was measured to plot surface tension and respective point of no significant surface tension variation.

3. Results and Discussion

Totally 40 bacterial isolates were isolated from oil contaminated soil from trichy location. 40X magnification microscopic examination conformed all the bacterial isolates were belongs to *Pseudomonas species.* To screen biosurfactant producing bacterial isolates were studied by Drop collapse assay, oil spreading assay, emulsification assay and Hydrocarbon Overlay Agar Assay. The results obtained from all the assays are depicted in Table No. 1-3.

3.1 Drop Collapse Assay

The drop collapse test was carried out for screening of potent bio-surfactant producing 40 bacterial isolates. This qualitative test is determined the surface and wetting activities [14] and it was considered as the indirect screening of bio-surfactant production study. From [Table No.1] 22 bacterial isolated showed positive drop collapse activity. The drop collapse activity of cell free supernatant samples were obtained by measured drop diameter.

Table No.1 : Drop Collapse activity of bacterial isolates

S.No.	Bacterial Isolates in (Codes)	Drop Collapse Activity
1.	BS1	+
2.	BS2	+
3.	BS3	+
4.	BS4	+
5.	BS5	+
6.	BS6	-
7.	BS7	+
8.	BS8	+
9.	BS9	+
10.	BS10	-
11.	BS11	+
12.	BS12	-
13.	BS13	+
14.	BS14	-
15.	BS15	-
16.	BS16	+
17.	BS17	-
18.	BS18	+
19.	BS19	+
20.	BS20	-
21.	BS21	-
22.	BS22	-
23.	BS23	-
24.	BS24	-
25.	BS25	-
26.	BS26	+
27.	BS27	-
28.	BS28	+
29.	BS29	+
30	BS30	-
31.	BS31	+
32.	BS32	-
33.	BS33	-
34.	BS34	-
35.	BS35	+
36.	BS36	+
37.	BS37	+
38.	BS38	+
39.	BS39	+
40.	BS40	-

3.2 Oil Spreading Assay

This method is used to observe clear zone during the addition of cell free supernatant samples on oil-water surface. A potent bio-surfactant cause reduction of surface tension property. The 20 isolates showed better Oil-Spreading activity against oil-surface surface.

Table No.2 : Oil Spreading assay exhibited by bacterial isolates

S.No.	Bacterial Isolates in (Codes)	Oil Spreading Assay
1.	BS1	-
2.	BS2	-
3.	BS3	+
4.	BS4	+
5.	BS5	+
6.	BS6	-
7.	BS7	+
8.	BS8	+
9.	BS9	+
10.	BS10	-
11.	BS11	+
12.	BS12	-
13.	BS13	+
14.	BS14	-
15.	BS15	-
16.	BS16	+
17.	BS17	-
18.	BS18	-
19.	BS19	+
20.	BS20	-
21.	BS21	-
22.	BS22	-
23.	BS23	-
24.	BS24	-
25.	BS25	-
26.	BS26	-
27.	BS27	-
28.	BS28	+
29.	BS29	+
30.	BS30	-
31.	BS31	+
32.	BS32	-
33.	BS33	-
34.	BS34	-
35.	BS35	+
36.	BS36	+

37.	BS37	+
38.	BS38	+
39.	BS39	+
40.	BS40	-

3.3 Emulsification Assay

The emulsification activity of the cell free supernatant of all isolates data was mentioned in Table No.3. From 40 isolates, 34 isolates showed significant emulsification activity with varying indexed values from 50 ± 0.1 %.

Table No.3 : Emulsfication index values of bacterial isolates

S.No.	Bacterial Isolates in (Codes)	Emulsification Index in (%)
1.	BS1	4
2.	BS2	2
3.	BS3	12
4.	BS4	3.0
5.	BS5	20
6.	BS6	9
7.	BS7	30
8.	BS8	50
9.	BS9	11
10.	BS10	1
11.	BS11	14
12.	BS12	0.3
13.	BS13	14
14.	BS14	3
15.	BS15	0.5
16.	BS16	19
17.	BS17	10
18.	BS18	11
19.	BS19	19.5
20.	BS20	10
21.	BS21	-
22.	BS22	-
23.	BS23	0.3
24.	BS24	-
25.	BS25	9
26.	BS26	12
27.	BS27	0.2
28.	BS28	12.4
29.	BS29	13
30.	BS30	4.2
31.	BS31	12.6

32.	BS32	-
33.	BS33	-
34.	BS34	-
35.	BS35	13.9
36.	BS36	49
37.	BS37	36
38.	BS38	23
39.	BS39	22.9
40.	BS40	0.1

4. Conclusion

Surfactants are chemically synthesized surface-active compounds widely used for large number of applications in various industries. During last few years there is increase demand of biological surface-active compounds or biosurfactants which are produced by large number of microorganisms as they exert biodegradability, low toxicity and widespread application compared to chemical surfactants. They can be used as emulsifiers, de-emulsifiers, wetting agents, spreading agents, foaming agents, functional food ingredients and detergents. Various studies at laboratory scale on sand-pack columns and field trials have successfully identified effeciency of biosurfactants property of oil recovery (MEOR) [15]. The results from our present study focused significant ability of bacterial isolates can be used as industrial scale bio-surfactant production in future. A screening methods has shown that an isolate BS8 was efficient strain for bio-surfactant production. Moreover further secondary screening, molecular identification of strains and analytical characterization studies are required for the complete study of bio-surfactant production.

References

1. Brumano LP, Soler MF, Silva SS. Recent advances in sustainable production and application of biosurfactants in Brazil and Latin America. Ind Biotechnol. 2016 doi: 10.1089/ind.2015.0027. [CrossRef] [Google Scholar] [Ref list].

2. Santos APP, Silva MDS, Costa EVL, Rufino RD, Santos VA, Ramos CS, Sarubbo LA, Porto AFL. Production and characterization of a biosurfactant produced by Streptomyces sp. DPUA 1559 isolated from lichens of the Amazon region. Braz J Med Biol Res. 2018 doi: 10.1590/1414-431x20176657. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list].

3. Shekhar S, Sundaramanickam A, Balasubramanian T. Biosurfactant producing microbes and their potential applications: a review. Crit Rev Environ Sci Technol. 2015 doi: 10.1080/10643389.2014.955631. [CrossRef] [Google Scholar] [Ref list].

4. Geetha SJ, Banat IM, Joshi SJ. Biosurfactants: production and potential applications in microbial enhanced oil recovery (MEOR) Biocatal Agric Biotechnol. 2018 doi: 10.1016/j.bcab.2018.01.010. [CrossRef] [Google Scholar] [Ref list].

5. Rismani E., Fooladi J., Ebrahimi Por G.H. Biosurfactant production in batch culture by a Bacillus licheniformis isolated from the Persian Gulf. Pak. J. Biol. Sci. 2006;9:2498–2502. doi: 10.3923/pjbs.2006.2498.2502. [CrossRef] [Google Scholar] [Ref list].

6. Abalos A, Vinas M, Sabate J, Manresa MA, Solanas AM. Enhanced biodegradation of Casablanca crude oil by a microbial consortium in presence of a rhamnolipid produced by Pseudomonas aeruginosa AT10. Biodegradation. 2004;15:249–260. doi: 10.1023/B:BIOD.0000042915.28757.fb. [PubMed] [CrossRef] [Google Scholar] [Ref list].

7. Pathak KV, Keharia H. Application of extracellular lipopeptide biosurfactant produced by endophytic Bacillus subtilis K1 isolated from aerial roots of banyan (Ficus benghalensis) in microbially enhanced oil recovery (MEOR) 3 Biotech. 2014;4:41–48. doi: 10.1007/s13205-013-0119-3. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list].

8. Kumar AP, Janardhan A, Radha S, Viswanath B, Narasimha G. Statistical approach to optimize production of biosurfactant by Pseudomonas aeruginosa 2297. 3 Biotech. 2015;5:71–79. doi: 10.1007/s13205-014-0203-3. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list].

9. Mani P, Dineshkumar G, Jayaseelan T, Deepalakshmi K, Ganesh Kumar C, Senthil Balan S. Antimicrobial activities of a promising glycolipid biosurfactant from a novel marine Staphylococcus saprophyticus SBPS 15. 3 Biotech. 2016;6:163. doi: 10.1007/s13205-016-0478-7. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list].

10. Mulligan CN. Environmental applications for biosurfactants. Environ Pollut. 2005;133:183–198. doi: 10.1016/j.envpol.2004.06.009. [PubMed] [CrossRef] [Google Scholar] [Ref list].

11. Santos D. K. F., Rufino R. D., Luna J. M., Santos V. A., Sarubbo L. A. (2016). Biosurfactants: multifunctional biomolecules of the 21st century. Int. J. Mol. Sci. 17:401. 10.3390/ijms17030401 [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list].

12. Silva R. C. F. S., Almeida D. G., Luna J. M., Rufino R. D., Santos V. A., Sarubbo L. A. (2014). Applications of biosurfactants in the petroleum industry and the remediation of oil spills. Int. J. Mol. Sci. 15, 12523–12542. 10.3390/ijms150712523 [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list].

13. Marchant R., Banat I. M. (2012). Biosurfactants: a sustainable replacement for chemical surfactants? Biotechnol. Lett. 34, 1597–1605. 10.1007/s10529-012-0956-x [PubMed] [CrossRef] [Google Scholar] [Ref list].

14. N. H. Youssef, K. E. Duncan, D. P. Nagle, K. N. Savage, R. M. Knapp, and M. J. McInerney, "Comparison of methods to detect biosurfactant production by diverse microorganisms," Journal of Microbiological Methods, vol. 56, no. 3, pp. 339–347, 2004.View at: Publisher Site | Google Scholar.

15. Khire J.M. (2010) Bacterial Biosurfactants, and Their Role in Microbial Enhanced Oil Recovery (MEOR). In: Sen R. (eds) Biosurfactants. Advances in Experimental Medicine and Biology, vol 672. Springer, New York, NY.