

Separation and characterization of biosurfactant from *P.aeruginosa sp1* isolated from oil mill area MIDC, Parbhani (M.S.)

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Abstract

Biosurfactant are an amphiphilic compound that reduces surface and interfacial tensions by accumulating at the interface of immiscible fluids and increase the surface areas of insoluble compounds leading to increased mobility. The extraction of the biosurfactant from the cell free supernatant using the solvent extraction procedure and the quantitative analysis has been discussed. The application of the biosurfactant includes biomedical, cosmetics and bioremediation rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa sp* showed significant applications in the bioremediation of hydrocarbon in gasoline spilled oil and petroleum oily sludge in this review we discuss the potential roles and applications of biosurfactant, mainly focusing on areas such as food and food related industries, biomedicine and therapeutics.

Keywords: Biosurfactant, Rhamnolipid, Bioremediation, Emulsification, Qualitative analysis, Quantitative analysis.

INTRODUCTION

Biosurfactant are amphiphilic biological compounds produced extracellular or as part of the cell membrane by a variety of yeast, bacteria and filamentous fungi [1] from various substances in wastes, most known biosurfactant are glycolipids. They are carbohydrates in combination with long chain aliphatic acids / hydroxyl aliphatic acids. The unique properties of biosurfactant allow their use and possible replacement of chemically synthesized surfactant in a number of industrial operations [2] Biosurfactant reduce surface tension, critical micelle concentration (CMC) and industrial tension in both aqueous solutions and hydrocarbon mixtures [3 and 4].

MATERIALS AND METHODS

Separation of Biosurfactant

The culture broth was centrifuged [10,000g., 15 min] to remove the cells and there after sterilized with milipore membrane filter. The clear sterile supernatant served as the sources of the crude. The biosurfactant was recovered from the cell free culture supernatant by cold acetone precipitation. Three volumes of chilled acetone was added and allowed to stand for 10h. at 4°C. The precipitate was collected by centrifugation and evaporated to dryness to remove residual acetone after which it was re dissolved in sterile water [5].

Biosurfactant Characterization

Qualitative test

Estimation of carbohydrate by Molisch's test

2-3 drops of α -Naphthol solution was added to 2ml of the test solution. 1ml. conc. H_2SO_4 was added along the side of the test tube so that the two distinct layers are formed.

Structural characterization

Rhamnose test

The presence of carbohydrate groups in the biosurfactant molecule was assayed by rhamnose test using the method of [6] A volume of 0.5 ml of cell supernatant was mixed with 0.5ml. of 5% phenol solution and 2.5ml. of sulfuric acid, and incubated for 15 min. before measuring absorbance at 490nm.

Activity characterization

Foaming and emulsifying properties

The foam was produced by hand shaking a 5g/l of crude biosurfactant solution for two minutes. The stability of the foam was monitored by observing it for 2h. The ability of biosurfactant to emulsify some liquid hydrocarbons, such as diesel oil, kerosene, n-heptane and sunflower oil was determined. The sterile biosurfactant 2ml was added into each test tube [in a set of three] containing the substrate 2ml the content of the tubes were vortexed at high speed for 2 min. and left undisturbed for 24h.

$$E_{24} = \frac{\text{Height of Emulsion}}{\text{Total Height}} \times 100$$

Stability characterization

Determination of the effect of temperature, pH and NaCl on the activity of the biosurfactant to determine the thermal stability of the biosurfactant cell free broth was also maintained at a constant temperature range of 20 – 100 °C for 16 min, and cooled at room temperature. To determine the effect of pH on activity the pH of the

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biosurfactant was adjusted (2.0 - 11) prior to filter sterilization. The effect of addition of different concentration of NaCl on the activity of the biosurfactant was investigated. The biosurfactant was re-dissolved after purification with distilled water containing the specific concentration of NaCl (5 – 20% W/V). The surface tension and E 24 values of each treatment were performed as described above. [7]

Extraction and Identification of rhamnolipids

After fermentation, the culture medium was centrifuged at 350g for 20 min and isolated supernatant was adjusted to pH of 2.0 by adding 5 mol/l H₂SO₄ for rhamnolipid precipitation. The precipitate was extracted with two volumes of diethyl ether/ methanol (1:1, v/v) mixture. Evaporation of the solvent yielded crude rhamnolipid. 300 mg. pellets were applied on to thin layer chromatography plates and developed with, a solvent mixture containing chloroform, methanol, and water (65:25:4, v/v/v) rhamnolipid spots were detected by using orcinol reagent.

RESULTS

Biosurfactant separation and characterization.

The biosurfactant was extracted from supernatant by liquid extraction method. A yield of approximately 1.3g/l was found by *P.aeruginosa sp1*. The powdered form of biosurfactant was dissolved in distilled water used for characterization. Same results were obtained by [8] Screening and production of rhamnolipids by *P.aeruginosa sp1* 47T2 NCIB 40044 from waste frying oils.

Qualitative analysis of carbohydrate

Molischs test :- Purple color obtained, Positive test. [9]

Rhamnose test :- Quantitive analysis of carbohydrate

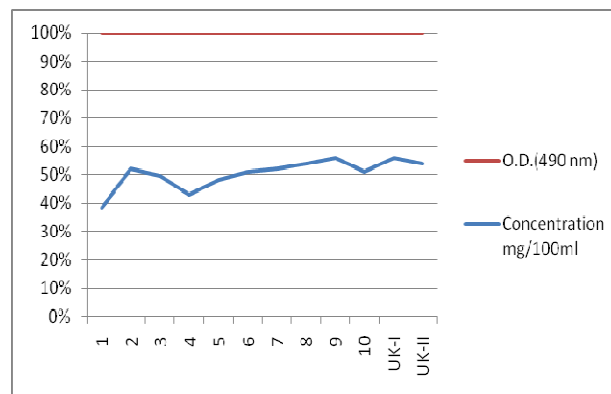
From table 1 and graph 1 it is clear that optical density increase with increasing the concentration of supernatant confirms the rhamnose test positive and separated biosurfactant could be of glycolipid type.

Quantitative estimation of carbohydrate by phenol sulfuric acid method [6]

Table 1 shows the carbohydrate estimation by phenol sulfuric acid method. The amount of carbohydrate contain in supernatant.

Table 1. Quantitive estimation of carbohydrate by phenol sulfuric acid method.

Sr.No.	Concentration (mg/100ml)	O.D.(490 nm)
1	0.2	0.324
2	0.4	0.372
3	0.6	0.610
4	0.8	1.064
5	1.0	1.086
6	1.2	1.16
7	1.4	1.297
8	1.6	1.375
9	1.8	1.430
10	2.0	1.931
UK-I	1.20	0.951
UK-II	0.90	0.780
Control	-	-



Graph 1. Concentration of supernatant v/s optical density

From graph The concentration of UK I :- 1.20 mg/100ml, UK II :- 0.90 mg/100ml

Rhamnose test was positive which indicate that *P.aeruginosa* strain produced glycolipid biosurfactant.

Biosurfactant nature

The preliminary analysis of the biosurfactant produced by the *Pseudomonas aeruginosa* indicated the presence of glycolipid or neutral lipids on the basis of Rf value 0.58.

CONCLUSION

Activity characterization of fermented media shows that the biosurfactant produced by *P.aeruginosa* was shown to be thermostable. The surface tension reduction and emulsification activity were quite stable at the temperature used. At alkaline pH was showed the activity and production of biosurfactant was maximum. The strain best actively at 20% w/v sodium chloride. Rhamnose test was positive it shows given biosurfactant was glycolipid type. The quantitative estimation shows that the given solution of biosurfactant contains 1.20 mg. of carbohydrate.

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