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Potentials of Biosurfactant Producing P*seudomonas sp* from automobile workshop

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ABSTRACT

In the present study soil sample from automobile workshop located at Gandhigram, Dindigul, Tamil Nadu, India was collected, serially diluted and the dilutions 10^{-5} and 10^{-6} were plated on to nutrient agar plates. The predominant colony was selected, identified by Biochemical tests. Then the isolated culture was mass multiplied in mineral salt medium by adding carbon sources such as sunflower oil and whey individually for the production of biosurfactant compounds. The produced product was extracted by acetone precipitation method. After extraction it was analysed physically, chemically and the product thus extracted was identified as biosurfactant compounds Rhamnolipid. Further emulsification index of rhamnolipid was calculated by the ability of biosurfactant compound to emulsify carbon sources like diesel oil, petrol and kerosene. The study concludes that the biosurfactant compound produced by Peudomonas sp was rhamnolipid which is having a higher emulsifying index of 50 with both sunflower oil and whey as carbon sources.

Key words: Biosurfactants, *Peudomonas sp*, Rhamnolipid , Emulsifying index.

INTRODUCTION

Biosurfactants are amphiphilic compounds which are produced by bacteria, yeast and fungi. It has the ability to reduce the interfacial tension. They have the properties of emulsification, foaming, wetting, dispersing or solubilization (1).

Rhamnolipids are class of glycolipids produced commercially by the *P.aeruginosa*, which has ability to produce both mono and di rhamnolipid. (2).

Rhamnolipids has both hydrophilic end, which attracts water and a hydrophobic end, which repels water and attracts non polar chemicals. It can withstand a temperature upto and over boiling point of water. It contains rhamnose sugar. (3).

MATERIALS AND METHODS

Collection of sample and isolation of bacteria:
The soil sample was collected from automobile workshop at Gandhigram and transported immediately to the laboratory.

The soil sample was serially diluted and from which 10^{-5} and 10^{-6} were plated on nutrient agar plates and incubated at 37°C for 24 hrs. From the plates one predominant colony was selected for identification (4)

Identification of bacterial isolate:

The isolated bacteria was identified based on various biochemical tests (4)

Preparation of inoculum and mass multiplication in mineral salt medium:
The identified bacterial isolate was pure cultured and the pure culture was inoculated in mineral salt medium enriched with carbon sources (Sunflower oil and whey). 5ml of inoculum was inoculated in mineral salt medium and incubated at 37°C for 24 hrs in shaker incubator. (5)

Extraction and identification of Biosurfactant:
The culture broth was centrifuged at 10,000 rpm for 15 min and the supernatant was collected. The biosurfactant compound was recovered from supernatant by cold acetone precipitation method. (6)

For identifying the extracted biosurfactant compound various analysis like analysis of Aminoacid, analysis of carbohydrate, and analysis of lipid (7) was done.

Activity characterization:
The sterile biosurfactant compound (2 ml) was added to the test tube containing the substrate (Diesel oil, petrol, kerosene (2ml)). The content of the tubes were vortexed at high speed for 2 mins and incubate for 7 days at 37 C and the results were observed.

$$\text{Emulsification index (E24)} = \frac{\text{height of emulsified layer}}{\text{Total height of the liquid column}} \times 100$$

RESULTS

The bacteria isolated and identified from soil collected from automobile workshop is screened for the production of biosurfactant compound rhamnolipid and the results were given in the table 1, 2, 3, 4, 5 and 6.

DISCUSSION

Biosurfactants are extracellular compounds which are produced by the living organisms. These biosurfactants used to convert insoluble to soluble polycyclic aromatic hydrocarbons (8). In the present study bacteria was isolated and identified from automobile workshop and screen for the production of biosurfactant (5). The *Pseudomonas sp* thus identified was cultivated in mineral salt medium enriched with sunflower oil and whey as carbon sources. The organism shows a good growth in medium containing whey as carbon source. (9.) Then the biosurfactant was extracted and the extracted biosurfactant was tested for the presence of amino acid, carbohydrate, and lipid and it was found to contain aminoacids and lipid which confirms the presence of Rhamnolipid as biosurfactant.(2). Finally the emulsification index was calculated and it was found to be higher with whey as carbon source. (10).

Table 1: Identification of bacterial isolate:

S. No	G	M	I	MR	VP	C	NRT	HSPT	SH	LH	CH	GH	Result
1	- , rod	+	-	-	-	+	+	_	+	+	+	+	*Pseudomonas sp*

Note: G – Gram's Staining, M – Motility, I- Indole, MR – Methyl Red, VP- Voges Proskaner, C- Citrate Test, NRT – Nitrate Reduction Test, SH – Starch Hydrolysis, LP- Lipid Hydrolysis, CH – Catalase Hydrolysis, GH- Gelatin Hydrolysis

Table2: Identification of Biosurfactant compound:

S.No	Test	Colour change	Result
1	**Aminoacid** : Ninhydrin test	 No color change	 -ve
2	**Carbohydrate**: Anthrone test Iodine test Berfoed's test Bial's test	 Bluish green color - Formation of red precipitate -	 +ve -ve +ve -ve
3	**Lipid :** Solubility test Saponification	 Insoluble in water but soluble in organic solvents. Formation of soap bubbles	 +ve +ve

Table 3 Emulsification activity in sample treated with biosurfactant compound (Rhamnolipid) using sunflower oil as carbon source:

Sample	Height of hydrocarbon(mm)	Height of biosurfactant (mm)	Height of emulsified compound(mm) / Day						
			D1	D2	D3	D4	D5	D6	D7
Petrol	0.6	0.6	0.7	0.6	0.7	0.7	0.8	0.9	1.0
Diesel	0.6	0.6	0.6	0.7	0.7	0.8	0.8	0.9	1.0
Kerosene	0.6	0.6	0.6	0.7	0.8	0.8	0.8	0.9	1.0

Note: D- Day

Table 4 Emulsification activity in sample treated with biosurfactant (Rhamnolipid) using whey as carbon source:

S. No	Sample	Height of hydrocarbon(mm)	Height of biosurfactant (mm)	Height of emulsified compound(mm) / Day						
				D1	D2	D3	D4	D5	D6	D7
1	Petrol	0.6	0.6	0.6	0.7	0.7	0.8	0.9	1.0	1.1
2	Diesel	0.6	0.6	0.6	0.6	0.7	0.8	0.9	1.0	1.1
3	Kerosene	0.6	0.6	0.6	0.7	0.7	0.8	0.9	0.9	1.0

Table 5 Emulsification index E_{24} of the samples treated with Rhamnolipid using Sunflower oil as carbon source:

S.No	Sample	Emulsification index $E_{24/}$						
		D1	D2	D3	D4	D5	D6	D7
1	Petrol	0	0	8.3	8.3	16.6	25	33.3
2	Diesel	0	8.3	8.3	16.6	16.6	25	33.3
3	Kerosene	0	8.3	16.6	16.6	16.6	25	33.3

Table 6: Emulsification index E_{24} of the samples treated with Rhamnolipid using whey as carbon source:

S.No	Sample	Emulsification index E_{24}						
		D1	D2	D3	D4	D5	D6	D7
1	Petrol	0	8.3	8.3	16.6	25	33.3	41.6
2	Diesel	0	0	8.3	16.6	25	33.3	41.6
3	Kerosene	0	8.3	8.3	16.6	25	25	33.3

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