IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 5 Ver. I (Sep. – Oct. 2017), PP 17-20
www.iosrjournals.org

Isolation and characterization of Biosurfactant Produced by Soil Isolates *Pseudomonas* species and *Rhodococcus sp*

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Abstract: *Biosurfactants due to their biocompatibility, biodegradability, nontoxicity and versatility over synthetic surfactants are gaining much importance over synthetic surfactants. The present study involves isolation of bacterial strains from hydrocarbon polluted areas of Hubli city and Karwar port and identified. The isolates were cultured on Bushnell Haas medium. Biosurfactant production was carried out in nutrient medium supplemented with hydrocarbon to minimise contamination. Efficiency of biosurfactants against different hydrocarbons was checked by Oil spread technique, Emulsification index and Emulsification activity. In the present study, soil isolate Pseudomonas sp was efficient in oil displacement and emulsification activity as compared to other isolates. While another soil isolate Rhodococcus sp was efficient in Emulsification index .The percentage yield of crude biosurfactant was also done which showed Pseudomonas sp to be the highest producer of biosurfactant. It is evident from the results that biosurfactants can be used in place of synthetic surfactants due to their efficiency and eco friendly nature.*

Keywords; *Biosurfactants, Pseudomonas species, Rhodococcus sp , Emulsification index,*

Date of Submission: 10-08-2017 Date of acceptance: 08-09-2017

I. Introduction

Pollution caused by petroleum hydrocarbons has significant ecological and social problems in terrestrial and aquatic environment. Chemical and physical cleaning processes used to decontaminate the oil polluted areas have been limited in their application (Plaza *et al*., 2006; Thavasi *et al*.,2011). Organic compounds like petroleum hydrocarbons with limited water solubility and low availability to microbial cells, biodegrade very slowly. Surfactants are chemicals that change the properties of water and other fluids. The lower the surface tension and helps in the formation of emulsions between different liquids. Reducing the surface tension allows the water to spread out, capable of degradation of oil droplets or other pollutants. Synthetic surfactants are widely used in many industries such as cosmetic, pharmaceutical and food industry but are expensive and toxic in many aspects. Therefore microbial surfactants used instead of synthetic surfactants.(Nasrin*et al*.,2007, Thavasi *et al.*, 2011, Bayoumi *et al*., 2011).

Biosurfactants are biologically produced surfactants which are naturally produced by bacteria, fungi and yeast. Biosurfactants are amphiphilic chemical compounds containing both hydrophilic (polar- proteins, peptides and carbohydrates) and hydrophobic (non polarsaturated, unsaturated and fatty alcohol or hydroxylated fatty acids.) moieties which can lower the surface tension of a liquid, interfacial tension between two liquids ,or that between a liquid and solid (Nasr *et al*., 2009, Nelly *et al*.,2003). The availability of slightly soluble organic compounds can be enhanced by microbial surfactants which increase aqueous dispersion by many orders of magnitude.(Afuwale *et al*., 2012) Due to environment friendly activity and demand of current market for cost competitiveness, isolation and screening of biosurfactant producing organisms has gained a wide attention. Therefore the present investigation was undertaken to isolate biosurfactant producing bacteria from soil and to check the efficiency of biosurfactant in oil degeradation,

II. Materials and methods

Isolation of Biosurfactant producing microbes:

Soil samples from oil spilled areas of Hubli city and Karwar port were collected in sterilized polythene bags .Samples were serially diluted using 0.9% NaCl and cultured on Bushnell Haas medium (BHM) containing 500µl kerosene as carbon source, the pH was adjusted to 7. The culture was incubated for 6 days at 25-300C temperature this would facilitate only the growth of hydrocarbon degrading microorganisms (Sonali *et al*., 2011).

Identification of Isolates:
Isolates were identified by morphological and biochemical characterization. Kanicka Sharma].Identification was done according to Bergey's Manual of systematic bacteriology. (Sharma 2007)-

Optimizing culture medium:
Selected isolates were sub cultured and maintained on optimized nutrient agar slants (2ml of Kerosene in 100ml). For mass production of surfactant and to minimize the contamination, hydrocarbon (2ml of Kerosene in 100ml) was added to nutrient broth and incubated at 25 – 30°C at pH-7, for 48 hours.

Innoculum preparation and Mass production:

Pure Biosurfactant producing bacterial strains *Bacillus subtilis*, *Bacillus cereus* and *Pseudomonas thivervalensis* were procured from Microbiology department, Karnataka University, Dharwad were sub-cultured on nutrient agar slants in triplicates and maintained at 4^oC. Each type single colony from selective medium (BHM) and pure cultures were inoculated in 5ml optimized nutrient and incubated for one hour at room temperature. After incubation inoculums were transferred to 95ml of nutrient broth separately. Incubated on Rotary shaker at 30^0C, 150rpm for 3 days. Viability of cultured cells was checked by taking optical density at 660nm.

Efficiency of crude extracts

The activity of crude extracts of isolates were checked and compared with synthetic surfactants and crude extracts of pure cultures using the efficiency assays which include oil spread technique(Bayoumi *et al*., 2001, Techaoei *et al*., 2011,)emulsification index (% E24) (Afuwale *et al*.,2012**;**Techaoei *et al*.,2011, Lobna *et al*.,2013, Sonali *et al*.,2011) and emulsification activity (Lobna *et al*.,2013).

III. Results

Total of 58 colonies were screened out of which two distinct morphological bacterial isolates were isolated by using the initial screening on BHM supplemented with kerosene as sole carbon source. Screened individual isolates from different sample were identified as *Pseudomonas* species and *Rhodococcus sp* based on morphology and biochemical characterization. Supernatant containing biosurfactant were centrifuged at 8000xg, and extracted by Chloroform: Methanol (2:1) method. The yield of crude biosurfactant by each isolates is as shown in the table no.1.)

IV. Discussion

Widespread environmental contamination has occurred due to extensive production and use of petroleum hydrocarbons as important energy resource used in our daily life and industries. (Bayoumi *et al.,* 2011; Magdalene *et al*., Due to their hydrophobic nature, they exhibit low availability to microbial cell for biodegradation; their availability can be enhanced by microbially produced biosurfactants. Biosurfactant play a key role in biodegradation by increasing the aqueous dispersion of organic compound. (Afuwale *et al.,* 2012*;*Magdalene *et al*., 2011)As per earlier studies, highest percentage of biosurfactant producing microorganism are found at hydrocarbon contaminated areas. These microbes produces surface active compound with unique biochemical properties.(Afuwale *et al.*,2012; Bayoumi *et al.,* 2011).

Biosurfactants are leading group with wide application in food, agricultural, cosmetic and petrochemical industries. Also, have certain advantages such as nontoxic, biodegradable, specificity over synthetic surfactant (Bayoumi *et al*., 2001; Thavasi *et al*,.2011; Vandana *et al*,. 2012; Erum *et al*,.2013)According to earlier studies, *Bacillus* species and *Pseudomonas* are the major producers of biosurfactant (Bayoumi *et al* 2011). In the present study *Pseudomonas sp* was found to be highest producer of biosurfactant Oil displacement test, Emulsification index and Emulsification activity tests are better predictor of efficiency assay tests for biosurfactant as they need small amount sample, does not require specialized equipment, easy, rapid and sensitive tests (Techoaei *et al*., 2011, Youssef *et al*., 2004) In oil displacement test there is direct relationship between diameter of clear zone and the concentration of biosurfactant (Mounira and Abdelhad 2015). Efficiency assay confirmed the presence of emulsifying agent (biosurfactant) which may not only solubilize the hydrocarbons but also make it available to be utilized as carbon source. (Afuwale *et al*,. 2012; Bayoumi *et al*.,2011). After optimization of nutrient broth, *Pseudomonas* species showed high yield 0.257g/100ml of biosurfactant . According earlier studies, *Pseudomonas aerugenosa* OCD-0.98mg/ml (Sonali *et al., 2011*) and 73.5mg/ml which were less compared to 7.1mg/ml (Vandana 2012).

V. Conclusion

The present study emphasizes on the production biosurfactants from the soil isolates of various oil polluted areas. It also focuses on the yield of biosurfactants by the isolates. The experimental data obtained indicates *Pseudomonas species* and *Rhodococcus sp* as a promising organisms showing highest activity as compared to the other isolates and also with the chemical surfactants. Biosurfactant efficiency assays, Oil spread technique, Emulsification index and Emulsification activity were used to measure the efficiency of crude extract biosurfactant and compared with synthetic biosuractant. Comparative studies for percentage yield and efficiency**.** Biosurfactant produced by isolates and its efficiency were compared with that of synthetic surfactant and biosurfactant produced by pure cultures.

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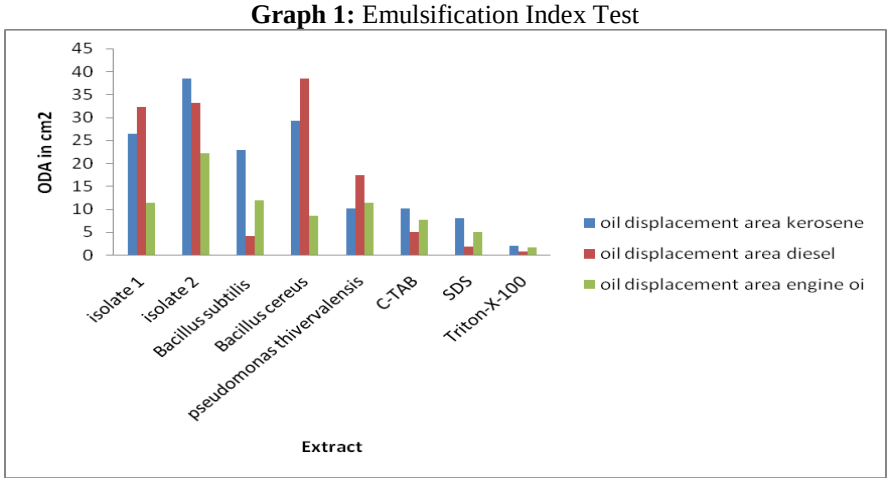
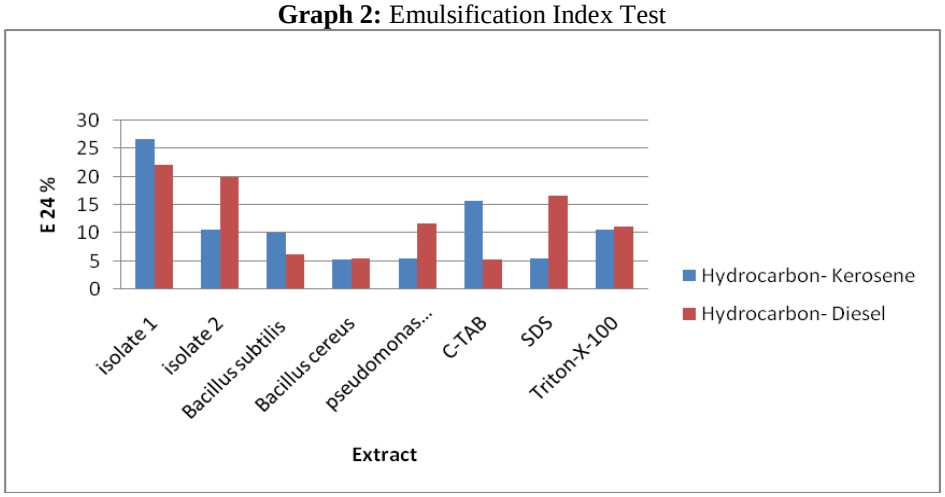
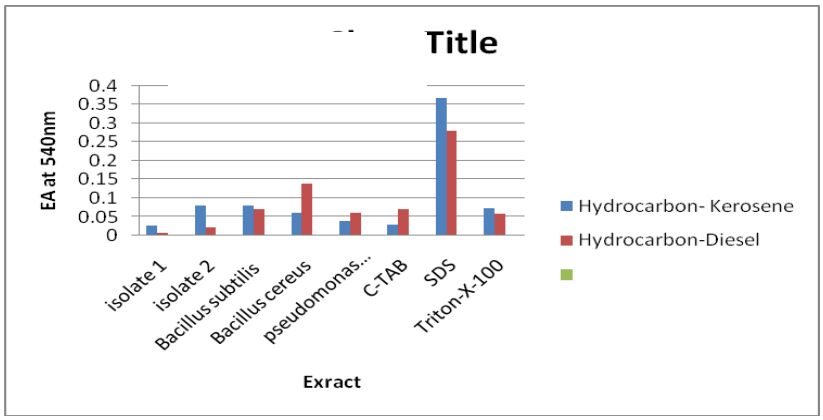
Graph 1: Emulsification Index Test



Graph 2: Emulsification Index Test



Graph 3: Emulsification Activity Test



Piechart-1: Percentage yield of Biosurfactants from Crude extracts of Isolates and Pure Culture.

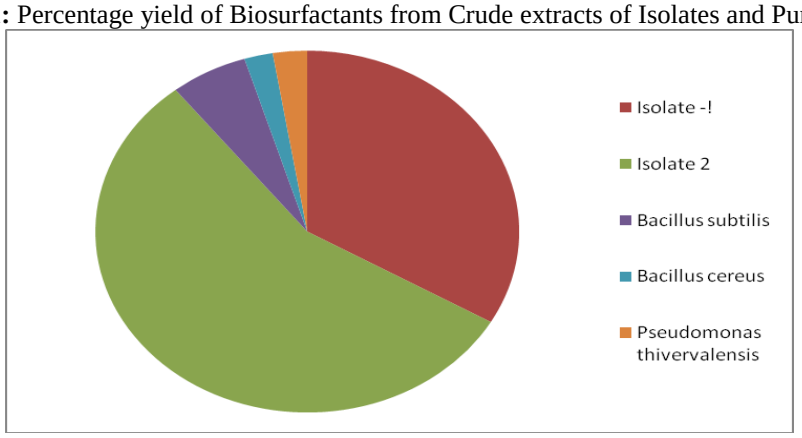


Table 1: Biochemical characterization

Biochemical tests	Isolate-3
Indole Test	--
MR test	--
VP test	--
Citrate test	++
Carbohydrate test	++
Catalase test	++
Name of Isolate	Pseudomonas sp

Table 2: Oil Spread technique-

Surfactants	Particulars	Oil displacement area(ODA) (cm^2)		
Crude extract of Isolates		Hydrocarbon source		
		Kerosene	Diesel	Engine oil
	Saccharomyces cerevisiae	7.069	30.194	2.216
	Isolate 1	26.424	32.174	11.342
	Isolate 3	38.489	33.187	22.064

Table 3: Emulsification index test

Surfactants	Particulars	Emulsification index (%) at E_{24} (After24hours)	
Crude extract of Isolates		Hydrocarbon sources	
		Kerosene	Diesel
	Isolate 1	26.666	22.222
	Isolate 3	10.526	20.000

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