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Bioremediation of heavy metals using biosurfactants

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Abstract- Industrial waste and sewage pollute more than 2/3 of India's water resources. Stream pollution is a serious and growing problem in most developing countries where there is little waste water treatment. Increasing contamination of aquatic resources with pollution including heavy metals (like chromium, lead, cadmium, zinc, nickel etc.) endangers aquatic biota and declines water quality. Bioremediation is a process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition. Biological methods for the removal of heavy metals from industrial waste may provide an attractive alternative to the physico-chemical process; biosurfactants are one of the compounds that aid in alleviating the heavy metals. Microorganisms while trying to utilize substrates like hydrocarbon as carbon source facilitate the diffusion into cell by producing a variety of substances called biosurfactants. Several microbes such as *Bacillus sp., Pseudomonas sp., Acinetiobacter sp. and Arthobacter sp.* are reported to produce biosufactants. Compared to synthetic compounds, biosurfactants offer the advantages of little or no environmental impact and the possibility of in situ production. Studies in recent past have demonstrated the successful use of biosurfactants for facilitating the degradation of organic pollutants in soil and water. In the light of above the present study is aimed to carry out the assessment of efficiency of biosurfactants (Rhamnolipid) producing microorganisms (*Pseudomonas sp*.) isolated from heavy metal contaminated site.
Keywords- Bioremediation, Biosurfactants, *Pseudomonas, Bacillus, Acinetiobacter*, Arthobacter, Rhamnolipid, Heavy metals

Introduction

Stream pollution is a serious and growing problem in most developing countries where there is little waste water treatment. Surfactants are one of the compounds that aid in alleviating the non degradable pollutants. Surfactants are compounds consisting of a hydrophilic and a hydrophobic domain. Usually hydrophobic domain is hydrocarbon and hydrophilic group can be non ionic, positively or negatively charged or amphoteric. Because of the presence of hydrophobic and hydrophilic groups, surfactants partition preferentially at the interface between fluid phase of different degrees of polarity and hydrogen bonding. These amphillic compounds have functional properties like surface and interface activity, emulsification, wetting, foaming, detergency, phase dispersing, solublization, and density reduction of heavy hydrophobic compounds and find wide application in industries. The bipolar nature of the compounds causes them to aggregate at interfaces between fluids with different polarities such as water and hydrocarbons. These biomolecules reduce the surface tension and interfacial tension between individual molecules at the surface and interface respectively. Biosurfactants typically promote the solubilization of hydrophobic chemicals buy forming molecular aggregates called micelles, which contain hydrophobic domains where the chemicals are incorporated. Microorganisms are ubiquitous in nature. They survive in varying environments which is based on the biochemical characteristics of the organisms. Some of the bacteria are found in the metal contaminated site, oil spills, and even in mines. The survival of bacteria in those places is linked with the utilization of metal ions, hydrocarbons and other toxic substances by the microbes by solublization, bioabsorption, bioaccumulation or any such mechanism for their growth and reproduction.

Materials & Methods

Collection of sample

This study aimed at isolating the bacterium and determining its ability to produce biosurfactant. The sample was collected from metal contaminated areas in and around ambur(leather city) and used for the study.

Isolation of microorganism

Microorganism was isolated by serial dilution method and spread plate technique.

Cultural & biochemical characterization

The isolated microorganisms were subjected to staining and various biochemical procedures for its characterization. Such as

- Gram's staining
- Motility test
- Indole test
- Methyl red test
- Voges-proskauer test
- Citrate utilization test
- Triple sugar ion test
- Catalase test
- Oxidase test
- Mannitol fermentation test
- Urea hydrolysis test
- Starch hydrolysis test

Result and Discussion

Screening for biosufactant production

The production of biosurfactant by the isolated bacterium can be determined by the ability of the bacterium to lyses the erythrocytes. The blood agar plates were prepared, sterilized and poured into the sterile Petri plates. Then the organism was inoculated the medium and incubated at 37^0c for 24hrs and then the haemolysis was measured.

Production of biosurfactants

The isolated organism was used for the production of biosurfactant by growing the organism in a specific medium. The choice of carbon source used for the production plays an important role on the yield and structure of microbial surfactants. There are microorganisms which produce biosurfactants only when grown on hydrocarbons and others which require simple, water soluble substrates.

Production of rhamnolipid from isolates

Pseudomonas sp. Produce surface active agents whether grown with water soluble or water insoluble substrates, although in later case the production is higher. In this study the production of biosurfactant was carried out in water insoluble medium containing 1.5% (V/V) Cooked vegetable as substrate. The medium was enriched with some addition nutrients like.

- $MgSO_4.7H_2O$
- KH_2PO_4
- $NaNO_3$
- Yeast extract
- Peptone

The cultures were grown in 500ml Erlenmeyer flasks with 100ml of medium. The trace element solution was filter sterilized and added to the medium, which have been autoclaved and allowed to cool. Then about 2ml of the culture of the medium and incubated at 30^0c for 48-72hrs.

Extraction of Rhamnolipid

During incubation the surfactants was produced and released into the medium. This was extracted by the acid precipitation method. First, the medium was centrifuged at 5000rpm for 15 min. the cell free broth containing surfactant was collected in a separate tube. The surfactant in the broth was precipitated at pH 2.0 by adding conc. HCl. The broth was again centrifuged at 5000rpm for 15 min; the surfactant was extracted with dichloromethane. Further, purification was achieved by recrystilization. The dichloromethane extract was dissolved in distilled water containing sufficient NaOH to give pH 7.0. This solution was filter with through whattman no.4 filter paper and reduced to pH 2.0 with conc. HCl. The white solid was collected as a pellet after centrifugation.

Effect of Biosurfactant on metal removal

The extracted biosurfactants was used for the removal of metals such as chromium and zinc. The nutrient broth medium containing the salts of chromium sulphate and zinc sulphate was prepared and sterilized. The salts of chromium and zinc were added to the medium at conc. of 10mg, 20mg, 30mg, 40mg, and 50ml per lts respectively. The pH of the medium was adjusted to 7.0 to 7.2 and sterilized in an autoclave at 15lbs pressure for 15min. then the extracted biosurfactant (about 50µl/ml) was inoculated in the medium and incubated at 30^0c for 24hrs. The medium with rhamnolipid was kept as treatment 1 and medium without biosurfactants and organism served as control. The treatment methods were same for both the metals used. Then the tubes were analyzed for the conc. of metals present after treatment in an atomic absorption spectrophotometer.

Conclusion

Biosurfactants are produced by diverse microorganisms and hence have varied chemical structures and surface properties. Therefore they have different natural role in the growth of the organism producing them. As their chemical structures and are so different, one group of biosurfactants would have a specific ecological niche, where as anther group would be more appropriate for a different niche. The identification and characterization of biosurfactant produced by various microorganisms have been extensively reviewed. The organism was isolated from heavy metal contaminated site and biochemically characterized by performing std. biochemical test. The biosurfactant produced by the isolates was conformed as rhamnolipid by observing dark blue halos around colonies when cultured in blue agar medium. Rhamnolipids are the group of surfactants produced by *Pseudomonas aeruginosa* then the strain was inoculated with various concentrations of chromium and zinc. The result indicates activity of surfactants in the removal of metals.

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Table I- Biochemical characterization of Pseudomonas sp. isolated from contaminated soil

S.No	Test	Observation	Result
1	Gram staining	Pink color	gram negative
2	Motility	Tumbling movement	Motile
3	Indole	No red ring on the top	Negative
4	Methyl red	Yellow color	Negative
5	Vogas-proskauer	Faint brown color	Negative
6	Citrate utilization	Persian blue	Positive
7	Triple sugar iron	Alkaline slant and butt	Positive
8	Catalase production	Production of bubbles	Positive
9	Oxidase	Purple color formation	Positive
10	Urea hydrolysis	No color change	Negative

Table II- Efficiency of biosurfactant produced by microorganisms by Pseudomonas sp. On the reduction of chromium

	Concentration of chromium(mg/L)	
S.No	Initial conc.	After treatment
1	10	5.3
2	20	10.7
3	30	16.9
4	40	20.8
5	50	27.1

Table III- Efficiency of biosurfactant production by Pseudomonas sp. On the reduction of zinc

	Concentration of zinc(mg/L)	
S.No	Initial conc.	After treatment
1	10	5.2
2	20	10.6
3	30	16.4
4	40	21.6
5	50	28

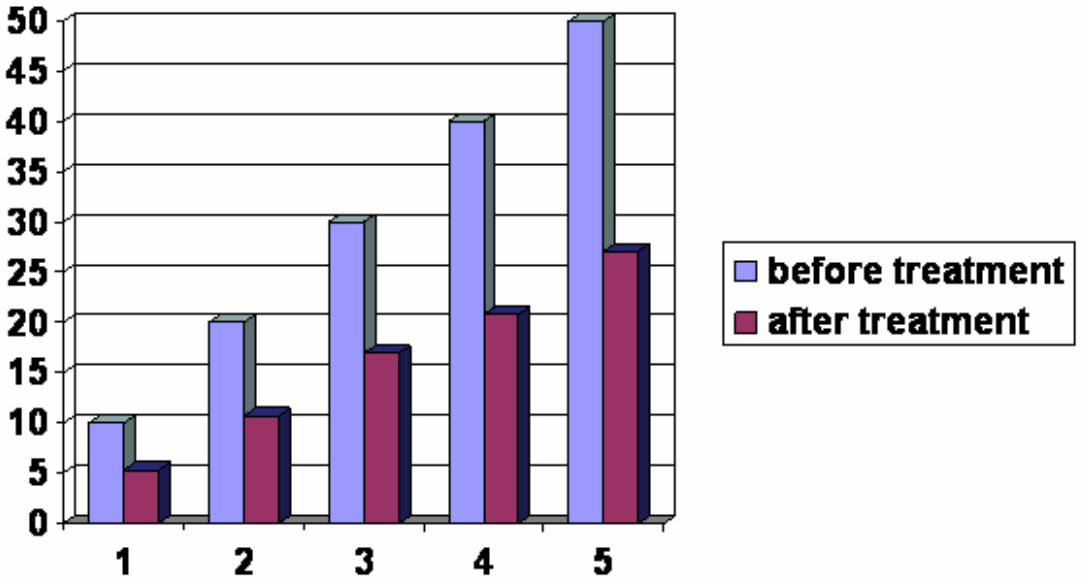

Fig. 1-Graph for chromium

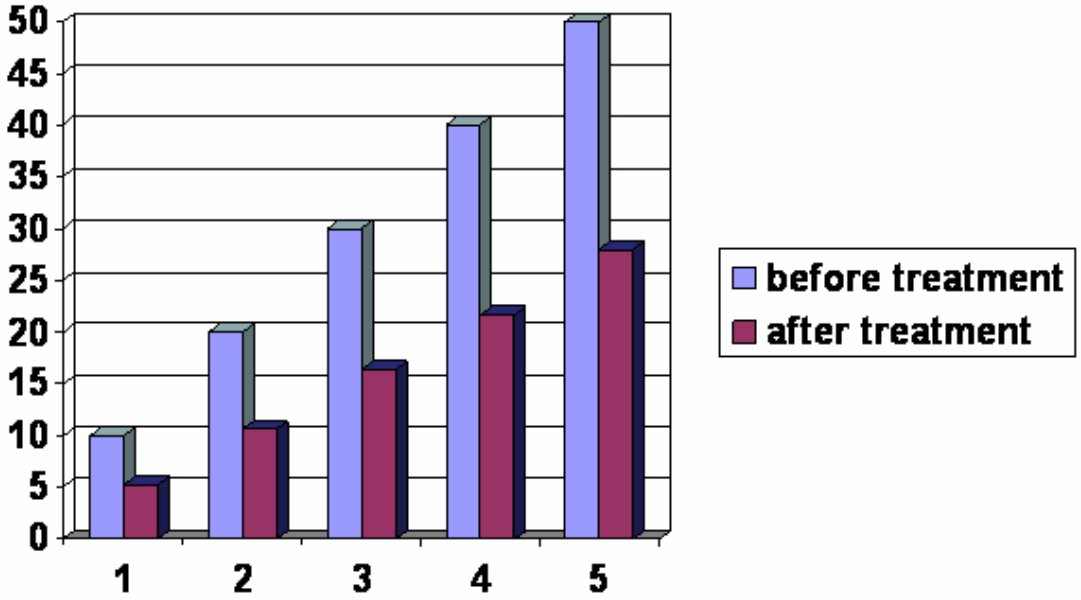

Fig. 2-Graph for zinc