BIOSURFACTANT PRODUCTION BY BACTERIAL SPECIES ISOLATED FROM PETROLEU M OIL CONTAMINATED SOIL.

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Abstract: Surfactants are chemical products widely used in our daily life in toothpaste and other personal hygiene and cosmetic products, and in several industries. Biosurfactants are surfactants of biological origin that can be produced by microorganisms and have many advantages, such as low toxicity and high biodegradability, compared to synthetic counterparts. Unfortunately, high production costs limit the use of biosurfactants. Low-cost production is the most important factor for biosurfactants to be able to compete in the global market place. Effort was made to study the biosurfactants from bacterial species isolated from petroleum contaminated sites.

Keywords: **Biosurfactants, *Pseudomonas aeruginosa*, Surfactants, Rhamnolipids.**

I. INTRODUCTION:

The main aim is increase the biosurfactants production with low-cost, for this purpose, different raw material, wastes, production methods and culture conditions are used in studies and the results are vary according to these variable factors. Despite the all studies and promising features of biosurfactants, the economics of their production is a major problem for commercialization. There is still no economically technology for purifying biosurfactants at industrial scale and also accessing cheap substrate is a barrier for low-cost production has drawn attention to important issues. Some of the important criteria that need to be considered for production on industrial scale are as follows; the need for cost effective raw material and supply raw material with same composition , potential bacterial species with enhanced production capacity, economical production technologies and purification methods.

Rhamnolipids have a big potential, especially in environmental applications for the remediation of contaminated soils due to their biodegradability and low toxicity and in medical fields due to their antimicrobial activities(Abouseoud M, Maachi R, Amrane A, Boudergua S, Nabi A. 2008) (Banat IM. 1995) (Benincasa M, Contiero J, Manresa MA, Moraes IO. 2002) (Bharali P, Saikia JP, Raya A, Konwarc BK. 2013). Rhamnolipids are an alternative to synthetic surfactants, but their industrial use is still limited because of high costs. Low-cost production and discovery of novel rhamnolipid-producing strains characterized by better yields are the most important keys for rhamnolipids to have a corner on the global market of surfactants (Abdel-Mawgoud A, Aboulwafa M, Hassouna N. 2009)(Dubey, K., Charde PN, Meshram SU, Yadav SK, Singh S, Juwarkar AA. 2012) (Dubey K, Juwarkar A, 2001). As with all biosurfactants, the composition and yield of rhamnolipid depends on the culture conditions as well as the producer strain. There are a number of studies in the literature about effects of various factors on rhamnolipid production, especially on yield. The carbon and nitrogen source, the amount of ions used in the medium and the culture conditions, such as pH, temperature, and agitation, influence the quality and quantity of rhamnolipids. Many microorganisms synthesize biosurfactants using different carbon sources. Studies indicate that the yield of a biosurfactant varies depending on the carbon source and the nutrient medium. (Abdel-Mawgoud A, Aboulwafa M, Hassouna N. 2009) (Babu PS, Vaidya AN, Bal AS, Kapur R, Juwarkar A, Khanna P. 1996) Crude oil, glucose, sucrose, and glycerol have been reported as good carbon sources for biosurfactant production (Babu PS, Vaidya AN, Bal AS, Kapur R, Juwarkar A, Khanna P. 1996) (Benincasa M, Contiero J, Manresa MA, Moraes IO. 2002) (Benincasa M, Abolos A, Oliveira I, Manresa A. 2004)

Carbon sources used in biosurfactant production can be divided into three categories, including carbohydrates, hydrocarbons, and vegetable oils. Water-soluble carbon sources, such as glycerol, glucose, mannitol, and ethanol, have been recommended for rhamnolipid production by *Pseudomonas* spp. (Banat IM. 1995) (Benincasa M, Abolos A, Oliveira I, Manresa A. 2004) (Bharali P, Saikia JP, Raya A, Konwarc BK. 2013) (Camilios-Neto D, Bugay C) (Santana-Filho AP, Joslin T, De Souza LM, Sassaki GL, et al. 2011) Nitrogen is an essential component for microbial growth and enzyme production for fermentation processes and hence an

important factor for biosurfactant production. Different nitrogen sources have been used for the production, such as peptone, urea, ammonium sulfate, ammonium nitrate, sodium nitrate, meat extract, and malt extract. (Costa SG, Nitschke M, Haddad R, Eberlin MN, Contiero J. 2006, Dubey K, Juwarkar A, 2001) observed that nitrate was the best nitrogen source for the biosurfactant production by *Pseudomonas* strain 44T1.

A wide variety of culture conditions have been tested for biosurfactant production to obtain large quantities of the product of interest. According to various studies the maximum rhamnolipid yield is obtained in the pH range from 6.0 to 6.5, and the optimum temperature ranges have been identified to be 30-37 °C.

II. MATERIAL AND METHODS:

2.1. Biosurfactant Production:

Biosurfactants are amphiphilic molecules mainly produced by bacteria. They possess both hydrophilic and hydrophobic moieties therefore they are able to display a wide range of surface activities which assist them to solubilise hydrophobic substrates. One of the major potential applications is their use as replacements for synthetic surfactants in many existing industrial and environmental applications.

2.2. Production of Biosurfactants:

Out of 188 bacterial isolates 17 bacterial isolates viz., IHD3, IHD13, IHD19, IHD21, IHD36, IHD44, IHD58, IHD80, IHD89, IHD96, IHD112, IHD148, IHD152, IHD157, IHD176, IHD178, IHD188 were selected on the basis of their efficiency to produce biosurfactant. For this the inoculum of the bacterial isolates are poured in the Minimal Salt Medium along with 2 ml engine oil with pH 7 and incubated at shaker incubator at 33˚C at 120 rpm for 7 days. The biosurfactant obtained after the bacterial strains was cultivated in MSM Medium with 2% Glucose.

2.3. Extraction of Biosurfactants:

The bacterial culture was inoculated in 50 ml of MSM broth with 1 ml of engine oil. The bacterial culture was incubated at 37˚C for 7 days with shaking condition in shaker incubator. After incubation the bacterial cells were removed and biosurfactant was extracted from the culture medium by centrifugation at 12,500 rpm at 4˚C for 30 minutes. The supernatant was taken and into it equal volume of was added chloroform: methanol in 2:1 ratio was added. This mixture was shaken well to ensure proper mixing. This mixture was further left overnight for evaporation. White colored/ brown colored sediment which was obtained as a result was the biosurfactant which was further analyzed by FTIR, Thin Layer Chromatography plate and the dry weight of the biosurfactant was determined.

2.4. Purification of Biosurfactants:

The purification technique consisted of Ammonium Sulphate Precipitation Method. Floating material that was obtained after the treatment of 40% of Ammonium Sulphate was collected by the centrifugation. The biosurfactant formed was carefully taken out with the help of micropipette and kept in eppendorf tubes. 1ml distilled was added to the eppendorf containing biosurfactant. These were centrifuged at 7000rpm, 4°C for 30 minutes. It was further dissolved in small amount of distilled water. Cold solution of acetone was added to it so as to remove the proteins. It was washed with distilled water and dried and then weighed.. The supernatant was discarded and the pellet was allowed to dry at 105˚C for 24 hours. The dry pellet thus obtained was the crude extract of biosurfactant.

2.5. Dry Weight of Biosurfactants

Sterile petri plate was taken and the weight of the plate was measured. Now the sediment was poured on the plates. They were placed on the hot air oven for drying at 1000 ºC for 30 minutes. After drying the plates were weighted. The dry weight of the biosurfactants was calculated by the following formula:
Dry weight of biosurfactants = Weight of the plate after drying - Weight of the empty plate)

2.6. Preliminary Characterization of Biosurfactants using Thin Layer Chromatography

Preliminary characterization of the biosurfactant was done by Thin Layer Chromatography method. Silica gel plates were prepared by weighing 30gm of silica gel and add 60-65 ml of chloroform or distilled water to form slurry. Transfer this to the applicator for spreading on clean TLC glass plates. A spot of crude biosurfactant was placed on the silica gel plate. The biosurfactant was separated on the plate Chloroform: Methanol: Water (70:10:0.5v/v/v) which is the developing solvent system. Rf value were calculated by measuring the distance travelled by the solvents vs. the distance covered by the spot.

III. RESULTS AND DISCUSSIONS

The success of biosurfactant production depends on producer microorganisms, development process, and raw materials. Production of biosurfactant was analysed by measuring the emulsification index and the growth kinetics was studied by measuring optical density of the fermentation medium. From the current study it is observed that bacterial isolate IHD 19 and IHD 152 showed 89% and 88% of emulsification activity. The dry weights of the biosurfactants were measured and estimated (Table: 1). All Bacterial strains produced spots on TLC plates, indicating that strains could produce biosurfactants.

Table 3.1: Production of biosurfactants

Temperature: 37 ºC. (Shaking Conditions) Inoculum = 1 vol%, engine oil = 2 vol%, Incubation time = 7days, shaking speed = 125 r.p.m, pH = 7.00																
Bacterial isolates	IHD 3	IHD 13	IHD 19	IHD 21	IHD 36	IHD 44	IHD 58	IHD 80	IHD 88	IHD 96	IHD 112	IHD 152	IHD 157	IHD 176	IHD 178	IHD 188
EI %	50	86	89	48	80	74	48	74	42	68	58	68	88	80	56	80
O.D 600 nm (growth kinetics)	0.62	2	2.2	0.58	1.8	1.6	0.578	1.6	0.43	1.24	0.89	2.14	1.22	1.8	0.78	1.8

Table no 3.2: Dry weight of biosurfactants

Sr.no.	Bacterial Isolates	Weight of Plate (g)	After drying of biosurfactants in the plate (g)	Dry weight of biosurfactants (g)
1	IHD3	47.31	50.438	3.128
2	IHD13	47.31	49.45	2.14
3	IHD19	47.3	52.02	4.72
4	IHD21	47.3	49.88	2.57
5	IHD36	47.3	50.7	3.4
6	IHD44	47.3	49.22	1.92
7	IHD58	46.9	50.06	3.16
8	IHD80	47	48.43	1.43
9	IHD88	47.1	49.2	2.1
10	IHD96	47	51.05	4.05
11	IHD112	47.8	49.76	1.96
12	IHD152	47.3	52.56	5.26
13	IHD157	45.8	50.2	4.4
14	IHD176	48.01	50	1.99
15	IHD178	48.01	50.22	2.21
16	IHD188	48	49.45	1.45

Table 3.3: Preliminary Characterization of Biosurfactants using Thin Layer Chromatography

Bacterial isolates (IHD)	**Distance travelled by solute (cm)**	**Distance traveled by solvent (cm)**	**Rf value**
IHD3	1.9	3.2	0.593
IHD13	0.8	2.0	0.4
IHD19	0.5	2.8	0.1785
IHD21	0.6	2.6	0.230
IHD36	0.6	2.4	0.25
IHD44	0.7	1.6	0.4375
IHD58	0.8	1.6	0.5
IHD80	0.9	1.9	0.4736
IHD88	0.7	2.3	0.3043
IHD96	0.6	3.5	0.1714
IHD112	0.8	2.6	0.307
IHD152	0.6	1.3	0.4615
IHD157	1.0	1.8	0.5555

IHD176	1.1	2.7	0.4074
IHD178	0.7	1.9	0.3684
IHD188	1.2	3.5	0.3428

IV. Conclusion:

The interest for biosurfactant production is increasing day by day as it can be used for bioremediation. Hydrocarbons contain high organic matter; it can be assimilated by the bacteria as a carbon source. From the present study it can be concluded that Bacterial isolate IHD 19 and IHD 152 are good producer of biosurfactant. . Further research on human cells to validate the applications of biosurfactants in various health related areas in the medical science arena waiting to be fully exploited.

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