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BIOSURFACTANT PRODUCING BACTERIA FROM ARABIAN SEA MUMBAI

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ABSTRACT

An extensive investigation was conducted to isolate indigenous bacterial strains with outstanding performance for biosurfactant production from soil and water samples of Arabian Sea, Mumbai. Biosurfactants or surface-active compounds are produced by microorganisms reduce surface tension of both aqueous solution and hydrocarbon mixtures. Increase in sea pollution by various harmful chemicals and oils causes a drastic change in environment. Marine biosurfactants produced by some marine microorganisms have been paid more attention, particularly for the bioremediation of the sea polluted by harmful chemicals and oils. Hemolytic activity, emulsification activity towards n-hexadecane, drop-collapsing test, emulsion of oils as well as oil displacement test were used to determine biosurfactants producing activity of marine bacteria. Isolates were identified primarily as strains of Bacillus sp and *Pseudomonas* sp.

KEYWORDS: A Bioremediation, biosurfactant and emulsification.

INTRODUCTION

Pollution of the sea by various harmful oils and chemicals, mostly caused by various ship operations and discard of various harmful wastes directly into the sea from various sources, which is the one of the urgent and serious environmental issues over the world. Ship operations produce wastes that are collected in the lowest part of the hull, called the bilge area. The oil-containing bilge waste must be managed properly to avoid environmental pollution [1]. The microbial cell itself is a biosurfactant and adheres to hydrocarbon. Those biosurfactants are capable of increasing the bioavailability of poorly soluble polycyclic aromatic hydrocarbons such as phenanthrene [1-2] and resins [3]. Clean up these spillages by synthetic detergents led to more destruction of environment. Therefore, the use of biosurfactant should be a promising means to emulsify the polluted oils prior to biodegradation [4-5] screened biosurfactant-producing microorganisms using hemolysis of RBCs. In this study, isolation and identification of biosurfactant producers has been carried out from marine soil and water sample, which were connected and unconnected to any hydrocarbon contamination.

MATERIAL AND METHODS

Sources of environmental samples

Samples for this study were collected from Arabian sea and marine drive from Mumbai. These sites were contaminated by various harmful chemicals and oils. Samples were taken from the upper surface of water and soil and stored at 4c until use.

Media used

Blood agar plates, Nutrient agar plates were used for screening and isolation of potential biosurfactant producing bacteria [6-7].

Isolation of biosurfactant producing bacteria

Isolation of biosurfactant –producing bacteria were carried out from soil and water samples from Arabian Sea, Mumbai. The samples were spread on nutrient agar dissolved in distilled water and incubated at Room temperature and for soil samples 1gm of soil sample by serial dilution method. Isolated colonies were inoculated into 100ml of Marine Broth 2216 and Nutrient Broth containing 2-3 drops of kerosene with petrol oil [8] and incubated with continuous shaking (200 rpm) for 24-48 hr at room temperature using a shaker.

Identification of bacteria

On the basis of biochemical tests such as gram staining, carbohydrate fermentation test, H_2S production test, indole production test, methyl red test, voges-proskauer test, citrate utilization test, urease test, catalase test, oxidase test, litmus milk reaction, starch hydrolysis test ,gelatin hydrolysis test, lipid hydrolysis test etc.

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Extraction of Biosurfactant

Each culture was centrifuged at 8000rpm, 4°C for10 min. The culture supernatant was taken and the pH was lowered to 2 with 5M HCL and incubated at 4°C for 24 hrs. The precipitate was separated by centrifugation at 8000rpm for 20 min. This white precipitate formed culture was selected [9].

Biosurfactant Activity tests

By Haemolytic activity [4], Drop collapsing test [10], Emulsification measurement [11], Oil displacement test [12].

Measurement of surface tension

Preculture of bacteria strains were again prepared in Nutrient Broth in OD600=1.1ml of Inocula were added to 100ml MSS and 1% filtered oil as hydrocarbon source. The mixtures with control samples were incubated at 30°C on shaker at 150 rpm for 3 days. The surface tension was measured using a F6 tensiometer [13].

Investigation of salt different concentrations effect on surface tension

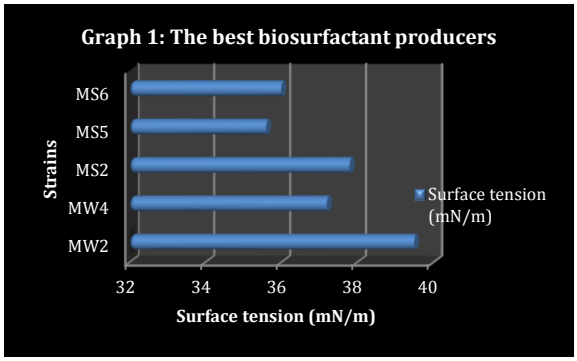
The effect of salinity on surface tension was determined by adding different concentrations (1-13%) of NaCl to the minimal cooper's medium. The mixtures were incubated at 30°C on shaker at 150 rpm for 3 days [14].

RESULTS

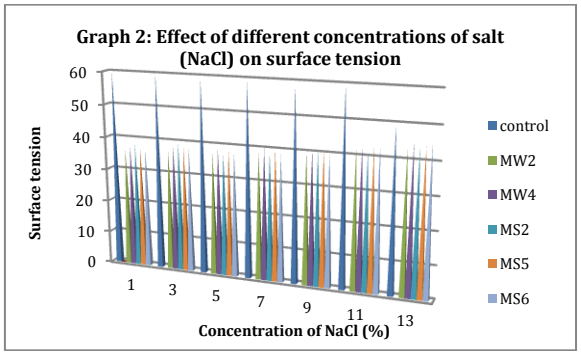
In the present work, it was found that microbially produced surface active compounds can control over the soil and water pollution which was increased by various human activities. Chemical methods are not so successful due to various harmful side effects. Thus, biological treatments are necessary because they are biodegradable. The degradation of various harmful chemicals by various microorganisms was observed by using various methods i.e. hemolytic activity, emulsification test, drop collapsing test, oil displacement test etc. Biosurfactant activity of various isolated microorganisms was also reported. Study revealed that five strains out of twenty isolated strains which were showing the higher biosurfactant activity in which MW2 (*Pseudomonas* sp.) strain showed the highest (70.5+ 0.55) (Fig. 1) emulsification activity, (3.14+ 0.02) (Fig. 2) oil displacement test.

Figure 1. P*seudomonas* Sp (MW2)

Figure 2. Various microbial strains grown in presence of oil



Graph 1. The best biosurfactant producers



Graph 2. Effect of different concentration of salt (NaCl) on surface tension

According to this study MW2 was found to be more effective in emulsifying the mixtures (kerosene + petrol + diesel in equal amounts) of oils. Emulsification activity and drop collapsing tests results are summarized in table 1.

Table 1. Oil Displacement Activity, Emulsification Activity & Drop Collapsing Test of cultural supernatant from different strains

Strains	Emulsification activity (%)	Oil displacement test (cm2)	Drop collapsing test
MW2	70.5±0.55a	3.14±0.02a	+
MW4	65.0±0.50b	2.55±0.03b	+
MS2	60.0±0.30d	2.30±0.03d	+
MS5	65.0±0.36b	2.20±0.03e	+
MS6	50.0±0.30d	1.14±0.02f	+

• MW (Marine Water sample from Mumbai),
• MS (Marine Soil sample from Mumbai).
• Different letters in the same column indicates significant differences ($p<0.05$).

Effect of different concentrations of salt

Surface tension reduced in 1-13% concentration, but suitable concentrations for reducing surface tension were 1, 3, and 5% as shown in Table 2 and 3.

DISCUSSION

In the present research, biosurfactant are known to be produced by hydrocarbonoclistic method by microorganisms during own growth on hydrocarbons and carbohydrates.

Table 2. The best biosurfactant producers

Strains	Surface tension (mN/m)
MW2	39.5
MW4	37.2
MS2	37.8
MS5	35.6
MS6	36

Table 3. Effect of different concentrations of salt on surface tension

NaCl (%)	1	3	5	7	9	11	13
Control	60	60	60	59.6	59	59.2	49.8
MW2	36	36.5	37.2	38.7	40.0	42	43.8
MW4	37.2	38.2	38.6	40.0	40.6	42.6	45.0
MS2	38	39	37.5	38.8	40.8	42.6	44.0
MS5	37.2	38	38.5	40.2	41	42.8	44.5
MS6	37	37.8	38.2	38.8	40.1	43.2	45.5

In this study microorganism capable of producing biosurfactant were successfully isolated and characterized. 20 microorganisms isolated from various samples, out of these 15 strains have shown biosurfactant activity, in which 5 strains were greatly efficient and could completely emulsify the oil. Among all the 15 strains MW2, MW4, MW5, MS2, MS6 strains exhibited the clear halos on blood agar plates and emulsified oil in Marine broth 2216 and Nutrient Broth during cultivation. Only strains exhibiting hemolytic activity test and emulsifying activity showed positive result with drop collapsing test, emulsification activity and oil displacement test. From the result highest activity for both oil displacement test towards oil (3.14cm2) and emulsification activity against mixture of oils (Petrol+ kerosene+ diesel) (70.5%). Identification of biosurfactant producing bacteria can be further confirmed by measurement of surface tension. Reduction of surface tension measurements by isolated bacteria from Arabian Sea, Mumbai indicates the production of surface- active compounds. Similar results obtained by Banat *et al* (1991) [15]. Salt concentration also affected biosurfactant production depending on its effect of cellular activity which is very near to the results obtained by Yakimov *et al* (1995). He isolated *Bacillus licheniformis* BAS50 which grew and produced a lipopeptide surfactant when cultured on a variety of substrate at salinities of 13% NaCl. Depicted results showed that the biosurfactant production was optimal at 5%NaCl. According to surface tension reducing and emulsification characteristics of strain 2 (MW2) and its stability over a wide range of high salt concentrations suggest that strain 2 (MW2) is suitable for use as MEOR and removal of oil pollutions from different oil polluted areas [16].

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