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Identification of biosurfactant producing bacterial isolates from garage soil and marine water

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Abstract - Biosurfactants that are nontoxic, easily biodegradable and eco-friendly with high stability have wide variety of industrial application. This makes them highly useful group of chemical compound. A variety of organisms are known to produce biosurfactants. Biosurfactant producing bacteria from different environments need to be screened for their ability to produce potentially useful novel compounds. In this study, soil samples contaminated with engine oil & diesel oil and marine water were used to isolate organisms by enrichment technique. Isolates were further screened for their ability to produce biosurfactant using hemolysis assay. Total 13 bacterial isolates were obtained. Out of these 13 isolates, 3 isolates were from the soil contaminated with engine oil, 4 isolates were from soil contaminated with diesel and six isolates were from marine water. Identification of isolates was done using cultural, biochemical tests and by MALDI-TOF. Isolates were identified as *Bacillus subtilis, Pseudomonas aeruginosa, Achromobacter xylosoxidans, Micrococcus luteus, Staphylococcus sciuri, Bacillus Cereus* and *Serratia marcescens*. Out of 13 isolates 3 were identified as *Pseudomonas aeruginosa*, 2 were identified as *Bacillus cereus*, 3 were identified as *Serratia marcescens*. One isolate (IS6) from diesel contaminated soil is yet to be identified. Biosurfactant production by these isolates was evaluated by oil spreading test and emulsification index test. Oil spreading technique and emulsification index was observed in all the 13 isolates. IS6 showed maximum oil displacement compared to others and highest emulsification activity with paraffin oil as well as engine oil. These isolates are important source to investigate further for future industrial applications.

Key Words: Biosurfactant, Oil contamination, Screening, Isolation, Hemolytic activity

1. INTRODUCTION

Biosurfactant producing bacteria from different environments need to be screened for their ability to produce potentially useful novel compounds [1]. Oil contaminated regions are reported to be a suitable place for the isolation of biosurfactant producing bacterial strains [2].

Biosurfactants are surface active agents produced by certain specialized microorganisms including bacteria, fungi and yeast [3]. These microbes produce biosurfactant, either secreted extracellularly or attached to parts of the cell membrane, predominantly during growth phase [4]. Besides being non-toxic and biodegradable, biosurfactants are amphiphilic molecules with high specificity [5, 6]. They are highly stable at extremities of temperature, pH and salt concentrations [7]. These molecules have the ability to decrease the surface and interfacial tension [8]. In addition, biosurfactants are promising natural surfactants that offer several advantages over chemically synthesized surfactants, such as lower toxicity, biodegradability and ecological acceptability [9, 10]. Biosurfactants are thus used as an alternative for chemical surfactants [11]. Biosurfactants belong to various classes including glycolipids, lipopeptides, fatty acids, phospholipids, neutral lipids and lipopoly- saccharides [12].

The range of industrial applications of biosurfactants includes excellent detergency, emulsification, foaming, wetting, penetrating, thickening, metal sequestering and resource recovering [13]. Most crucial property of biosurfactants, which has captivated the researchers of today, is its use in bioremediation of pollutants, health care and food processing [14].

Since the last decade, increasing attention has been paid to the isolation of biosurfactant producing organisms. However, the high production costs and low strain productivities, limit its wide application [15]. With this scope, the present study proposes an effective methodology to search for biosurfactant producing bacteria from different environments.

2. MATERIALS AND METHODS

Sampling Area

For the isolation of biosurfactant producing bacteria, the soil sample contaminated with engine oil & diesel oil and marine water were collected from different places.

Enrichment and Isolation of Bacterial Isolates

Samples were inoculated in 50 ml Nutrient Broth with Minimal salt medium (MSM) and 2 % oil as described earlier [16]. Inoculated media were incubated for 4 -5 days at 37^0C on rotary shaker. Enrichment was repeated 3 times in the same medium. After incubation, enriched broth cultures were serially diluted upto 10^{-8} and last three dilutions were used. 0.1ml culture was spread on blood agar plates to isolate biosurfactant producers. These plates were incubated at 37^0Cfor 2 days. After incubation heamolysis on plates was observed. Morphologically distinct well grown heamolytic colonies were selected & transferred on NA agar plates. Purity of the isolates was confirmed by repeated sub-culturing on fresh agar plates of the isolation media, followed by microscopic examinations [17]. The selected bacterial isolates were stored on NA agar slants and kept under refrigerated conditions for further screening.

Bacterial Identification

Identification of isolates was done using cultural, biochemical tests and by MALDI-TOF (18).

Screening for Biosurfactant Production

The isolated strains were tested for biosurfactant production using following methods.

Oil Spreading Technique

The isolated isolates were tested for oil spreading technique. 10 µl supernatant was added to the surface of oil as described by Nasar, *et al*., [19]. Event of clear zone was a sign of biosurfactant production. 10 µl of culture media without any growth was taken as a control.

Emulsification Index

2 ml of culture supernatant was added to 2 ml of oil for determining Emulsification index of bacterial isolates. The mixture was vortexed for 2 min and incubated for 24 hours for emulsification activity E_{24} (%) was calculated using following equation:

$$E_{24} = \frac{h_{emulsion}}{h_{total}} \times 100\%$$

Where, h= Height

3. RESULTS

Isolation of Bacterial isolates

Total 13 bacterial isolates that showed hemolytic activity (Fig. 1) were obtained for further screening. Out of these 13 isolates, 3 isolates were from the soil contaminated with engine oil, 4 isolates were from soil contaminated with diesel and six isolates were from marine water. All 13 bacterial strains were purified and screened for the biosurfactant production. The isolates were coded as IS-1 to IS-13.

Bacterial Identification

Identification of isolates was done using cultural, biochemical tests and by MALDI-TOF (Table 1). Isolates were identified as *Bacillus subtilis, Pseudomonas aeruginosa, Achromobacter xylosoxidans, Micrococcus luteus, Staphylococcus sciuri, Bacillus Cereus* and *Serratia marcescens*. Out of 13 isolates 3 were identified as *Pseudomonas aeruginosa*, 2 were identified as *Bacillus cereus*, 3 were identified as *Serratia marcescens.* One isolate (IS6) from diesel contaminated soil is yet to be identified. We are performing 16S rRNA PCR for the identification of the isolate using sequencing of the amplicon obtained (Data not shown).

Screening for Biosurfactant Production

Following results are obtained after screening tests.

Oil Spreading Technique

Overnight cultures of these isolates were centrifuged and added to oil containing plates. All 13 isolates showed the clear zone by being able to displace the oil around the colony indicating biosurfactant production. Out of the 13 isolates IS6 showed maximum oil displacement (Fig. 2). No clear zone was watched with control.

Emulsification index

All 13 isolates showed emulsification (Data not shown). Out of the 13 Isolates IS-6 showed maximum emulsification index viz. 79.37% with paraffin oil and 71.43% with engine oil when compared to others (Fig.3).

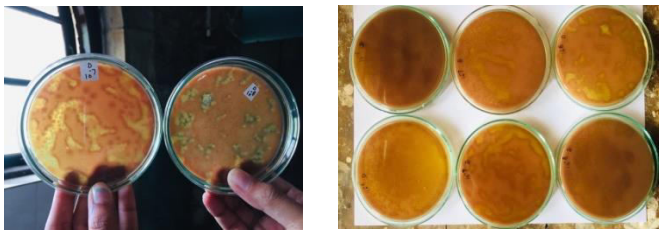

Fig.1 Results of Hemolytic activity

Table 1

Isolates	Source	Identification
IS - 1	Soil contaminated with engine oil	*Bacillus subtilis*
IS - 2	Soil contaminated with engine oil	*Pseudomonas aeruginosa*
IS - 3	Soil contaminated with engine oil	*Achromobacter xylosoxidans*
IS - 4	Soil contaminated with diesel	*Pseudomonas aeruginosa*
IS - 5	Soil contaminated with diesel	*Micrococcus luteus*
IS - 6	Soil contaminated with diesel	Not yet identified
IS - 7	Soil contaminated with diesel	*Pseudomonas aeruginosa*
IS - 8	Marine water	*Staphylococcus sciuri*
IS - 9	Marine water	*Bacillus Cereus*
IS - 10	Marine water	*Serratia marcescens*
IS - 11	Marine water	*Serratia marcescens*
IS - 12	Marine water	*Bacillus Cereus*
IS - 13	Marine water	*Serratia marcescens*

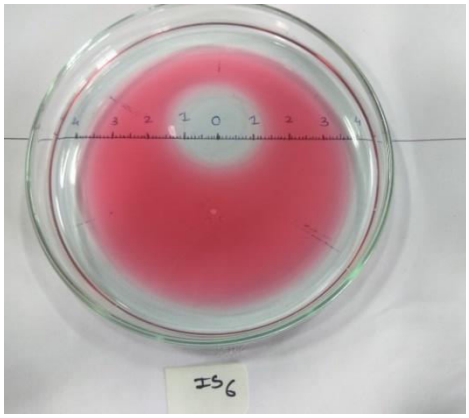

Fig.2 Result of oil displacement assay

Fig.3 Emulsification Index

4. CONCLUSIONS

In recent years plenty of research is being carried out on the potential use of biosurfactants in the industries. The focus of the present study is to isolate and characterize indigenous biosurfactant producing bacterial isolates so as to obtain suitable biosurfactant producers. Garages are the largest small quantity generators of hazardous waste.

Biosurfactant producing microorganisms are naturally present in the oil and hydrocarbon contaminated soils [20]. We were able to isolate bacteria with the ability to produce biosurfactants from the different environments. Biosurfactant production was confirmed by the conventional screening methods including oil spreading technique and emulsification index.

Isolates were tested for haemolytic activity which was used as a preliminary method for screening of biosurfactant producers [21, 22].

Oil spreading technique was used as reported by Urum and Pekdemir [23] and all 13 isolates were found to displace the oil successfully indicating production of biosurfactant. IS6 was more efficient in oil displacement compared to others. Displacement of oil is a sign of extra-cellular surfactants present in the supernatant of cultures.

Emulsification index was used as reported earlier [23]. All 13 isolates could successfully showed emulsification index. IS6 showed highest emulsification index for paraffin oil and engine oil.

As a result of screening tests it can be noticed that all the isolates are showed potential to be utilized for molecular and chemical investigations.

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