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Isolation of Biosurfactant Producing Spore-Forming Bacteria from Oman: Potential Applications in Bioremediation

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***Abstract*—**Environmental pollution is a global problem and best possible solution is identifying and utilizing native microorganisms. One possible application of microbial product -biosurfactant is in bioremediation of hydrocarbon contaminated sites. We have screened forty two different petroleum contaminated sites from Oman, for biosurfactant producing spore-forming bacterial isolates. Initial screening showed that out of 42 soil samples, three showed reduction in surface tension (ST) and interfacial tension (IFT) within 24h of incubation at 40°C. Out of those 3 soil samples, one was further selected for isolation of bacteria and 14 different bacteria were isolated in pure form. Of those 14 spore-forming, rod shaped bacteria, two showed highest reduction in ST and IFT in the range of 70mN/m to <35mN/m and 26.69mN/m to <9mN/m, respectively within 24h. These bacterial biosurfactants may be utilized for bioremediation of oil-spills.

***Keywords*—**Bioremediation, biosurfactant, hydrocarbon pollution, spore-forming bacteria.

I. Introduction

WORLDWIDE usage of crude oil-products, oil spills, waste chemical compounds and increased industrialization lead to a worst environmental problem – pollution. Currently various methods are used to tackle the problem of pollution, like microbial or phytoremediation. Biosurfactants amongst different microbial products play a vital role in bioremediation. Different types of biosurfactants are reported to enhance oil recovery, biodegradation of oil/hydrocarbon products, heavy metals etc. [1]-[3]. Biosurfactants are biochemical compound produced by microorganisms, which has two ends - a polar end (hydrophilic) and non-polar (hydrophobic) end. Biosurfactant has the ability to cause emulsification of oil-in-water/water-in-oil mixtures. The polar end (hydrophilic) mixes with water and the non-polar (hydrophobic) end mixes with oil, so it holds oil and water together and causes a decrease in the surface tension (ST)/Interfacial tension (IFT) between oil and water. Amongst different types of biosurfactant low molecular biosurfactants produced by *Bacilli* group are reported to be highly potent and play an important role in petroleum field, medicinal field and environmental field, amongst others [4].

Crude oil spills is a global problem and the magnitude of oil spill internationally is overwhelming and it is very frequent in the Arabian Gulf and the Gulf of Oman. More than 40% of world's oil is produced in the Gulf and transported internationally through Musandam and the Gulf of Oman. One of the major sources of oil pollution in Oman arose from operational discharges of oil from passing vessels traffic. Methods for cleaning up include bioremediation using oil degrading microorganisms, oil dispersants (chemical or biological surfactants). These bacteria occur naturally and can produce biological compounds like emulsifiers or biosurfactants. Joshi et al. [5] reported occurrence of biosurfactant producing *Bacillus* spp., from diverse habitats, and majority of isolated bacteria were clusterd with the *Bacillus licheniformis* and *B. subtilis* group, based on amplified 16S rDNA restriction analysis. Several spore-forming bacteria were isolated from soil samples contaminated with 'petroleum/crude oil', from local garages/oil reservoir at different locations in Oman. The spore-forming bacteria were selectively isolated by boiling the soil samples. The samples and isolates were screened for biosurfactant production and reduction in ST/IFT.

II. Materials and Methods

Several oil contaminated soil samples (42) were collected from garages, auto repair centers and oil wells in Oman. The soil samples (100g each) were collected in sterile plastic sample collectors and stored at 15°C in a portable incubator-freezer, while transferring to laboratory. To isolate 'spore-forming bacteria', 5g of each soil samples were added in 30ml of sterile distilled water, mixed by vortexing vigorously and boiled at 85°C for 30min, in a boiling water bath.

Two media were used as an inoculum and production media - Luria Bertaini (LB) broth and Cooper's minimal media [6], [7], respectively. For primary screening of soil samples containing biosurfactant producing bacteria, 2ml from each of the pre-boiled samples were added in 50ml sterile LB broth. After overnight incubation at 40°C, 160rpm, it was transferred (2% v/v) to Cooper's minimal medium (50ml in 250ml Erlenmeyer flask). The composition of Cooper's minimal media (g/l): NH_4NO_3, 4.002; KH_2PO_4, 4.083; $NaHPO_4$, 7.119; $MgSO_4$, 0.197; Glucose, 20.00; Trace mineral solution,

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1mL/L. The flasks were incubated at 40°C, 160rpm and samples were collected at 24 and 48h, and analyzed for growth (OD_{660}), change in pH and biosurfactant production (surface tension and interfacial tension). For secondary screening the bacteria were isolated from selected soil samples, and further studied individually for biosurfactant production.

To analyze surface tension (ST) and interfacial tension (IFT), Drop shape analyzer (Pendant Drop Tensiometer, Kruss, Germany) was used [8]. IFT was measured between biosurfactant solution and n-Hexadecane at 26°C.

III. Results and Discussion

It is expected that availability of oil-degrading or bacteria helpful for bioremediation will be comparatively higher from samples pre-exposed to hydrocarbon/crude oil. So, 42 soil samples were collected from different garages, car service stations and oil reservoirs of Oman (Fig. 1). The frequency of sample collection was higher for Al-Mabaila, because of its proximity to our research laboratory. The soil samples were boiled to destroy all non-spore forming bacteria, and only spore-forming bacteria were screened for biosurfactant production. Biosurfactants are reported to play an important role in microbial enhanced oil recovery, bioremediation of hydrocarbon or heavy metal contaminated sites [9]. Surfactins and lichenysins are well-studied biosurfactants produced by spore-forming *Bacilli*, and are most potent biosurfactant reported so far [5], [10], [11].

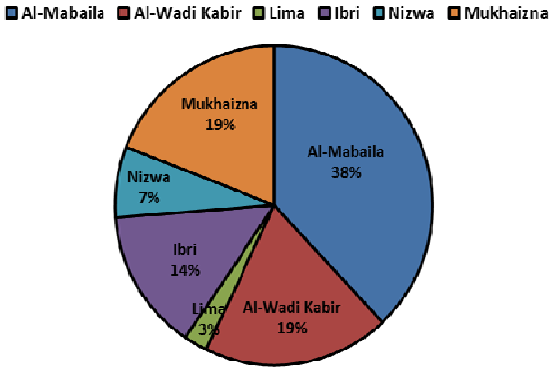

Fig. 1 Frequency distribution of soil sample collection from different locations in Oman

In this study, the contaminated soil samples to eliminate non-spore forming bacteria and screened all the samples for biosurfactant production activity in glucose based minimal medium. Out of 42 samples analyzed, sample number – 11, 12 and 35 showed reduction in ST from 70mN/m to <35mN/m (Fig. 2), and sample number 11 and 35 also showed reduction in IFT from 26.69mN/m to <9mN/m (Fig. 3), which are considered to be a selection criterion for biosurfactant production [4]. Maximum growth was observed in sample number – 4, 6, 8, 15, 21 and 30 (Fig. 4).

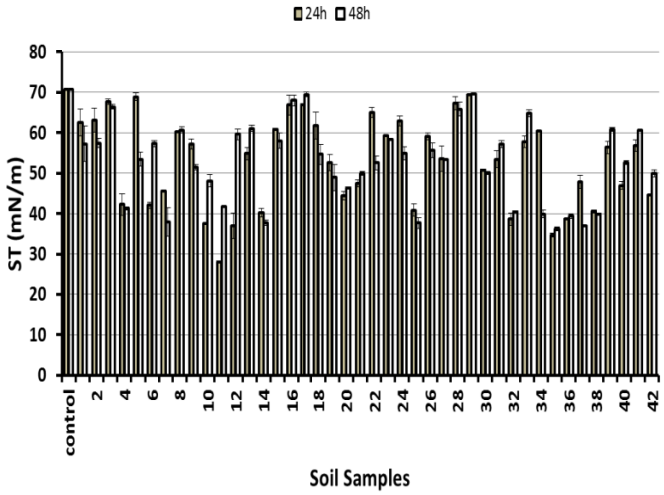

Fig. 2 Biosurfactant production (ST) from soil samples collected from different locations in Oman

Based on the results from primary screening of soil samples, sample number 11 was selected for isolation of single colonies of bacteria and further screening of biosurfactant producing bacteria. Several Gram positive, rod shaped bacteria were isolated on LB agar plates, and were screened for biosurfactant production in Cooper's minimal medium. Samples were analyzed at 24h for growth (OD_{660}), and biosurfactant production (ST and IFT). Fourteen different bacteria were isolated and studied, of which isolates 11/2 and 11/3 showed highest reduction in ST and IFT - <35mN/m and <9mN/m respectively (Fig. 5). Other researchers have also reported reduction in ST and IFT in this range within 24h [5], [7]. These isolated spore-forming bacteria needs to be further characterized and their biosurfactant needs to be studied further for bioremediation applications.

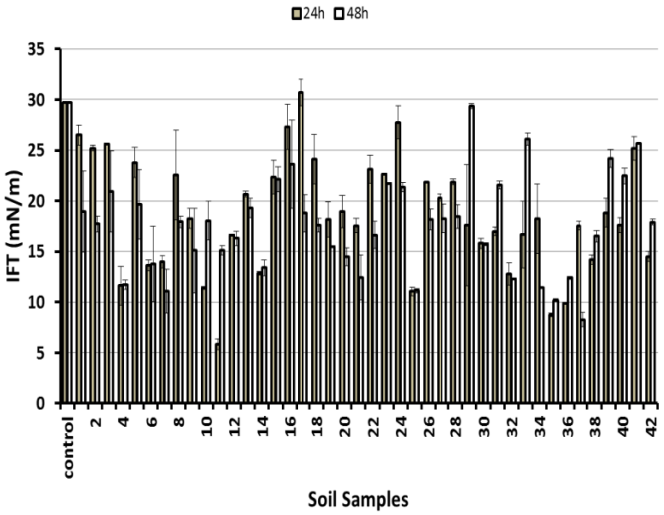

Fig. 3 Biosurfactant production (ST) from soil samples collected from different locations in Oman

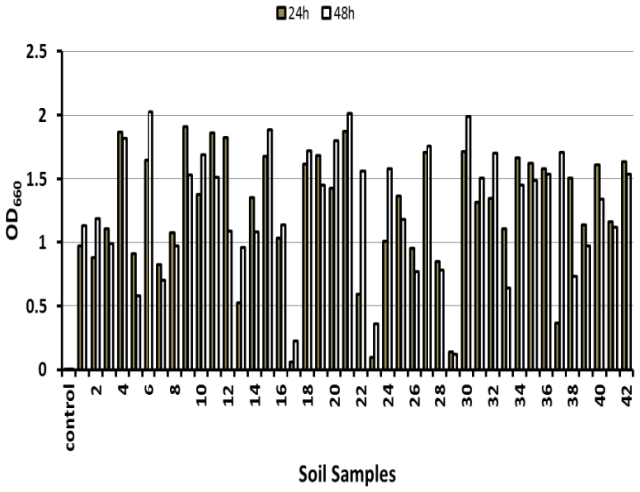

Fig. 4 Biosurfactant production (ST) from soil samples collected from different locations in Oman

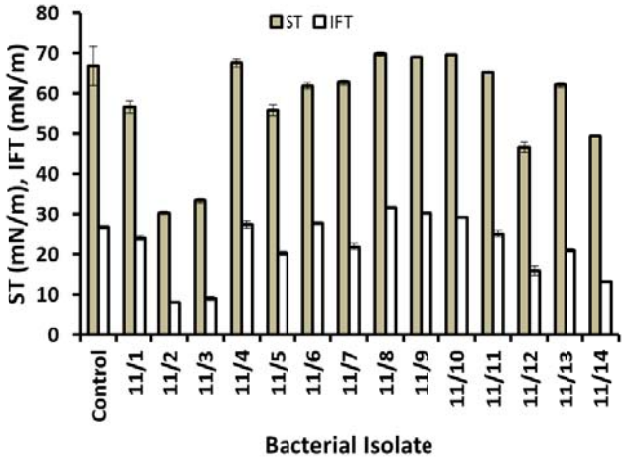

Fig. 5 Biosurfactant production (ST) from soil samples collected from different locations in Oman

IV. Conclusion

We have reported screening of oil-contaminated soil samples for isolation of spore-forming biosurfactant bacterial isolates from Oman, using glucose based minimal production medium. Our results from screening of 42 soil-samples showed that one sample contained spore-forming bacteria which reduced ST and IFT substantially within 24h. Further studies are needed to identify the bacteria and the role of biosurfactant molecule in bioremediation. Present study showed that isolation of spore-forming bacteria from oil-contaminated sites will lead to potent biosurfactant producing microbes, which may have potential role to play in bioremediation of those particular sites.

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