Screening of biosurfactant producing microorganisms from oil contaminated soil samples in Coimbatore, Tamilnadu, India

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Abstract

Increasing contamination of soil with benzene, toluene, and xylene (BTX) due to activities of the chemical and oil refinery industry has prompted severe environmental pollution. The biodegradation of BTEX compounds by microorganisms has shown promising results to overcome this problem. In the present study, we found stabilized bacterial consortium from the oil contaminated soil samples in Coimbatore, Tamilnadu, India. Among the 113 isolates, 65%, 67.2% and 55% of isolates observed from Benzene, Xylene and Toluene respectively. To confirm the ability of isolates to produce biosurfactant, agar blue plate, hemolysis, oil spreading test, drop collapsed assay and emulsification test were conducted. Among the 16 isolates of biosurfactant producers, 56% of were gram-negative isolates and 44% of were gram-positive isolates were observed. These isolates are valuable source to investigate further for future industrial applications.

Key worlds: Benzene, Toluene, and Xylene**,** Biosurfactant,

I. INTRODUCTION

One of the major environmental issues today is hydrocarbon contamination resulting from the activities related to the petrochemical industry. If these products are exposed to the environment they can be dangerous to the surroundings as well as to life forms. A million liters of petrochemicals enter into the environment from both natural and anthropogenic sources every year (Shallu *et al.*,2014). Petroleum compounds can slash the provision of water, oxygen and vitamins and minerals within the soil, which consequently, may decline the price of seed germination.

Among the hydrocarbons, benzene, toluene and xylene are common pollutants (BTX) and which are classified as main pollutants by the US environmental protection agency (Shim *et al.*,2002). These aromatic compounds are highly toxic, cause cancer and decrease the agricultural productivity of the soil (Bijay *et al.*,2012). Mandri *et al.*,2007 reported significant increases in tumors of kidney, liver, and other tissues and organs following exposure to BTX.

Among the hydrocarbons BTX compounds were not easily degraded because they lack an activating oxygen or nitrogen substituent group which would make oxidation of the ring more energetically feasible (Rajni and Mary, 2010). The number of technologies were commonly used for soil remediation, among them biodegradation of BTEX compounds by microorganisms has shown promising results to overcome this problem.

A huge array of microorganisms including fungi, algae and bacteria are known to degrade PAHs. Nonetheless, micro organisms play by using far the principal role in whole mineralization. Number of studies reported that most of the biosurfactant produced by bacteria especially Pseudomonas species (Krivobok *et al.*,2003). Many studies have been conducted to investigate microbial enhanced oil recovery, but not much work has been reported from Coimbatore area on a screening of biosurfactant producing isolates. Therefore, the present study was conducted to screen the biosurfactant producing isolates from the crude oil contaminated soil and investigation of the degradation of hydrocarbons in laboratory conditions.

II.METHODS

2.1Collection of soil samples

Sample of oil contaminated area were collected from 60 locations of Coimbatore where the chances of oil spillage. The soil sample was collected from an oil contaminating site of Coimbatore, Tamilnadu, India. The collected samples were transferred to pre-sterilized, labeled plastic bags and transported to the laboratory and maintained at 4 °C until microbiological analysis.

2.2 Isolation of bacterial isolates from oil contaminated soil

The serially diluted soil samples were spread on each of BHA plates overlaid with 100 µl of Hydrocarbon (Benzene, Toluene and Xylene) and were incubated at 25 °C for 14 days. After the incubation period, observed the colonies and sub cultured into nutrient agar for further analysis.

2.3 Screening of biosurfactant producing isolates

All isolates were subjected to screening of biosurfactant production with various methods including blue agar plate, Blood hemolysis, Drop collapse, oil spreading test, Emulsification test and gravimetric method (Rehman *et al.*,2014).

2.4 Characterization and Identification of isolates

All isolates were identified based on their cultural, morphological and physiological characteristics in accordance with the taxonomic scheme of Barrow and Feltham (1993) and reference to Holt *et al.*,(1992). The tests performed include Gram stain, spore stain, motility test, catalase test, oxidase, coagulase, urease, indole production, hydrogen sulphide production, nitrate reduction, methyl red, Voges-Proskauer, oxidative/fermentative test and utilization of carbon sources.

III.RESULTS AND DISCUSSION

Oil contamination became a serious concern nowadays, particularly for the soil and water because these are prime sites of oil spill. Currently, scientists are looking in the direction of some cleanup technology for removal of hydrocarbons, which is able to rid us from this long-term harmful result. Among many cleanup methods, the most preferred one is the bioremediation (Islam and Rahman 2017). Against these backdrops, this study was aimed at isolating and screening of biosurfactants producing bacterial isolates from oil contaminated soil samples.

In the present study, 113 bacterial isolates were isolated from petrochemical contaminated soil samples. Among them, 65% of were grown on Benzene containing medium and followed by 67.2% of were grown on Xylene and 55% of were grown on Toluene containing a medium, which isolates were carried out to further studies. Very few authors were isolated the BTX degrading isolates from soil samples (Rajni *et al.*,2010; Kim *et al.*,2008). The previous study of Marcelo (2005) and Wang (2008) were also observed the hydrocarbon degrading bacteria with BTX containing BH media.

Presently, all isolates were subjected to screening of biosurfactant production with various screening methods. Based on the screening test, 32 isolates were showed positive in blue agar test, hemolysis, oil spreading test and drop collapse test. Furthermore, these isolate were subjected to emulsification test for determinate the emulsifying activity, among them, 16 of were showed better activity. The previous study of Thavasi *et al.*, 2008 and Saravanan *et. al.*, 2012 also observed the biosurfactant producing isolates through blue agar, hemolysis, oil spreading, drop collapse, etc. Satpute *et al.* (2008) that more than one screening methods should be included in the primary screening to identify potential biosurfactant producers. These preliminary screenings step used to be primary for effective biodegradation due to the fact that the selected microorganisms were adapted to BTX mixture.

In the current study, 56% of were gram-negative isolates and 44% of were gram-positive isolates were observed. The gram negative isolates have mainly belonged to 3 genera repeatedly viz. *Serratia sp, Pseudomonas aeruginosa and Klebsiella pneumoniae* accounting to 3(19%), 5(31.2%) and 1(6.2%) respectively. The gram-positive isolates have belonged to 2 genera viz, *Bacillus subtilis*, *Bacillus cereus* and *Micrococcus luteus* accounting for 3(19%), 2(12.5%) and 2(12.5%) respectively. The dominance of gram - negative bacterial isolates in oil contaminant soil in this report goes against the general recognition that gram -positive bacterial isolates are the main group of oil pollutant degrades (Lin *et al.*,2007). The results of screening procedures consistently showed the biosurfactant-producing property of the isolates.

From this study, an efficient BTX tolerating bacterial consortium was screened through various methods from the oil contaminated soil. These isolates were an ability to create emulsions with used engine oil and this result suggests that isolates could be applied towards the bioremediation of engine oil from contaminated soil via the use of biosurfactants.

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