



Isolation and Characterization of Oil Degrading Bacteria from Soil of Oil Contaminated Sites

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ABSTRACT

Oil spills have been a major issue across decades. Bioremediation is considered to be more economical and safe method. This study was aimed to isolate the oil degrading bacteria from oil contaminated soil. The soil samples were collected from oil-contaminated areas from Islamapur, Maharashtra, India. The collected soil samples were subjected for isolation of oil-degrading bacteria by enrichment culture technique. The isolates were studied for their cultural, morphological, and biochemical properties and oil degrading properties. Two isolates were obtained having the promising oil-degrading ability and were found to be Bacillus spp.

Key words: Bacillus spp., Bioremediation, oil-degrading bacteria

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INTRODUCTION

Oil spills have been a major issue across decades. One of the famous two oil spills in the worlds are as follows one which was s in Taylor Energy well in Gulf of Mexico; U.S.A caused due to Hurricane (set 16, 2004 till present date) and almost 0.03-0.05 tones oil/ per day is estimated to leak. Second oil spills in Mumbai (India) and caused due to the leakage in Mumbai-Uran pipeline dated January 21, 2011 and about 55 tons of oil was leaked in Arabian Sea. Used engine oil can be considered as one of the sources responsible for polluting the soil. Motor oil typically has much higher concentrations of PAHS (polycyclic aromatic hydrocarbons). Naphthalene, a constituent in used motor oil, include changes in the liver and harmful effects on the kidneys, heart, lungs and nervous system. Due to their relative persistence and potential for various chronic effects like carcinogenicity PAHs and particularly the alkyl PAHs can contribute to long term chronic hazards of jet fuels in contaminated soils, sediments, and ground waters [1].Workshop seepage of used engine oil impacts on loss of soil fertility, water holding capacity, permeability and binding capacity [2].Bioremediation method is considered to be more economical and safe method for the treatment of oil contaminated site. Petroleum hydrocarbons cause damages to the surrounding ecosystems. Soil contaminated with diesel and engine oil is the major environmental problem today which caused due to increase in the consumption of petroleum hydrocarbon products. Damage by oil spills cause very serious adverse effects and are long lasting. Components of petroleum hydrocarb on their uncontrolled release into environment causes serious impact to human and animal health effects such as, carcinogenic, mutagenic and immune toxicants. Microorganisms are known to convert harmful substances to non-toxic substances. In this view we isolated and characterized oil degrading bacteria from contaminated oil soil.

MATERIALS AND METHODS

ISOLATION OF OIL DEGRADING BACTERIA [1]

Collection of Soil Samples

The six soil samples of oil – contaminated sites were collected from various locations such as three each from Industrial area and Automobile Garage from Islamapur. Maharashtra, India location, in separate plastic bags and were brought to the laboratory.

Enrichment of Oil Degrading Microorganisms

Each soil sample was serially diluted and enriched with 2 % industrial machine oil and incubated for 10 days at 30°C on the shaker at 175 rpm. The 1-g of each sample was incubated separately in 100 mL of sterile mineral salt enrichment broth with 2 % industrial machine oil as sole carbon source.

Isolation of Oil Degrading Bacteria

The 0.1 mL of enrichment sample was 1:10 diluted spread inoculated in triplicates on sterile mineral salt agar with 2% industrial machine oil. The plates were incubated at 30°C for 10 days. After incubation, the representative colonies were picked and maintained on sterile mineral salt agar slants in triplicates at refrigeration and designated as B1 and B-2.

Study of Cultural, Morphological and Biochemical Characteristics of Isolates

The two isolates were studied for their colony characters, Gram's and spore staining.

Study of Biochemical Characteristics

The biochemical tests included IMVic tests, production of showing hydrolysis of starch, gelatin and production of catalase, oxidase and urease enzyme with reference to [3, 4].

RESULTS AND DISCUSSION

Morphological Identification / Characteristic

As per colony characteristics, morphological, Gram and spore staining and biochemical tests (Tables-1 and 2), aerobic nature of isolates, and with reference to [3] the two isolates were identified as *Bacillus Spp.* [5, 6, 7, 8] have reported on the role of *Bacillus spp* as oil degraders. In hydrocarbon bioremediation, *Bacillus spp.* occurring in extreme environment and being the predominant isolates of all the crude oil utilizing bacteria characterized from highly polluted soil sample [9].

Photoplate-1: Gram's staining: *Bacillus spp*

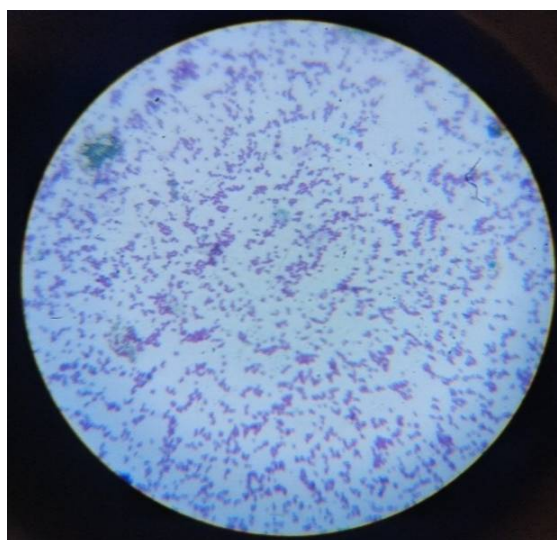


Table- I: Morphology

Colony morphology B-1 isolate B-2 isolate	
Size	2.0 mm 2.5 mm
Shape	Circular Irregular
Color	Cream white white
Elevation	Elevated Elevated
Margin	Wavy Lobate
Opacity	Opaque Opaque
Consistency	Moist Moist
Gram nature	Positive, rods Positive, rods
Motility	Motile Motile

Table 2: Biochemical Characteristics of Isolates

Biochemical tests	Oil Degrading Isolates	
	Isolate B- I	Isolate B-II
1) Indole Production Test	Positive	Positive
2) Citrate Utilization Test	Positive	Negative
3) Catalase Test	Positive	Positive
4) Oxidase Test	Positive	Positive
5) Gelatin Hydrolysis Test	Positive	Negative
6) Urease Test	Positive	Negative
7) Starch Hydrolysis Test	Positive	Positive
8) Methyl red test	Positive	Positive
9) Vogus Proskauer Test	Positive	Positive

CONCLUSION

Microbial degradation is the most significant natural mechanism for removal of hydrocarbon pollutants from the environment. Bacteria can be used to detoxify pollutants owing to their metabolic capabilities. It has been postulated that *Bacillus spp.* are more tolerant of high levels of hydrocarbons in soil due to their resistance endospore. There is growing evidence that isolates belong to two *Bacillus* spp. and could be used effectively in clearing oil spills.

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