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Isolation and Characterization of Lubricant Oil Degrading Microorganisms from Oil Contaminated Soils

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ABSTRACT
Lubricant oils are manufactured in various formulations for different applications. They majorly contain a complex mixture of hydrocarbons. In present work, soil samples were collected from three different petrol filling stations situated in Karad, Maharashtra, India. Three different isolates were obtained from all the soil samples. The detailed study of the isolates resembled with Bacillus subtilis, Micrococcus luteus and Staphylococcus aureus. The lubricant oil concentration of 0.5% seemed to be tolerable by all the isolates. Under the optimum concentration of lubricant oil, M. luteus was found to be the most efficient degrader.
Key words: *Oil contaminated soils, Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Lubricant oils, Oil degradation.*

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INTRODUCTION

Oil reservoirs are characterized by high toxicity, low water activity with hydrophobicity, high temperature, pressure and salinity [1] and are extreme environments for microbial life [2]. Butthey offer a variousniches for a large number of bacteria and archaea, such as sulfate- reducers, nitrate- reducers, iron-reducers, methanogens, fermenters and acetogens [2,3]. Petroleum is amixture of thousands of compounds, 50–98% of which is hydrocarbons. The indigenous microorganisms transform or mineralize the organic contaminants by petroleum hydrocarbons biodegradation. Many different factors of contaminated soil such as pH, total nitrogen, electrical conductivity and heavy metal, characterize effect of petroleum hydrocarbons bacterial biodegradation. Eight hydrocarbon degrading bacteria were specifically detected as *Bacillus*spp, *Alcaligens*pp, *Corynebacterium*spp, *Chromobacterium*spp, *Aeromonas*spp, *Pseudomonas*spp, *Serratia*spp and *Flavobacterium* spp. [4].

A large number of oil pollutants are produced while the production, processing, transportation, and utilization of oil [5, 6] which has caused a serious soil pollution. Thus,it is very vital to repair the oil-contaminated soil. As compared with physical and chemical repairs, bioremediation has been commonly used because of its advantages of easy operation, low cost, good effect, rapid degradation rate and lack of causing secondary pollution [7, 8]. Bioremediation is divided into two types: *in-situ* and *ex-situ*. *In-situ* remediation techniques include bio-culture, microorganism addition, land tillage and bio-ventilation. *Ex-situ* remediation technologies include bioreactors and prefabricated beds [9, 10].

MATERIALS AND METHODS [5]

1. Collection of Soil Samples

The oil – contaminated soil samples were collected from various petrol – filling stations and auto – mechanical workshops situated at the local areas of Karad, Maharashtra, India. Total three samples from three different areas were collected in separate plastic bags and were brought to the laboratory.

2. Enrichment of Oil Degrading Microorganisms

Each soil sample was serially diluted and enriched with 1% lubricant oil and incubated for 8 days at 30^0c on the shaker at 150 rpm.1 g of each sample was incubated in 100 mL of sterile enrichment broth separately. The mineral salt medium broth was used for enrichment using 0.5% lubricant oil as the sole carbon source and energy source.

3. Isolation of Lubricant Oil Degraders

From the enrichment sample, 0.1 mL of sample was spread inoculated in tripliceson sterile mineral salt agar with 1% lubricant oil. The plates were incubated at 30^0c for 7 days. After incubation, colonies with different morphological features were purified by four – quadrant streaking plates of mineral salt agar.
Total three isolates obtained were maintained on nutrient agar slant in triplicates and designated as I-1, I-2 and I-3. These were maintained under refrigeration till use.

4. Study of Cultural and Morphological Characteristics

The isolates were studied for their colony characters. The morphological characteristics were iby Gram' staining. The isolates were subjected to Dorner's staining method to detect the presence of endospores.

5. Study of Biochemical Characteristics

Different biochemical tests were performed to check the presence of certain enzymes characteristics of bacteria and to test their ability to metabolize some of the common carbohydrates such as glucose, sucrose, lactose, maltose and mannitol with reference to Holt *et al*., 1994; Cruickshank, 1985.

6. Study of Effect of Physiological Factors on Isolates[12]

A loopful culture of each isolate was inoculated in separate 5 mL of sterile mineral salt medium broth tubes and incubated at 0°C, 4°C, 15°C, 30°C, 37°C and 45°C for 5 days. After incubation tubes were observed for growth of organisms in terms of turbidity which is measured at 660 nm by using colorimeter.
A loopful culture of each isolate was inoculated in separate 5 mL of sterile mineral salt medium broth tubes of various initial pH values such as 3, 5, 7, 9 and 11 and incubated at room temperature for 5 days. A loopful culture of each isolate was inoculated in separate 5 mL of sterile mineral salt medium broth tubes with different lubricant oil concentrations such as 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL. These tubes incubated at room temperature for 5 days. After incubation, tubes were observed for growth of organisms in terms of turbidity which is measured at 660 nm by using colorimeter.

RESULTS AND DISCUSSION

1. Isolation of Lubricant Oil Degrading Bacteria

Isolation of lubricant oil degrading bacteria was carried out from soil samples collected from petrol filling stations and auto-mechanic workshops from Karad area. Total three isolates were obtained, designated as I1, I2 and I3 (Fig-1).

2. Cultural and Morphological Characterization of Isolates

The colony characters and morphological characteristics of the isolates are given in Tables 1 and 2 respectively. The Fig. 1 shows photographs of all the three isolates. All the three isolates were found to be Gram positive. The isolates I-2 and I-3 were cocci while I-1 was rods. The isolate I-1 was arranged singly, I-2 was in regular packets and large cells while I-3 was found to be in grape like bunches/clusters with small cells, when observed under microscope. The isolate I-1 was found to be motile while I-2 and I-3 were non-motile. Also, I-1 showed centrally located endospores.

3. Biochemical Characterizations of Isolates

Biochemical characterizations of isolates are given in Table 3. It can be seen from the table that all the isolates were catalase positive. Isolate I-2 was oxidase positive while I-1 and I-3 were oxidase negative. All the isolates gave negative tests for urease, caseinase and indole production test. Isolate I-1 was lipase positive while I-2 and I-3 were lipase negative. Gelatin and starch were hydrolyzed only by I-1. I-2 and I-3 showed positive nitrate reduction test. Only I-3 gave H_2S evolution test positive. I-1 and I-3 utilized glucose. I-1 and I-2 utilized lactose. Only I-3 utilized mannitol. No any isolate was able to utilize sucrose. I-1 and I-3 showed positive Vogus – Proskauer test. I-2 and I-3 showed growth on 7.5% NaCl.

4. Tentative Identification of Isolates

From all the colony characters, morphological and biochemical characteristics, all the isolates were tentatively identified as I-1-*Bacillus subtilis, I-2-Micrococcus luteus and I-3-Staphylococcus aureus* aslisted in Table 4.

5. Study of Effect of Physiological Factors on Isolates

The effects of various temperatures on the growth of isolates are shown in Table 5. The curves obtained are shown in Fig. 2. All the isolates showed growth at all the temperatures. The optimum temperature for all the isolates was found to be room temperature, i.e.30°C.
The effects of various pH on the growth of isolates are shown in Table 6. The curves obtained are shown in Fig. 3. At acidic pH, all isolates showed maximum growth. The growth increased with increase in pH to reach the maximum value at pH 7.0 and remained steady thereafter in the alkaline range.
Table 7 and Fig. 4 represent the results of growth of isolates at different lubricant oil concentrations. It was observed that effective lubricant oil concentration was 0.5%. At higher oil concentrations the growth of three isolates was decreased.

Total three isolates obtained were maintained on nutrient agar slant in triplicates and designated as I-1, I-2 and I-3. These were maintained under refrigeration till use.

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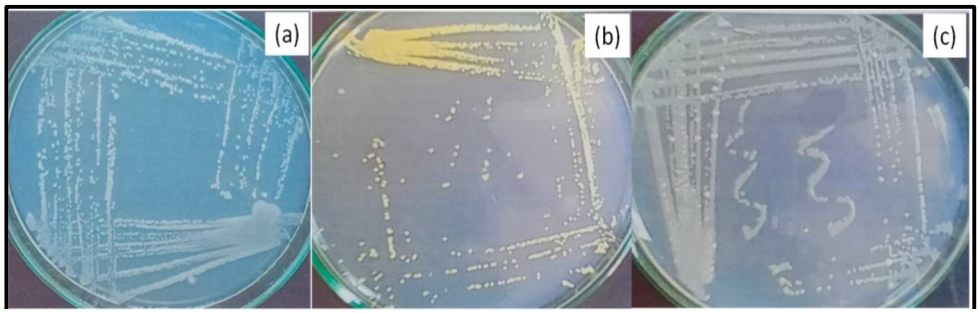

Fig. 1: Photographs of Isolation of Isolates I-1- (a), I-2-(b) and I-3-(c).

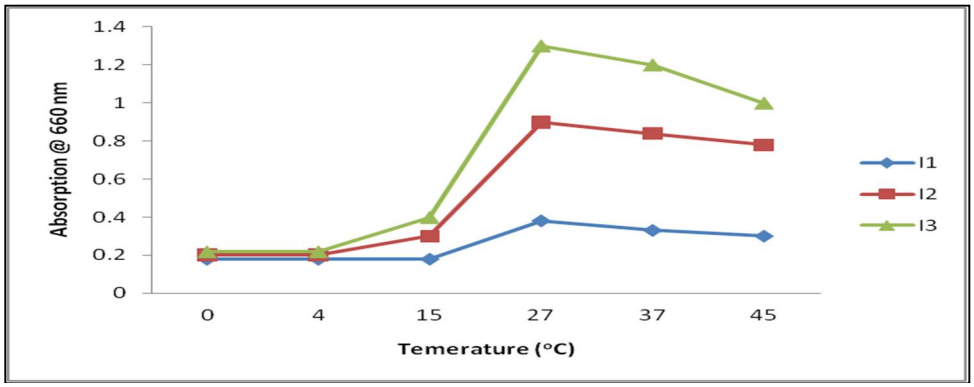

Fig. 2: Effect of Temperature on Growth of Isolates

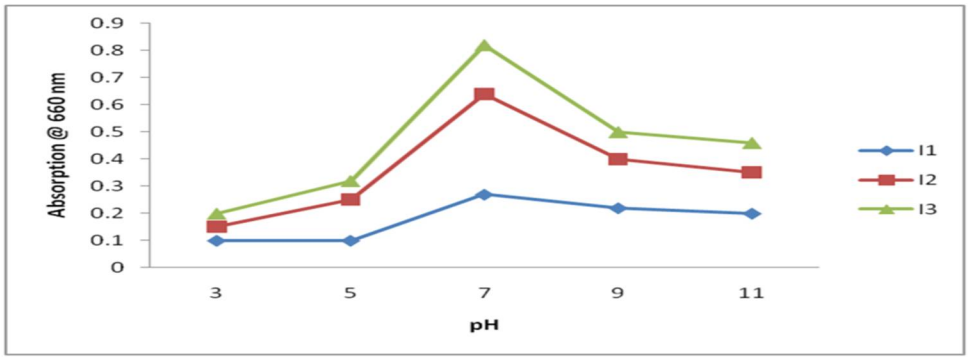

Fig.3: Effect of pH on Growth of Isolates

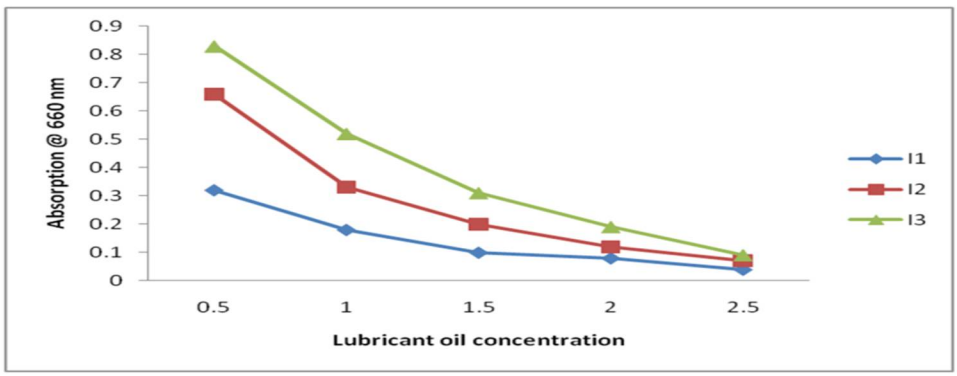

Fig. 4: Effect of Lubricant Oil Concentration on Growth of Isolates

Table 1: Colony characters of isolates

Isolate	Diameter (mm)	Shape	Color	Elevation	Margin	Opacity	Consistency
I-1	1	Circular	White	Low Convex	Regular	Opaque	Moist
I-2	1	Circular	Yellow	Low Convex	Regular	Opaque	Moist
I-3	1	Circular	White	Flat	Regular	Opaque	Moist

Table 2: Morphological characteristics of isolates

Isolate	Gram nature	Motility	Endospore staining
I-1	Gram positive rods	Motile	+
I-2	Gram positive cocci	Non-motile	-
I-3	Gram positive cocci	Non-motile	-

Table 3: Biochemical characteristics of isolates

Sr. No.	Characteristics	Isolates		
		I-1	I-2	I-3
1	Oxidase	-	+	-
2	Catalase	+	+	+
3	Urease	-	+	+
4	Lipase	+	-	-
5	Caseinase	+	-	-
6	Gelatin hydrolysis	+	+	-
7	Starch hydrolysis	+	-	-
8	Nitrate reduction	+	-	+
9	H_2S production	-	-	+
10	Indol production	-	-	-
11	Glucose	+	-	+
12	Lactose	-	-	+
13	Mannitol	+	-	+
14	Xylose	+	-	-
15	Sucrose	-	-	+
16	VP test	+	-	+
17	Growth on 7.5% NaCl	+	+	+

Table 4: Tentative identification of isolates

Isolate	Tentatively identified organism
I1	*Bacillus subtilis*
I2	*Micrococcus luteus*
I3	*Staphylococcus aureus*

Table 5: Effect of temperature on growth of isolates

Isolate	Growth (OD at 660 nm),Temperature ^{o}C					
	0	4	15	30	37	45
I1	0.06	0.09	0.10	0.35	0.32	0.25
I2	0.05	0.07	0.13	0.54	0.45	0.37
I3	0.08	0.09	0.12	0.42	0.35	0.30

Table 6: Effect of pH on growth of isolates

Isolate	pH and Growth (OD at 660 nm)				
	3	**5**	**7**	**9**	**11**
I1	0.06	0.09	0.10	0.35	0.32
I2	0.05	0.07	0.13	0.54	0.45
I3	0.08	0.09	0.12	0.42	0.35

Table 7: Effect of lubricant oil concentration on growth of isolates

Isolate	Lubricant oil concentration (%) and Growth (OD at 660 nm				
	0.5	1.0	1.5	2.0	2.5
I1	0.27	0.18	0.10	0.08	0.05
I2	0.35	0.20	0.13	0.09	0.09
I3	0.20	0.15	0.12	0.06	0.06

CONCLUSIONS

Soil samples collected from petrol – filling stations found to have presence of lubricant oil degarders. All the three isolates were Gram positive in nature, the isolates were found to be*, Bacillus subtilis, Micrococcus luteus*and *Staphylococcus aureus.* The growth of isolates was affected by various physiological conditions. The maximum growth was obtained at room temperature(30^{o}C) at pH 7 with lubricant oil concentration 0.5%. Out of the three isolates, *Micrococcus luteus* was found to be the most efficient degrader of oil.

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