



Isolation and Characterization of Oil Degrading Bacteria from Soil Sample in Lucknow, India

Naveen kumar Sharma*, Rajiv Rai, Vicky Agarwal, Narendr Kumar

Department of Biotechnology, Saroj Institute of Technology & Management, Lucknow (Up)-India.

ABSTRACT

As the usage of petroleum hydrocarbon products increases, soil contamination with diesel and engine oil is becoming one of the major environmental problems. Investigating the countermeasure to remediate soils polluted with oil, has indicated that bioremediation is an effective and useful technique to speed up the cleanup processes. Human and animal health has been seriously threatened by un-controlled release of petroleum hydrocarbons into the groundwater and soil. The oiled area is covered with "fertilizers" that contains microorganisms, like bacteria, in bioremediation process. These microorganisms speed the natural degradation processes already at work. It is thought that the more microorganisms at work, the faster the oil will be removed. In this study, three microbial isolates capable of using engine oil as carbon source were isolated from contaminated soils.

Keywords: Engine Oil, Bioremediation, Contaminated Soil, *Pseudomonas Aeruginosa*.

Corresponding author: Naveen kumar Sharma

e-mail ✉ adi13sept@gmail.com

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(Alexander, 1999). In mechanic workshops, various forms of petroleum products such as engine oil, petrol, diesel and kerosene are used every day.

1. INTRODUCTION

Engine oil which is used to lubricate the parts of a car engine to keep everything working smoothly (Hagwell et al., 1992), is a complex combination of hydrocarbons and other organic compounds, including some organic metallic components (Butler and Mason, 1997). Viscosity is the most important property of the lubricating oil for automotive use. Much of the metals and heavy polycyclic aromatic hydrocarbons (PAHs) which are found in used motor oil would lead to chronic dangers including mutagenicity and carcinogenicity (Keith and Telliard, 1979), Hagwell et al., 1992; Boonchan et al, 2000). Liver or kidney diseases, possible damage to the bone marrow, and an increased risk of cancer may be developed by prolonged exposure and high oil concentration (Mishra et al., 2001; Lloyd and Cackette, 2001). In addition, PAHs occur widely in various ecosystems that contribute to the persistence of these compounds in the Afr. J. Biotechnol environment (Van Hamme et al., 2003). The illegal dumping of used motor oil is an environmental danger which has attracted the public attention worldwide (Roling et al., 2002). Mechanical methods to reduce hydrocarbon pollution are expensive and time consuming.

Hydrocarbons including PAHs have been long found as substrates supporting microbial growth (Bushnell and Haas, 1941; Speight, 1991). In bioremediation, indigenous oil-consuming microorganisms, called petrophiles, are enhanced and fertilized in their natural habitats. Petrophiles are very exclusive organisms that can be used to degrade large hydrocarbons naturally, and utilize them as a food source (Harder, 2004). These compounds which can be useful in cleaning up contaminated sites are degraded by microorganisms using enzymes in their metabolism

2. MATERIELS AND METHODS

The soil samples were collected from Gombi motors Sarsawa Arjunganj Lucknow randomly 5-10 cm beneath the surface using spatula and were packed in capped vials, and transferred to the laboratories. Then, 0.5 gm of soil sample was added to test tube A, and vortexed well. 0.5 ml of the solution from test tube A is added to test tube B, containing 4.5 ml of sterile saline solution, which gives 1-10 dilution of original sample, i.e. original sample is diluted to 1/10.

Isolation of wild type microbes from oil contaminated soil: Spread plate technique (Obtaining mixed culture):

Sterilized NA (Nutrient Agar) was poured carefully to the sterilized petri plates under aseptic conditions of laminar air flow. It was then allowed to solidify. 20 μ l of diluted soil sample was taken from test tube B and spread over solidified agar medium using spreader. Similarly, all the diluted inoculums of soil samples ranging from 10⁻⁴ to 10⁻⁵ were spread over their respective petri plates. Finally, the plates were incubated in an inverted position at 37 °C for 24 hours.

Purification of obtained mixed culture:

Streak plate method: In this method, a sterilized loop of transfer needle is dipped into suitable diluted suspension of organism which is then streaked on the surface of an already solidified agar plate to make series or parallel and non-overlapping forms.

Staining and other biochemical tests of the obtained pure culture:

Gram staining:

The gram staining was used to identify the gram positive or gram negative bacteria.

Catalase test:

Approximately two drops of hydrogen peroxide solution were placed over a clean glass slide. To be tested, the colony N1 and N2 were picked carefully using inoculation loop. The colony was rubbed above the surface of hydrogen peroxide solution over glass slide. The bubble formation was observed over the glass slide.

Mannitol fermentation test:

A 5 ml of mannitol fermentation broth was poured in 3 test tubes and autoclaved. It was then cooled up to room temperature. All the test tubes, except the blank, were inoculated with N1 and N2. Tubes were incubated at 37°C for 48 hrs, in a shaking incubator. Change in colour was observed after 48 hrs.

Glucose fermentation test:

Approximately 50 ml of glucose fermentation broth was prepared, and its pH was maintained up to 7.1. 0.75 ml of bromo-thymol blue was added to the glucose fermentation broth. Approximately 10 ml of glucose fermentation broth was transferred to two test tubes separately. The tubes were incubated at 37°C for 48 hrs, in a shaking incubator. Change in colour was observed after 48 hrs.

Oil degradation studies of *Pseudomonas aeruginosa*:

Sub-culturing of *P. aeruginosa*:

Pseudomonas aeruginosa strain available at DBT-SITM was sub-cultured by continuous streaking on solidified N.A. plates. The plates were maintained and sub-cultured in between, throughout the research work.

Oil degradation studies of *Pseudomonas aeruginosa*:

Material required: Conical flasks, N.B., unused 2T oil (Castrol), fresh *Pseudomonas aeruginosa* species broth culture, micropipettes, sterile tips, laminar air flow, autoclave, measuring scale were used in this study.

Procedure:

The components required for preparing 100 ml N.B. were weighed and dissolved in 75 ml of distilled water in a conical flask. 25 ml of unused 2T oil was also added to the same conical flask. The flask was then plugged well and autoclaved. After autoclaving, it was cooled up to room temp. and the length of oil and broth layer was measured using scale, in mm. The mixture of oil and nutrient medium was then inoculated with a 24 hrs old broth of *Pseudomonas aeruginosa* culture. It was then incubated at 120 rpm, 37°C for 10 days in a shaking incubator. Measurement of oil layer and nutrient broth layer was done at regular intervals.

Oil degradation studies of N1 & N2 species isolated from oil contaminated soil

The components required for preparing 100 ml N.B., were weighed and dissolved in 75 ml of distilled water inside two different conical flasks respectively. 25 ml of unused 2T oil was also added to each of the two conical flasks separately. The flasks were then plugged well and then autoclaved. The mixture of oil and N.B. was then inoculated with a 24 hrs old broth of each of the two species in their respective flasks. They were then incubated at 120 rpm, 37°C for 10 days in a shaking incubator. Measurement of oil layer and nutrient broth layer was done at regular intervals.

3. RESULTS AND DISCUSSION

Table 1. Colony morphology of obtained mixed culture

Colony Morphology	N1	N2
Shape	Circular	Irregular
Margin	Discrete	Curl
Elevation	Flat	Raised
Pigmentation	Creamish	Creamish
Texture	Hard	Hard
Surface	Rough	Rough
Opacity	Opaque	Opaque

Staining and other biochemical tests for the obtained pure culture:

The results for gram staining of N1 & N2 species were observed as follow:

Table 2. Staining results of pure culture

	N1	N2
Gram Staining	Positive	Positive
Cellular morphology	<i>Pseudomonas</i>	<i>Pseudomonas</i>

Catalyse test:

Bubble formation was observed in N1 & N2 species. The results were as follow:

Table 3. Results of catalyse test

N1	N2
Positive	Positive

Mannitol fermentation test:

Colour changed from red to yellow.

Table 4. Results of Mannitol fermentation test

Species	N1	N2
Mannitol fermentation test	Positive	positive

Glucose fermentation test:

Changes in colour with respect to the blank one, i.e., blue, to yellow.

Table 5. Results of glucose fermentation test

Species	N1	N2
Glucose fermentation test	positive	Positive

Oil degradation studies of N1 & N2 species isolated from oil contaminated soil:

Oil degradation studies were carried out in NB media and unused 2T (Castrol) oil, and the degradation of oil by inoculating N1 & N2 species culture was studied by

decreasing oil layer. The decrease in oil layer can be observed in the table and figure below:

Table 6. Decreasing oil layer

Culture	Day zero		Day five		Day ten	
	N.B. layer (mm)	Oil layer (mm)	N.B. layer (mm)	Oil layer (mm)	N.B. layer (mm)	Oil layer (mm)
N1	24	6	24	5.5	24	5
N2	24	6	24	5	24	4.5

4. CONCLUSION

Microbes show the maximum degradation of petroleum hydrocarbon in contaminated ecosystem (Leahy and Cowell 1990). Engine oil can be degraded by indigenous microorganisms (T. Mandari and J. Lin, 2007). *Pseudomonas* sp. is capable of utilizing petroleum (Emtiazi G, Shakarami H, Nahvi I & Mirdamadian S H. 2005). A bacterial isolate of *P. aeruginosa* was obtained from the contaminated soil sample collected from Gomti Motors Sarsawa Arjunganj, Lucknow. *Pseudomonas* is not only known to be abundant in soils but also its ability to utilize oil is well documented (Krylov et al, 1980). The isolation and identification was done by using serial dilution, gram staining and colony morphology, and the conformation of microbial activity (oil degradation), catalase test, Mannitol fermentation test and Glucose fermentation test. *P. aeruginosa* degraded about 80% engine oil (in the terms of oil degrading layer 4.75 mm of oil degrading layer).

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