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A Study on Oil Degradation Potential of *Bacillus megaterium* Isolated from Oil Contaminated Sites in Lucknow

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ABSTRACT

Oil-degrading microorganisms are ubiquitous. They can be isolated from contaminated soils where the oil seepage occurs. In this study, five different bacterial species were isolated from soil contaminated with used engine oil and their ability to degrade used engine oil in vitro was studied. The isolates were grown in mineral salt medium (MSM) *supplemented with used engine oil. Isolate MJAL1101 showed maximum oil degradation (27.37%) after 7 days incubation period. The isolate was tentatively identified as Bacillus megaterium based on Bergey's Manual.*

Key words: Biodegradation, Bioremediation, *Bacillus megaterium.*

INTRODUCTION

Oil pollution, whether due to seepage of crude oil or refined product, damages the environment in many different ways. Many biological processes of organisms are affected by the presence of low concentration of petroleum hydrocarbons. Oil toxicity is caused by the soluble fraction, which consists of low boiling point aromatic hydrocarbon which includes benzene, toluene, xylene, naphthalene and phenanthrene. Oil pollution has been recognized as one of the most serious problems for biotic life. Due to exposure of high oil concentrations, many liver or kidney diseases are developed and by the effect of this cancer risk is enhanced, and it also damages the bone marrow.

The illegal dumping of used motor oil is an environmental hazard with global ramifications. The release of oil into environment causes environmental concern and attracts the public attention. The traditional methods to cope with oil spills are mainly through physical containment and

collection using floating booms, applications of adsorbents such as straw, shredded polyurethane or porous volcanic glasses and/or dispersal by detergents [1].

One of the best approaches to restoring contaminated soil is to make use of bioremediation which is an attractive approach of cleaning up petroleum hydrocarbons because it is simple to maintain, applicable over large areas, cost-effective and leads to the complete destruction of the contaminant [2].

Taking into consideration previous research works on oil degradation using microflora [3-7] the demand of the time to have some alternatives of the traditional methods of remediating the oil contaminated sites and the effectiveness of bioremediation over them the present study was carried with an objective of isolating some potent oil degrading bacterial species from oil contaminated sites.

MATERIALS AND METHODS

Collection of soil samples-
Based on the previous studies reported on the presence of oil degrading microbes in oil contaminated soil, soil sample was collected from a mechanic workshop situated in Gomtinagar, Lucknow, India, that had heavy seepage of oil. Soil was collected randomly 5-10 cm beneath the surface using a sterile spatula and were packed in sterile polybags and transferred to the laboratory for further studies**.**

Isolation of oil degrading bacteria-
Bacterial species were isolated by serial dilution agar plate technique. The resulting mixed colonies were studied and five different colonies from the mixed plate were picked based on the differences in colony morphology and were named as MJAL1101, MJAL1102, MJAL1103, MJAL1104, MJAL1105. All the five colonies were purified by quadrant streaking on sterile NA plates and the purity was cross checked by Gram's staining procedure.

Sources of crude oil samples-
The used engine oil was obtained from the same mechanic workshop from where the soil was collected.

Screening of purified cultures for oil degradation-
Screening was carried out in laboratory by inoculating the purified cultures in MSM (minimal salt media) containing the following in g/L of distilled water; NaCl, 5.0; KCl, 0.2; NaH_2PO_4 ,2.8; $MgSO_4.7H_2O$, 0.1;Glucose,8.0; Na_2HPO_4,6.0 pH 7. **[8]** supplemented with 5% used engine oil, incubating the same at 37^oC for seven days in a shaking incubator at 120 rpm and tracking the growth of culture in the flask throughout the seven days incubation. As the media was minimal and will be supporting the culture for a few hours and after that the available source of carbon would be the hydrocarbons present in the engine oil, a growth throughout the incubation indicated that the culture was able to utilize hydrocarbon by degrading it into its basic components.

Also the oil degradation was quantified by recovering the left out oil after seven days by dispensing the culture broth from both the control and Test flasks in centrifuge tubes and spinning the same at 10000 rpm for 10 minutes, recovering and weighing the left out engine oil. The obtained values were put in the formula **[9]** given below to obtain the % oil degradation.
A control flask was also maintained along with the Test flasks having no inoculation, and % oil degradation was calculated. Screening experiments were performed in triplicates.

$$\% \text{ oil degradation} = \frac{\text{Weight of Oil on 0 day} - \text{Weight of Oil after 7 days}}{\text{Weight of Oil on 0 day}} \times 100$$

Identification of the culture showing maximum oil degradation potential-
The culture MJAL1101 showing maximum oil degradation potential was identified based on the key of Bergey's manual **[8]** by performing various staining (Gram's staining, Endospore staining) and biochemical activities (Catalase Test, Mannitol fermentation Test, Voges proskeurs Test).

RESULTS

Isolation of bacteria from soil sample
Bacterial species were isolated from soil samples and mixed cultures were named as MJAL1101, MJAL1102, MJAL1103, MJAL1104, MJAL1105 and were further purified by quadrant streaking.

Oil degradation studies of purified cultures:
Oil degradation potential of the purified cultures was studied based on their ability to grow in MSM supplemented with 5% oil for a period of seven days giving an indication of the capacity of cultures to utilize oil as a source of carbon and energy. **Table 1** below shows the results of the enumeration of the cultures carried out in MSM supplemented with 5% oil.

Table-1:- Oil Degradation Studies of Purified Culture

S. No.	Culture	No. of colonies on 4^{th} day	No. of colonies on 7^{th} day
1.	MJAL1101	Lawn	Lawn
2.	MJAL1102	Lawn	Lawn
3.	MJAL1103	Lawn	Lawn
4.	MJAL1104	Lawn	Lawn
5.	MJAL1105	Lawn	Lawn

Oil degradation was also quantified by calculating the percentage oil degradation by using the formula described earlier. **Table 2** below shows the percentage oil degradation by the samples after 7 days incubation.

Table 2: Oil Degradation % of Purified Cultures.

S.NO	CULTURE NO.	WEIGHT OF OIL ON ZERO DAY	WEIGHT. OF OIL ON 7th DAY	% OF OIL DEGRADATION
1.	CONTROL FLASK (without inoculation)	1.980	1.980	0%
2.	MJAL1101	1.980	1.438	27.37%
3.	MJAL1102	1.980	1.612	18.58%
4.	MJAL1103	1.980	1.436	27.17%
5.	MJAL1104	1.980	1.604	18.98%
6.	MJAL1105	1.980	1.650	16.81%

Identification of the culture showing maximum oil degradation (MJAL1101) during screening:

Table 3 below shows the results of staining and biochemical activities of the culture MJAL1101 showing maximum oil degradation potential, by comparing these results with Bergey's manual **[8]** the culture MJAL1101 was tentatively identified as *Bacillus megaterium.*

Table 3: Staining and Biochemical activities of MJAL1101

TEST	OBSEVATION/RESULT
Gram staining	Positive
Cellular morphology	Bacillus
Endospore Staining	Positive
Catalase Test	Positive
Mannitol Fermentation Test	Positive
VP Test	Negative

DISCUSSION

Soil sample was collected from oil contaminated sites as done earlier by [**10; 6].** Further microorganism was isolated by serial dilution agar plating method as done previously by [**11].**

Wild type isolates were purified in the present study, characterized by studying their morphological characteristics, Gram staining characteristics, Endospore staining and biochemical activities including Catalase Test, Mannitol Test,VP Test and the results obtained were compared with bergey's manual as done earlier by **[12-13].** The isolate showing maximum oil degradation abilities (MJAL1101) was found to Gram positive, *bacillus spp.* Catalase positive, Mannitol fermentation positive and VP positive and was identified as *Bacillus megaterium* earlier also oil degradation studies have been performed by using bacillus species by **[6; 14].**

Screening of purified cultures for oil degradation potential was done by enumerating the microbial growth in the minimal salt media supplemented with 5% used engine oil and also by calculating percentage oil degradation studies, a highest % oil degradation of 27.37% was obtained earlier oil degradation of **7- 15 %** has been obtained in *Nocardia simplex* **[9].**

CONCLUSION

Based on the present study it can be concluded that *Bacillus* species inhabiting the oil contaminated sites can be a good source for the degradation of oil. As oil pollution caused due to any mode is very much harmful for both plant and animal life, bioremediation which is a better mode than the traditional methods discussed earlier can be a very good mode for the cleaning up of environment contaminated with oil.

Future prospects of the current work includes the field oil degradation studies using the culture MJAL1101 wherein the culture would be used for oil degradation in oil contaminated soil and water bodies. If these field studies are successful than the culture can directly be used in the oil spills, mechanic workshops and other places causing oil pollution either in soil or water bodies.

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