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ISOLATION AND CHARACTERIZATION OF POTENT OIL DEGRADING MICROBES FROM OIL CONTAMINATED SOURCES

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ABSTRACT

The study was designed to evaluate the capability of bacterial strains to degrade oil under in vitro conditions. Two mechanic workshops within Lucknow were selected for collecting two different samples from each site. One isolate showed complete oil degradation capability within seven days incubation period. The results tend to perform certain characterization tests. In order to check expression of oil degrading gene, plasmid was isolated and transformed to *E. coli* cells.

Keywords: *E. coli*, Degradation, Crude oil.

INTRODUCTION

Petroleum hydrocarbon can be degraded by microorganisms such as bacteria, fungi, yeast, and microalgae [1][3]. Numerous studies have been conducted on microbial consortia and enrichment [2] and most bacterial petroleum hydrocarbon degraders have been isolated from heavily contaminated coastal areas [4][5][6].

Oil spills have been recognized as one of the most serious current problem particularly in industrialized and developing countries. Inevitable spillages, which occur during routine operations of oil products, refining or as a consequence of acute accidents, lead hydrocarbons to reach the water table before becoming immobilized in the soil. They spread horizontally on the ground surface, leading loss of soil fertility and water holding capacity. Because of great number of oil contaminated sites requiring cleanup and high cost involved with the conventional approaches for excavation and landfills, a need arises to develop new remediate

technologies, such as bioremediation that uses microorganisms to detoxify environmental pollutants and transform into simpler less toxic compounds.

The parameters typically measured in laboratory tests for bioremediation efficacy include enumeration of microbial populations, determination of fate of hydrocarbon degradation. Undoubtedly, the most direct measure of bioremediation efficacy is the monitoring of hydrocarbon degradation or disappearance rate. Undoubtedly, the most direct measure of bioremediation efficacy is the monitoring of hydrocarbon degradation or disappearance rates. Petroleum products such as petrol, engine oil, diesel and kerosene are used day to day in various forms at oil mechanic workshops. These products lead to hardening and change in colour of the solid which may have unseen and untold health hazard on the technicians, artisans and other co-workers. This study, thus was aimed to assess the unused oil biodegradation potential of selected bacterial strain under in vitro conditions.

The present study is carried out by isolation of oil degrading microbes from oil contaminated soil and expression of oil degrading genes in *E.coli*.

Materials and Method

Study Site

Study sites were two different mechanic workshops in Lucknow. The places were Vikas Nagar and Gomati Nagar.

Sample Collection

Soil samples were collected from specific location within the workshops that had heavy spoilage of engine oil. There were no grasses growing at the locations and soil sample were blackish in colour.

Isolation of Microbes

Soil sample was serially diluted and the culture media used was Nutrient Agar media which is an enrichment media for isolation of bacterial degrading organism.

5 gm soil sample was taken for serial dilution and the culture media used was NA media which is an enrichment media for isolation of bacterial degrading organism. Inoculation was done using 3 petri plates containing NA media of 10 ml in each plate into which 50 µl of serially diluted soil sample was spread all over and incubated at 37˚C for overnight.

Sub- culturing

The colonies on the basis of their morphology were chosen and streaking plate method was performed in order to obtain pure culture.

Inoculation of pure culture media into NB

80 ml NB media was prepared in 3 flasks and 20 ml of 2T oil was added, autoclaved it at 121˚C for 15 minutes. With the help of inoculum loop, inoculation of pure culture media was performed into NB. Incubation was done in shaker incubator at 37^0C for 7-10 days in order to check degradation of oil layer.

Expression of Oil Degrading Genes in *E. coli*

Plasmid Isolation

Plasmid was isolated from overnight grown bacterial culture with the help of Alkaline denaturation method, in order to observe the expression of oil degrading genes in *E. coli* followed by Agarose gel electrophoresis.

Competent cells Preparation

Competent cells are the cells which are ready to uptake foreign DNA and for transformation competent cells are required and in this work *E.coli* cells are used as a host cells.

Transformation

Transformation was done to transfer genetic material from one cell to another with the help of plasmid which acts as a vector. Further the activities of transformed and non-transformed cells were checked.

RESULTS AND DISCUSSION

Isolation

The serial dilution method was performed for 2 samples and the oil degrading capacity was obtained for only one culture.

Initially , Microbes were isolated from soil samples and mix cultures were obtained

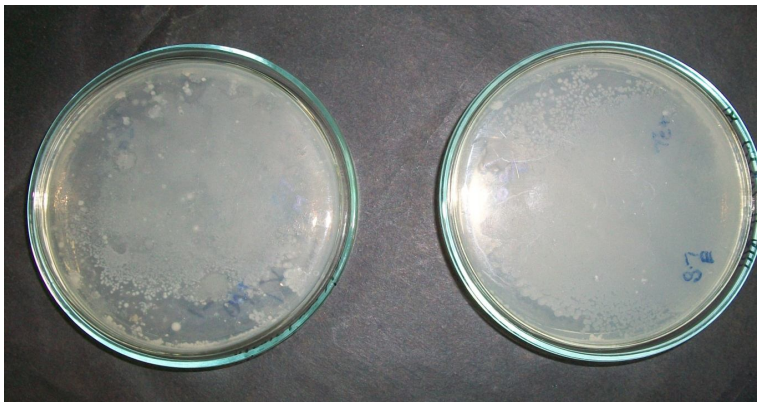

Figure 1 showed mix culture colonies

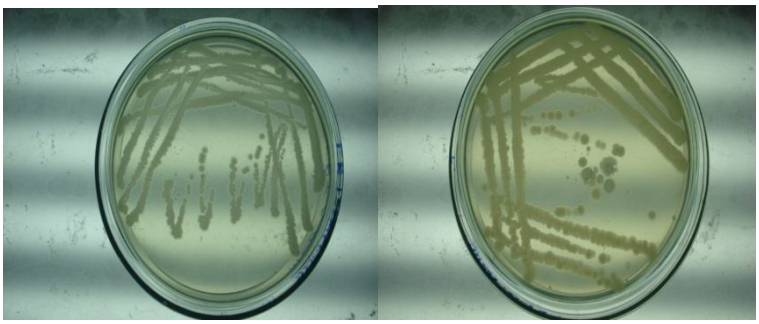

Figure 2 showed pure culture of bacteria

Oil degradation by pure culture bacteria

Oil degradation was performed by Turbidity method , to check the oil layer .Oil degradation by different bacteria when inoculated with NB and oil.

Figure 3: The middle flask contains *Pseudomonas* which was used as a control, other two flasks were inoculated with pure colonies of bacteria obtained.

Complete Degradation of oil

Figure 4 : showed the complete degradation of oil within 7 days.

The flasks were incubated for 7 days in shaker incubator to check the degradation of oil layer the measurement was done in each day.

Plasmid Isolation

Plasmid was isolated by Alkaline denaturation method process which were analysed by agarose gel electrophoresis.The plasmid was isolated for expression of oil degrading genes.

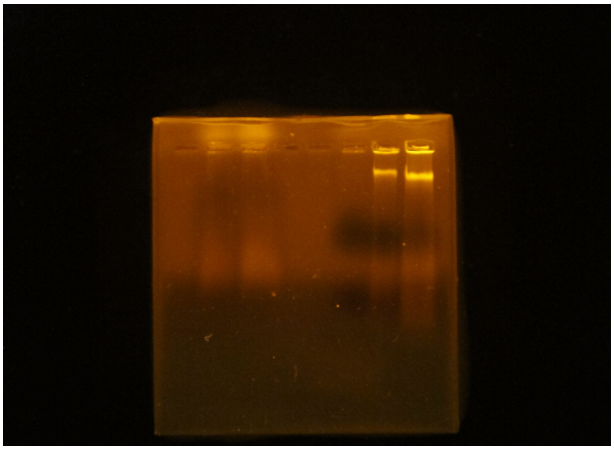

Fig 5: showed the plasmid bands in agarose gel

Competent cell preparation

Competent cells were prepared to uptake foreign DNA.In this work the *E.coli* cells were used as a host cells.

Transformation

Transformation was done in order to transfer genetic material from one cell to other cell with the help of plasmid which acts as vector.

Figure 6 showed ability of oil degradation of transformed and non – transformed cells *(E. coli)*

DISCUSSION

Soil samples were collected from two different mechanic workshops. Further microorganism was isolated by serial dilution method and nutrient agar plating method. In mechanic workshops, there is constant change in the soil micro-organisms as a result of spillage of engine oil. These change the ecology and biomass of the soil such that there were no grasses growing on them. The changes in the colour and texture of the soil, lead to different microbial flora establishment[7].

Purified culture was inoculated with NB and oil, leading to complete degradation. Further the plasmid was isolated, competent cells were prepared and transformation was done. The isolate showed the maximum oil degradation abilities, was gram positive and characterization marked the presence of Bacillus species.

The result clearly indicated that Bacillus spp. were comparatively better and potent hydrocarbon/oil degraders[8].

CONCLUSION

The oil degradation is major problem for environment and can be removed by process of bioremediation which include the uses of oil degrading microbes. The overall conclusion for this study was that microbeswhich was isolated from oil contaminated site were having

properties to degrade the oil layer and the test were performed for 2T Oil. One culture was characterized as a *Bacillus*.The Plasmid was isolated from *Bacillus* and transformed in to *E.coli* to check the oil degradation.

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