Original Research Article

Isolation of oil degrading bacteria isolated from the contaminated sites of Bhopal Madhya Pradesh

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Abstract

Oil spills and petroleum waste products are major source of pollution with their recalcitrant properties that cause major source of alarm due to their adverse effect on the environment. Rate of biodegradation depends greatly on the compositional properties, the concentration and type of hydrocarbon chain present along and their physical state. These xenobiotic compounds are recalcitrant and thus make it a great problem and requires attention. Bioaugmentation of these contaminated sites using cost effective methods for restoration of ecosystem is need of the hour. The main focus of our research is to isolate hydrocarbon degrading bacteria from the contaminated soil with petrol and diesel oil 10 isolates were isolated out of which five isolates were selected for study of oil degradation namely D2, D5, D7, D8, D10. The isolates were screened for their oil degrading capacity among them D5 showed the best growth with oil as a sole source of carbon.

Key Words: Xenobiotic, recalcitrant, bioaugmentation, biodegradation.

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INTRODUCTION

Hydrocarbons are amongst the top contaminants that pollute our environment due to their recalcitrant nature they cause environmental issues that cause hazardous pollution due to their physio-chemical and xenobiotic properties[4]. Hydrocarbons enter into the ecosystem mostly due to the intervention of humans due to dumping and accidents cause during storage and transportation of these substances. One of the major contributors of such hydrocarbons is mineral oils that are highly toxic and a major source of problem. Petroleum are highly mutagenic and cause immunological issue.[8] Oil spills and dumping of petroleum waste due to ship or tanker accidents, pipe-lines rupture or leakages has made land and water remediation a great problem that is highly costly and difficult to achieve. [5][18] Petroleum compounds are highly complex due to their long and branched chain properties and variable functional groups and ring and cyclic structure containing sulphur and nitrogen compounds and are mostly saturated make them highly complexes and versatile compounds that are not easily destroyed. Certain microbes have been isolated from contaminated sites that are capable to biodegrade mineral oils and are being employed to solve this problem through the process called Bioremediation[9,18] The main focus of our study was to isolate, Biochemically identify and screen such bacterial population that have the potential to degrade mineral oil under in vitro conditions.

MATERIAL AND METHOD

Sample Collection: Soil samples were collected from two different sites near Garage sites of Indrapuri, Bhopal. Samples were collected within a depth of 5cm from the surface of the soil and were bagged in sterile polythene bags and seal packed. These soil samples were used to isolate the Bacteria for test to be conducted for screening of oil degrading strains. Samples were collected at a depth within 5cm from the surface of the soil. They were

collected in sterile polythene bags and tightly packed and stored at 4°C[7,17,11]

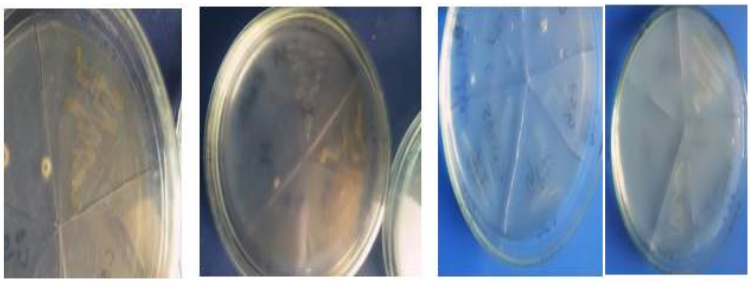
Isolation of Oil degrading Bacteria from soil samples: Serial dilution was prepared of the two sites followed by spreading of dilution 10^{-6} of sample A and 10^{-6} of sample B on Nutrient Agar Media (NAM) Plates and incubated at 35°C for 24 hrs[2,15,16]. After 24 hr colonies were enumerated and CFU was calculated using the formula (Table 1.): CFU/ml = (no. of colonies x dilution factor) / volume of culture plate. Then individual colonies isolated on NAM plates using quadrate streaking method.

Screening of oil degrading bacteria: Screening of Potential Oil degraders was done using Bushnell Haas (B and H) Agar Media plate supplemented with 1% engine oil. Sterilized Bushnell Haas Agar medium plates inoculated with the ten isolates were incubated at 35°C for 48 hours. Further screening performed by changing carbon sources using Petrol and Kerosene for comparison to isolate the best strain possible for mineral oil degradation[2,3,10](Table 2).

Colony Characterization, Staining and Biochemical tests: Colonies obtained from screening were then subjected to colony characterization, Grams staining, Endospore staining, Cell motility and capsular staining were performed and various biochemical tests were done like IMViC (Indole test, Methyl Red Test, Voges Proskauer test and Citrate test), Catalase test, Tipple Sugar Ion tests, Starch Hydrolysis Test, Gelatin hydrolysis, Urease and Casein hydrolysis tests, Lipases, Cetrimide test, Lipase test and Niterate reduction tests were also conducted as followed by Singh and Ravi[14][15] (Table 3.)

OBSERVATIONS AND RESULT

The screening media Bushnell Haas Agar Media was used to screen perspective oil degrading bacteria out of 10 isolates 5 isolates namely D2, D5, D7, D8, D10 showed positive growth on B and H media supplemented with sole Carbon sources namely Kerosine, Petrol and Engine oil as shown in Figure.1. Results of plates supplemented with petrol, Kerosene and Engine oil separately was tabulated in Table 2. It was observed that D5 isolate showed growth on all the three plates of B and H supplemented with Kerosene, Petrol, Engine oil respectively. While D8 showed growth on petrol and Engine Oil. D2, D5, D7, D8, D10 all showed positive growth on BandH Media supplemented with Engine Oil. These were then Morphologically and Biochemically characterized the results are depicted in Table 3 and Table 4.



a) Kerosene b) Petrol c) Engine Oil

Figure 1: Bushnell Haas Agar Plates a) represent growth of D5 on kerosene supplemented B and H plate, b) Represents growth of D5 and D8 isolate on BandH plates supplemented with Petrol and c) Represents growth of isolates D2,D5,D7,D8,D10 on two B and H plates supplemented with Engine oil

Table 1: CFU of bacteria colony that were isolated from oil contaminated sites of Garage soil, Indrapuri, Bhopal, MP.

Plate	Dilution	Number of colonies	CFU	Strains obtained
Plate 1 Site A	10^{-6}	780	$7.8x10^{6}$	D1, D2, D3, D4, D5, D6
Plate 2 Site B	10^{-6}	592	$5.9 x10^{6}$	D7, D8, D9, D10

Table 2: Growth of 10 colonies using varied carbon sources in Bushnell Haas Plates

SN	Isolate	Engine Oil	Petrol	Kerosene
1	D1	-	-	-
2	D2	+	-	-
3	D3	-	-	-
4	D4	-	-	-
5	D5	+	+	+
6	D6	-	-	-
7	D7	+	-	-
8	D8	+	+	-
9	D9	-	-	-
10	D10	+	-	-

(+ sign denotes growth and – sign denotes no growth)

Table 3: Colony Characteristics of bacterial isolates from oil contaminated sites of Garage soil, Indrapuri, Bhopal, MP.

SN	Isolate	Colony shape/Form	Surface	Elevation	Margin	Color
1	D2	Circular	Smooth	Flat	Entire	Creamy
2	D5	Circular	Smooth	Flat	Entire	Creamy
3	D7	Punctiform	Smooth	Convex	Undulated	White
4	D8	Irregular	Smooth	Flat	Curled	White
5	D10	Circular	Smooth	Convex	Entire	White

Table 4: Staining and Biochemical test

SN	TEST	D2	D5	D7	D8	D10

1	Grams Staining	-ive, Bacillus	-ive, Bacillus	-ive, Coccus	+ive, rods	-ive Bacillus
2	Capsular Staining	-	-	-	-	+
3	Motility	+	+	+	+	+
4	Endospore	-	-	+	+	-
5	Catalase test	+	+	-	+	+
6	Starch Hydrolysis	-	+	-	+	+
7	Urease	-	-	+	-	-
8	Casein hydrolysis	-	+	+	+	-
9	Gelatin hydrolysis	+	+	-	+	+
10	Indole	-	-	-	-	+
11	Methylene Red	-	-	+	-	+
12	Voges Proskauer	-	-	+	+	-
13	Cirate	+	+	+	+	-
14	H_2S gas production	-	-	-		-
15	Glucose Fermentation	-	-	+	+	+
16	Lactose Fermentation	-	-	+	-	+
17	Sucrose Fermentation	-	-	-	+	-
18	Gelatin Hydrolysis	+	+	-	+	-
19	Cetrimide test	+	+	-	-	-
20	Lipase	+	+	+	+	-
21	Nitrate test	-	+	-	+	+

DISCUSSION

Soil sample was collected from contaminated sites as done earlier by[6,12,13]. Colonies were obtained on petri-plates which were serially diluted upon being incubated and CFU was calculated (Table 1). Then ten colonies were isolated after pure culture isolation and were named D1, D2,D3,D4,D5,D6,D7,D8, D9,D10 respectively. These 10 isolates were then subjected to screening on Bushnell Haas Agar Media[2,3,10] and only five isolates grew on these plates out of the ten isolate namely D2, D5, D7, D8, D10. It was observed that D5 bacterial isolates showed best growth and oil degrading activity when these isolates were subjected to petrol and kerosene carbon sources. These were subjected to colony characteristics (Table 3.) followed by Grams staining in which various staining was done and biochemical analysis was carried out as done by[14,15,6] (Table 4.) of all the five isolates that were screened from Bushnell Haas Plates[1] were qualified as oil degraders as has been reported by preliminary screening method used by Ravi[14] This is one of the reports on this method of qualifying oil degradation abilities. Out of the five isolated D5 showed best growth ability upon being grown on Bushnell Haas Plates having varied carbon sources namely Petrol, Kerosene and Engine oil respectively.

CONCLUSION

Pollution has grown to an alarming scale with mostly due to human intervention at fault due to industrialization and thus poses a grave threat to all living creatures including human existence as well. To avoid the delirious after affects of such problem bioremediation using bacterial strains from contaminated site of Indrapuri, Bhopal has been done so as to isolate oil degraders. The soil sample was collected and various Biochemical and Bushnell Haas Media was used as Screening Media to identify the mineral oil degrading bacterial strain. Further tests are yet to be conducted to further identify these five strains and to quantify their oil degrading ability and use these strains in bioremediation of oil spilled areas through either *insitu* or *exsitu* methods that are cost-effective as well as environment friendly.

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