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Isolation of Mineral Oil Degraders Isolated from Ayodhya Bypass Petrol Pump Bhopal, Madhya Pradesh, India

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ABSTRACT

Petroleum hydrocarbons and their waste products are one of the major sources that are considered to harm natural domain. The dominance of petroleum and its products in the world economy produce the conditions for distributing large amounts of toxins that are xenobiotic in nature and are recalcitrant and thus persists into populated areas and ecosystems all around the globe resulting into pollution and destruction of ecological balance in the long run. The adverse effect of such pollution needs an urgent remediation. Mineral oil degrading bacteria (oil degraders) are a powerful solution to a problem. These are ubiquitous in nature and use such petroleum hydrocarbons as a source of carbon and metabolic energy. The main objective of this research was to isolate and screen oil degrading bacteria from the soil contaminated site. A total of 20 isolates were isolates were subjected to Bushnell and Haas Agar Media (B&H) supplemented with petrol, engine oil and kerosene respectively and out of the 20 isolates only AB-3 showed best result and utilized petrol, engine oil, kerosene as sole carbon sources when cultured in B&H media while AB-2 showed growth when cultured on B &H media supplemented with petrol and kerosene as soul carbon sources.

Keywords: Remediation, biodegradation, xenobiotic, petroleum, oil degraders

1. INTRODUCTION

The application of Petroleum hydrocarbons and their products have been drastically resulting in pollution (PHP). Being a fossil fuel the mass utilization and continuously growing demands of oil and oil based products has exceeded thus resulting in shortage and also pollution in the long run. This condition has lead to the harmful effects on both marine and terrestrial ecosystems (Tian et al., 2018). Crude oil contains asphaltenes, aromatics, resins, and alkanes as well as numerous organic chemicals made up of oxygen, nitrogen, and sulfur (Das and Chandran 2011). During harvesting of this crude oil from land or marine area results in mass spillage which can be detrimental to the flora and fauna in the surroundings especially the marine ecosystem. Despite of the various methods of removal and remediation of these compounds persist in the environment due old and ineffective solutions. This scientist and researchers are constantly working to degrade them. One such solution to this problem is by application of microorganism especially bacteria which can easily degrade these recalcitrant compounds. These various complex and simple petroleum hydrocarbons can be degraded using a number of native microorganisms that can breakdown particular molecules (Zanaroli et al. 2010) and these special class of bacterial consortia are called as mineral oil degraders. For example, Acinetobacter, Rhodococcus, Bacillus, Pseudomonas, and Sphingomonas that utilize saturated hydrocarbons (n-alkanes) during petroleum oil degradation (Hassanshahian et al. 2014). The process of bioremediation can be discribed as the use of microorganisms to remove pollutants owing to their diverse metabolic capabilities. It is a developing method for the degradation of many surrounding pollutants which include the products of petroleum industry (medina et al 2005). The successful outcome of petroleum bioremediation or degradation depends on one's ability to develop and maintain such conditions that favor enhanced oil biodegradation rates in the corrupt environment. This paper provides an approach to isolate, identify and screen such type of oil degrading bacterial population that can create a boom for bioremediation process to cure this problem in future (https://pubs.acs.org/doi/10.1021/acsestengg.0c00217)

2. MATERIAL AND METHOD

2.1. Sample collection

Soil sample was collected from oil spillage site from Ayodhya Bypass Petrol pump Bhopal, Madhya Pradesh(23.2698oN, 77.4685oE). Samples were collected in sterile zip lock polybags from depth of 5 cm from the surface of soil. The collected soil was sieved in order to remove unwanted residues (pebbles) from it.

2.2. Isolation of Bacteria

The collected soil samples were weighed properly and their serial dilution was prepared and isolation was done via spread plate method. This was followed by spreading of dilution 10-3 and dilution 10-4 in autoclaved Nutrient Agar Media (NAM) plates. These inoculated plates were kept for incubation at 35ºC for 24 h. After 24 h, colonies were enumerated and CFU (Colony Forming Unit) was calculated (Table 1).

CFU ml^{-1} = (No. of colonies x Dilution factor) / Volume of culture plate

Individual colonies were studied for their morphological characters and pure culture was obtained by streak plate method. (Murab, 2018). After 24 h the growth of bacteria was seen and its colony characteristics were recorded (Table 2).

2.3. Screening of Oil Degrading Bacteria

Bushnell Hass Agar Medium (B and H) was prepared with composition of Distilled Water –1000 ml., $MgSO_4$- 0.2 gram, $CaCl_2$ - 0.02 gram KH_2PO_4 - 1.0 gram ,K_2HPO_4 - 1.0 gram, NH_4NO_8 or $(NH_4)_2SO_4$ -1.0 gram, $FeCl_2$-2 drops conc. solution. pH of the medium was kept to 7±0.2 (Bushnell et al.,1940). Media was supplemented with petrol, engine oil and kerosene respectively as sole carbon source in individual plates and inoculated with the isolates obtained and incubated at 35ºC for 48 h. The growths of the bacterial isolates were then observed.

2.4. Colony and Biochemical Characterization

Bacterial colonies obtained from screening were subjected to colony characterization (color, margin, elevation, form) (Table 2). Grams staining (Jana et al, 2016), Endospore staining and capsular staining was also conducted (Table 4). Biochemical tests were also performed inoreder to biochemically characterize the screened isolates. The test included IMViC (Indole test, Methyl Red Test, Voges Proskauer test and Citrate test) (Dubey et al, 2002), Catalase test (Baron et al, 1994) and Oxidase test (Jurtshuk et al, 1976) (Table 4).

3. OBSERVATIONS AND RESULTS

Bushnell Haas Agar Media was used to screen isolated oil degrading bacteria. Out of 20 isolates 7 isolates were grown in the media. AB2, AB3, AB4 and AB6 showed positive growth on Bushnell and Haas media supplemented with 3 different Carbon sources namely Petrol and Engine oil and kerosene and the observations have been compiled in Table 3. Outcome of plates supplemented with petrol, Engine oil and kerosene separately were tabulated in Table 3. It was observed that isolate AB1, AB4, AB6 only showed growth on plate of Bushnell and Haas media supplemented with Petrol. While isolate AB3 showed maximum growth on petrol, Engine oil and kerosene. Isolate AB2 showed growth in both petrol and kerosene but not in Engine oil. Isolates AB5 and AB7 did not flourished in any of the hydrocarbon containing media. These isolates were then morphologically and Biochemically characterized and the results are depicted in Table 2 and Table 4 respectively.

TABLE 1: CFU of bacterial colonies isolated from petrol pump, Ayodhya Bypass, Bhopal (M.P.)

PLATE	DILUTION	NUMBER OF COLONIES	CFU	STRAINS OBTAINED
Plate 1	10-3	770	7.7 × 10-3	AB1, AB2, AB3, AB4
Plate 2	10-4	698	6.9 × 10-4	AB5, AB6, AB7

TABLE 2: Colony Characteristics of bacterial isolates from petrol pump of Ayodhya Bypass Bhopal

S. No.	ISOLATES	COLOR	MARGIN	ELEVATION	FORM
1	AB1	White	Entire	Raised	Punctiform
2	AB2	White	Entire	Pulvinate	Circular
3	AB3	White	Undulate	Crateriform	Irregular
4	AB4	White	Entire	Crateriform	Irregular
5	AB5	Yellow	Entire	Raised	Circular
6	AB6	Transparent	Undulate	Crateriform	Irregular
7	AB7	White	Entire	Crateriform	Irregular
8	AB8	Creamy white	Entire	Raised	Circular
9	AB9	Pink	Undulate	Convex	Irregular
10	AB10	Orange	Entire	Raised	Circular
11	AB11	Greenish	Entire	Convex	Punctiform
12	AB12	White	Entire	Pulvinate	Circular
13	AB13	White	Entire	Raised	Punctiform
14	AB14	Yellow	Irregular	Raised	Circular
15	AB15	Creamy white	Entire	Flat	Irregular

16	AB16	Yellowish white	Undulate	Raised	Irregular
17	AB17	White	Entire	Raised	Irregular
18	AB18	Yellow	Entire	Raised	Circular
19	AB19	White	Entire	Flat	Circular
20	AB20	White	Undulate	Convex	Punctiform

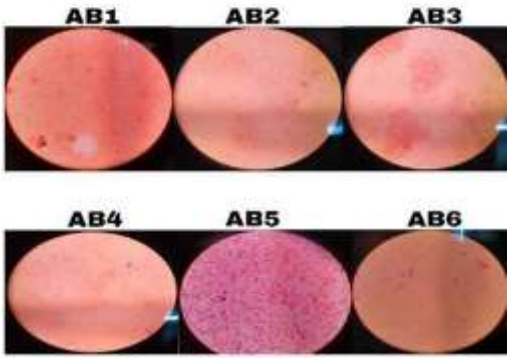
TABLE 3: Growth of bacterial colonies on Bushnell Haas Media

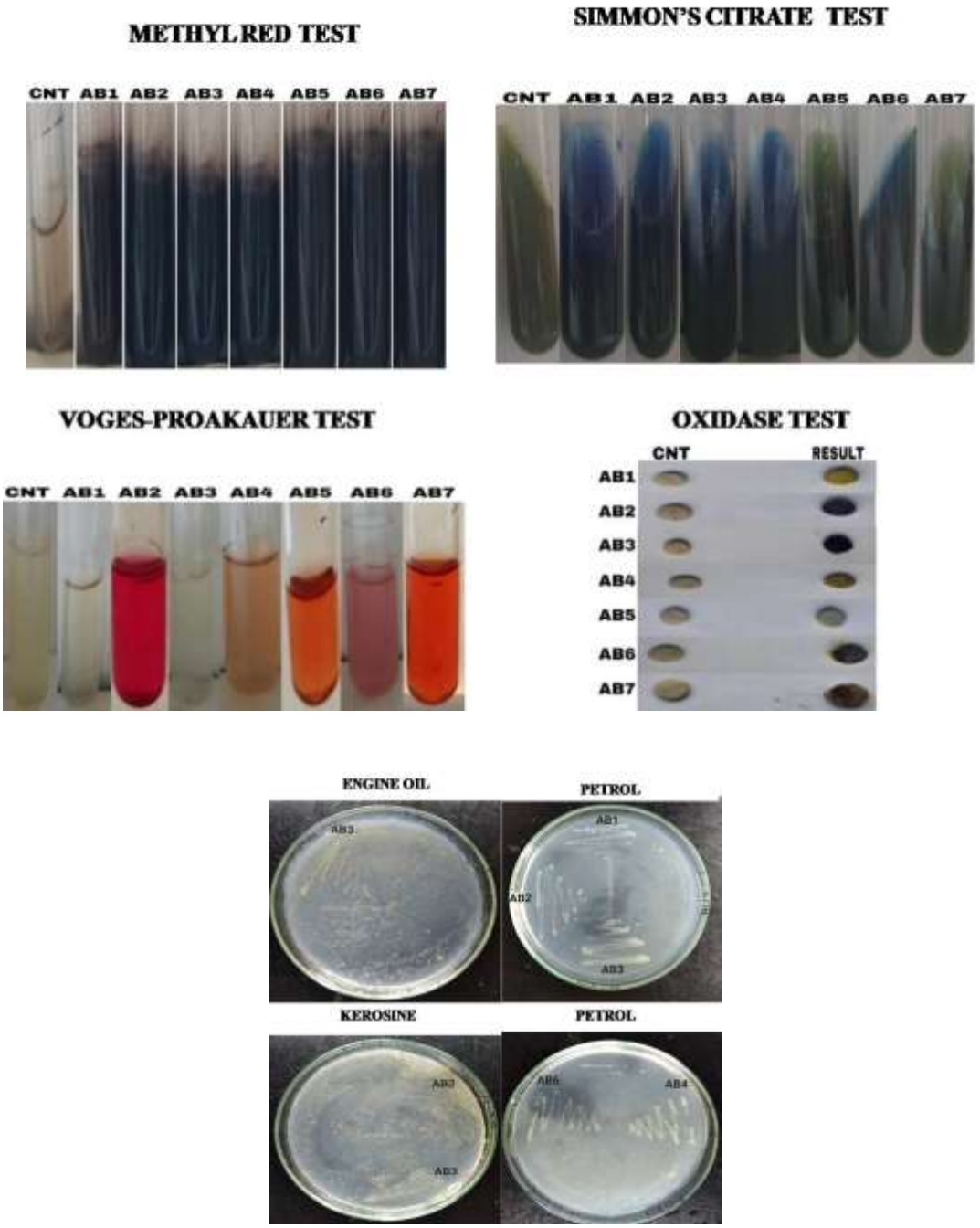
S. NO.	ISOLATE	PETROL	ENGINE OIL	KEROSENE
1.	AB1	+	-	-
2.	AB2	+	-	+
3.	AB3	+	+	+
4.	AB4	+	-	-
5.	AB5	-	-	-
6.	AB6	+	-	-
7.	AB7	-	-	-

TABLE 4: Biochemical Characterization of bacterial samples isolated from Ayodhya Bypass Petrol Pump,Bhopal, Madhya Pradesh.

S. No.	TEST	AB1	AB2	AB3	AB4	AB5	AB6	AB7
;l1.	Grams Staining	-	-	-	-	+	-	-
2.	Endospore staining	-	-	-	-	-	-	-
3.	Indole Test	-	-	-	-	-	-	-
4.	Methyl Red Test	-	+	-	-	+	-	+
5.	Voges Proskauer Test	-	-	-	-	-	-	-
6.	Simmon Citrate Test	+	+	+	+	-	+	-
7.	Catalase Test	+	-	-	-	+	+	-
8.	Oxidase Test	-	+	-	+	-	+	+

GRAMS STAINING





4. DISCUSSION

Soil sample was collected from site of petrol pump of Ayodhya Bypass, Bhopal, Madhya Pradesh. The soil was then sieved and serial dilution was prepared to isolate pure colonies of oil degrading bacterial colonies. The pure colonies isolated were named as AB1, AB2, AB3, AB4, AB5, AB6 and AB7. These isolates were then subjected to screening in Bushnell and Haas Media in which 1% of petrol, engine oil and kerosene were added respectively on individual plates as sole carbon source. Isolates AB2, AB3, AB4 and AB6 showed growth in B&H media supplemented individually with petrol, engine oil or kerosene respectively indicating their oil degrading behavior. Isolate AB3 showed maximum growth in comparison to other colonies of bacteria. AB2 showed growth in petrol as well as in kerosene but not in engine oil. Whereas isolate AB1 flourished in petrol but not in engine oil. Whereas isolate AB5 and AB6 showed no growth hence they are not oil degraders. Further screening of these oil degrading bacteria were performed such as Grams staining (Jana et al, 2016), endospore staining and capsular staining. Various biochemical tests were also performed such as IMViC (Indole test, Methyl Red Test, Voges Proskauer test and Citrate test) Catalase test and Oxidase test(Baron et al, 1994, Dubey et al, 2002).

5. CONCLUSION

Petroleum derived contaminants account for one of the most widespread sources of environmental degradation in the industrialized world. Hydrocarbon molecules that make up crude oil and petroleum products are highly noxious to many organisms. Hence their consumption on large scale is dangerous. Trace amount of sulfur and nitrogen compounds are also present in petroleum, which are dangerous on their own and can also react with the environment to construct other secondary poisonous chemicals. To decrease the after affects of such problem, bioremediation can be done using bacterial strains from petrol pump of Ayodhya Bypass Bhopal Madhya Pradesh. This has been done so as to isolate oil degraders which use petroleum as a source of energy. The soil sample was collected and various Biochemical tests were applied. Bushnell Haas Media was used as Screening Media to identify the mineral oil degrading bacteria. Further tests are yet to be done to further study these bacterial isolates and to further exploit their oil degrading capacity. Further studies are also done for bioremediation of oil spilled areas through either in-situ or ex-situ methods that are cost-effective as well as environment friendly.

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