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ISOLATION AND SCREENING OF KEROSENE OIL DEGRADING BACTERIA FROM CONTAMINATED SOIL OF MOTOR MECHANIC WORKSHOP

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ABSTRACT

Pollution of the environment by petroleum products poses a serious threat to all living organisms. Bioremediation is considered as one of the most promising technologies for the removal of polycyclic aromatic hydrocarbons. The present study was investigated to isolate bacteria from contaminated soil of motor mechanic workshop and screened for degradation of kerosene oil by DCPIP test, and gravimetric analysis. The results revealed that THBC, THUBC and THUBC % were found to be 41± 1.7 x 10^5 CFU/g, 14 ± 1x 10^5 CFU/g and 34.15 ± 1.3 %. In the primary screening by DCPIP assay the isolate PHDB3 exhibited highest percentage of degradation 21.42% was identified as Proteus sp. The results of the gravimetric analysis showed that kerosene oil degradation percentage of Proteus sp. was 75% after 30 days of incubation. The Proteus sp. isolated in this study could be a potential agent for effective biodegradation of kerosene oil and other polycyclic aromatic hydrocarbons.

KEY WORDS

Pollution, Bioremediation, Kerosene, DCPIP, Proteus, Hydrocarbons.

1.INTRODUCTION

Environmental pollution is the introduction of contaminants into the natural system that cause harm or discomfort to humans or other living organisms or that damage the environment. Petroleum products consist of extremely complex mixture of aliphatic and aromatic hydrocarbons [1,2]. Bioremediation is emerging as one of the most promising technologies for the removal of hydrocarbons from the environment [3,4]. Now more than 70 genera and 200 strains have been found that can oxidatively biodegrade one or more types of petroleum hydrocarbons [5,6]. In addition, some algae can biodegrade oils as well [7]. Bacteria degrade PAH compounds by an assimilative process where they gain carbon and energy for the growth, which typically leads to mineralization of the compound [8]. Bacteria generally use intracellular dioxygenase enzymes for the degradation of PAHs [9]. The ability to degrade hydrocarbon substrates is exhibited by a wide range of bacteria namely *Pseudomonas* sp. *Bacillus* sp, *Alcaligenes* sp, *Acinetobacter, Flavobacterium, Micrococcus* and *Corynebacterium* sp. [10,11].

Kerosene is a flammable hydrocarbon derived from fractional distillation of petroleum. Its toxicity varied from moderate to high acute toxicity [12]. It is used as solvent in paints, cleaners and pesticides and others etc. Petroleum products such as engine oil, petrol, diesel and kerosene used in various forms in mechanic workshops tend to harden or change the texture of the soil and may affect soil physicochemical properties. Therefore, there

is need to remove them from the environment. Hence the present study was aimed to isolate and identify bacteria from the hydrocarbon contaminated soil of motor vehicle workshop, Perambalur and to evaluate its hydrocarbon degradation potential.

2. MATERIALS AND METHODS

2.1 Collection of Sample

Hydrocarbon contaminated soil samples were collected from the motor mechanic workshop at Perambalur, Tamil Nadu, India. The Perambalur is situated between the latitude of 11.23° N and Longitude 78.88° E. Soil samples were taken up to 5 cm depth in a suitable container and were immediately transported to the laboratory using an ice box and stored at 4°C for further studies.

2.2 Isolation of hydrocarbon degrading bacteria

Soil suspension was prepared by dissolving 1gm of soil samples in 100ml of distilled water and kept in an orbital shaker incubator (120 rpm) at 37°C for 30 mins and serially diluted upto 10^{-5}. 0.1ml from 10^{-5} dilution was transferred to Nutrient agar plate by spread plate technique and incubated at 37°C for 24hrs [13] . Total Heterotrophic bacterial count (THBC) was enumerated as CFU/g. For Total Hydrocarbon Utilizing Bacterial Counts 0.1ml from 10-5 dilution was transferred to Mineral Salt Medium (MSM) agar as described by Balogun and Fagade [14] with Kerosene 1% carbon source and incubated at 37 °C for 5 days. The MSM consist of Basal Salt Medium (BSM) (g/L): K_2HPO4, 1.8; KH_2PO4, 1.2; NH_4CL, 4.0; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; yeast extract, 0.1 and $FeCl_2.4H2O$, 0.05. and trace element (g/L): H_3BO_3, 0.1; $ZnSO_4.7H_2O$, 0.1; $CuSO_4.5H_2O$, 0.05 and $MnSO_4.H_2O$, 0.04 at pH of 6.5.

Percentage of THUBC = (THUBC) / THBC ×100 [15]

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.3 Primary Screening of Hydrocarbon degradation by DCPIP test

Based on the morphology five distinct bacterial isolates (PHDB1 TO PHDB5) were investigated for their potential to utilize hydrocarbon by using DCPIP as redox indicator [16]. Five bacterial isolates 100µl (O.D. 0.5 at 600nm) were inoculated separately into 5ml of MSM medium incorporated with 50 µL of kerosene as hydrocarbon substrate. Then, 40 µL of 2,6-Dichlorophenol Indophenol (DCPIP) was added and incubated at 37°C for 5 days. The medium was observed for decolorization of blue colour. After incubation period the medium was centrifuged to separate the biomass and the absorbance of the supernatant was read at 609 nm using UV–VIS spectrophotometer (Systronics117) with control without inoculum and performed in triplicates [17].

2.4 Characterization of the isolates

The isolate PHDB3 which was selected on primary screening was identified by morphological and biochemical characteristics with reference to the to the Bergey's manual of determinative bacteriology [18].

2.5 Secondary Screening of Hydrocarbon degradation by gravimetric method

The isolate PHDB3 1% inoculum was inoculated onto 50ml of MSM medium with two gram of kerosene oil separately and incubated at room temperature for 30 days. At a regular interval of 10 days to assess residual concentrations of kerosene oil, the content was transferred to a separating funnel and extracted with of 5 ml of Benzene twice and organic phase was centrifuged at low speed for 10 mins. After the evaporation of benzene, the residual kerosene oil was quantified gravimetrically [19]. Degree of biodegradation was calculated as follows:

$$\text{\% of residual kerosene oil} = \frac{\text{Weight of residual kerosene oil}}{\text{Weight of kerosene oil added in the media}} \times 100$$

$$\text{(Hydrocarbon) degradation} = \frac{\text{\% of kerosene weight of kerosene oil degraded}}{\text{weight of kerosene oil added in the media}} \times 100$$

3 RESULTS AND DISCUSSION

3.1 Isolation of hydrocarbon degrading microbial strain

Generally, almost all of the microbial species in the environment have genetic ability to utilize the hydrocarbons as carbon source [20]. This property of microorganisms comes into expression when they are inhabiting hydrocarbon rich environment [21]. Therefore, soil from petroleum oil contaminated sites could be a potential source of microbes with hydrocarbon degradation capability. Hence, soil sample was collected from the hydrocarbon contaminated site motor mechanic workshop Perambalur, Tamilnadu. The total heterotrophic bacterial (THB) count, Total Hydrocarbon Utilizing Bacterial Count were found to be 41± 1.7 x 10^5 CFU/g and 14 ± 1x10^5CFU/g (fig.1). The THUBC% from the hydrocarbon contaminated soil sample was 34.15 ± 1.3 %.

3.2 Primary screening by DCPIP test

Based on the morphology five predominant bacterial isolates PHDB1 to PHDB5 were screened for their potential to utilize Kerosene as carbon source by DCPIP test. The percentage of degradation of the five isolates were 4.3 ± 1.43 %, 1.43 ± 0.28 %, 21.42 ± 0.71%, 10 ± 1.2 % and 7.14 ± 0.72% (Fig.2). The isolates PHDB3 showed highest percentage of degradation 21.42% among the other. Hence PHDB3 was selected for further biodegradation studies. The previous reports also showed that degradation of crude oil by the strain 2-IV was 77% [22]. The degrading potential of bacterial cultures in the complete reduction of DCPIP in 75 hrs for mineral oil, 87 hrs for used oil, 125 hrs semi-synthetic oil, and 138 hrs for synthetic oil have been reported [23] . Mariano et al. [24] also performed the DCPIP assay and reported that the color change from blue to colorless indicated the ability of bacteria to degrade crude oil.

3.3 Characterization of the isolates

The hydrocarbon degrading bacterial isolate PHDB3 was identified by gram staining, motility test, lactose fermentation, IMVIC test, hydrogen sulphide production test and urease test. The results are presented as in the table 2. The isolate was confirmed to be *Proteus* sp.

3.4 Secondary Screening of Hydrocarbon degradation by gravimetric method

The bacterial isolate PHDB3 *Proteus* sp. was screened for hydrocarbon degradation by gravimetric method. The *Proteus* sp. was grown on MSM medium supplemented with 2g kerosene and incubated at room temperature. At a regular interval of 10 days residual oil was extracted and percentage of kerosene oil degradation was calculated by gravimetric method. The results showed percentage of residual oil and percentage of kerosene oil degradation after 10, 20 and30 days of incubation were 70%,30%, 60%, 40% , 25% and 75% respectively (Table 2& fig 3). Jayashree et al. [25] reported that *Pseudomonas* degraded 90.2% of kerosene in 30 days followed by 82.3% of degradation by *Bacillus*, 78.8% of degradation by *Serratia* and 25.5% of oil degraded by *Staphylococcus.* Vinothini *et a*l. [26] studied the degradative potential of *Pseudomonas putida* and *Bacillus cereus* isolated from crude oil contaminated soil samples. They also reported *Pseudomonas putida* more efficiently degrade crude oil than *Bacillus cereus.* Similarly, *Streptococcus* sp. is the potential bacteria for petrol and engine oil degradation, degraded 89.6% of petrol and 84.6 % of engine oil. *Pseudomonas* sp. is the potential bacteria for diesel degradation, degraded 97.8% of diesel [19]. Many other bacterial species also have the potential to be good bioremediation agents as a result of their ability to degrade petroleum wastes and toxic organic solvents [27,28]. Different species of *Bacillus* are well known for their hydrocarbon degradation ability. *Bacillus anthracis* was found to be degrading 67, 57, 72 and 42% of diesel, kerosene, crude oil and used engine oil, respectively after 28 days [29].

Table 1. Identification of the bacterial isolate

Isolate	a	b	c	d	e	f	g	h	i	j
PHDB3	G-ve	Rods	motile	-	+	+	-	-	+	+

a. Gram staining, b.shape, c. motility, d. lactose fermentation, e. Indole, f.MR, g. VP, h. citrate, i. H_2S and j. urease.

Table 2 Hydrocarbon degradation of *Proteus* sp.by gravimetric method

Incubation days	Amount of kerosene oil added (g)	Amount of residual oil (g)	Amount of kerosene oil degraded (g)	% of residual oil	% of kerosene Degradation
10 days	2	1.4	0.6	70	30
20 days	2	1.2	0.8	60	40
30 days	2	0.5	1.5	25	75

Figure 1. Total Heterotrophic bacterial count (THBC), Total Hydrocarbon Utilizing Bacterial Count.

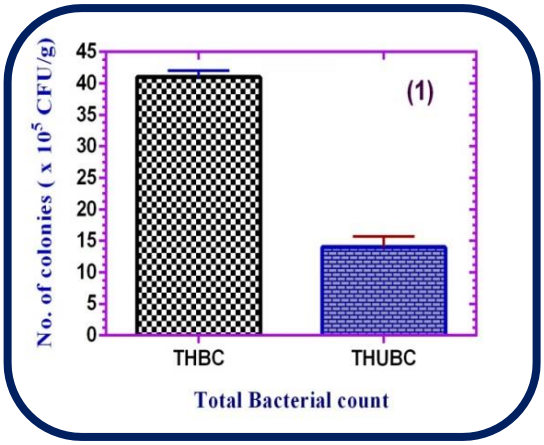


Figure 2 Primary Screening of hydrocarbon degradation by DCPIP test.

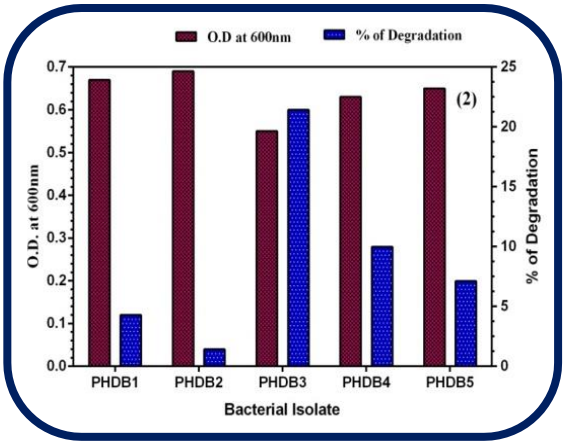
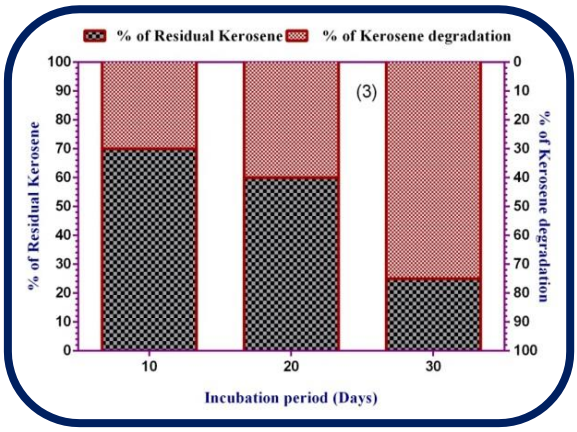


Figure 3. Hydrocarbon degradation of *Proteus* sp.by gravimetric method.



CONCLUSION

Bacteria are diverse and are capable of utilizing pollutants as energy and carbon source to survive in natural environment [30]. In the present study hydrocarbon utilizing bacteria was isolated from soil of motor mechanic workshop by serial dilution technique. Five bacterial isolates were screened for hydrocarbon degradation by DCPIP redox indicator test. The isolate PHDB3 which showed 21.42% degradation was identified as *Proteus* sp. and screened for the degradation of kerosene oil by gravimetric method in which it exhibited 75% of degradation. Hence the use of *Proteus sp*. will be effective and eco-friendly for the degradation of kerosene oil and other polycyclic aromatic hydrocarbons. Further research on *in situ* bioremediation approaches will reveal the potential applications of this bacteria.

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