Screening of hydrocarbon degrading bacteria isolated from oil contaminated soil

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Abstract
The use of indigenous microorganisms in the bioremediation of hydrocarbon pollutants to cleanup environment has become a valuable technique. The aim of the present study was to isolate bacteria from contaminated soil of motor vehicle workshop Perambalur. A total of eleven bacteria isolated was investigated for hydrocarbon tolerance in Bushnell Haas broth containing 1% (w/v) crude oil as sole carbon source. Four bacterial isolates exhibited growth of > 1.0 OD screened for hydrocarbon degradation by DCPIP method. The isolate HDB5 showed 27.5% of biodegradation was identified as Pseudomonas sp and investigated for biodegradation of petrol, diesel and engine oil by gravimetric method for 30 days of incubation revealed 76%, 83% and 69% of degradation. The Pseudomonas sp. isolated could be a potential candidate for the degradation of polycyclic aromatic hydrocarbons.

Keywords: Biodegradation, DCPIP, pseudomonas, hydrocarbons, gravimetric method

1. Introduction
Petroleum-based products are the major source of energy for industry and daily life. Petroleum products consist of extremely complex mixture of aliphatic and aromatic hydrocarbons [1]. Pollution of the environment by petroleum products is an inevitable consequence of oil production, transportation and distribution activities [2, 3]. The discharge of used oil from vehicles or motorcycles is a major source of oil pollution in mechanic workshop and its environments [4]. Various forms of petroleum products petrol, engine oil, diesel, lubricant oils and others are used in mechanic workshop [5]. These products tend to harden or change the texture of the soil, which may have effects on the microbiological and physicochemical properties of the contaminated soil [6]. Therefore, due to the increasing number of mechanic workshops and their indiscriminate disposal of used oil into the environment, there is need to consider options for their removal from the environment due to the environmental hazards associated with petroleum products.

Bioremediation is emerging as one of the most promising technologies for the removal of hydrocarbons from the environment. Microbial remediation of hydrocarbon contaminated site is performed with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil [7]. The first line of defense against oil pollution in the environment is the microbial population [8]. Many bacteria belonging more than 70 genera have the ability to degrade one or more types of petroleum hydrocarbons [9, 10] and gain energy [11]. In the present study hydrocarbon degrading potential of bacteria isolated from oil contaminated soil from motor vehicle workshop was investigated.

2. Materials and Methods
2.1 Isolation of hydrocarbon degrading bacteria
Hydrocarbon contaminated soil samples were collected from the motor vehicle workshops at Elambalur, Perambalur, Tamil Nadu, India. Soil sample 1gm was serially diluted in sterile saline up to 10^{-5} and 1ml of the suspension was transferred to 100ml of BH broth and incubated at 37 °C for one week. After incubation the culture suspension was spreaded on sterile nutrient agar plate and incubated at 37 °C for 48hrs. Predominant bacterial colonies grown on the NA plates were selected and transferred to BH broth containing 1% (w/v) crude oil as sole carbon source and incubated at 37 °C for one week. The growth of the culture was measured by reading optical density at 660nm [12]. The colony which exhibited > 1.0 O.D at 660nm was selected for further hydrocarbon degradation study.
2.2 Screening of hydrocarbon degradation by DCPIP method
Four bacterial isolates (HDB3, HDB5, HDB6 and HDB8) which exhibited growth > 1.0 O.D at 660nm were selected and each bacterial isolates 100μL (O.D. 0.5 at 600nm) were inoculated separately into 5ml of BHM medium incorporated with 50μL of petrol, diesel and engine oil (1:1:1) as hydrocarbon substrate. Then, 40μL of 2, 6-Dichlorophenol Indophenol (DCPIP) was added and incubated at 37 ºC for 5 days. The medium was observed for decolourisation of blue color. After incubation period of five days the medium was centrifuged to separate the biomass and the absorbance of the supernatant was read at 600 nm using ultraviolet-visible (UV-VIS) spectrophotometer (Systronics 117).

2.3 Identification of hydrocarbon degrading bacteria
The isolate HDB5 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 [15].

2.4 Hydrocarbon degradation by gravimetric method
The bacterial isolate selected was screened for degradation of different hydrocarbon petrol, diesel and engine oil. The isolate was grown on BHM medium supplemented with 2g/l of petrol, diesel and engine oil separately and incubated at room temperature for 30days. At a regular interval of 10 days to assess residual concentrations of each oil, the content was transferred to a separating funnel and extracted with 5 ml of Benzene twice and organic phase was centrifuged at low speed for 10 mins. After the evaporation of benzene the amount of each residual oil was measured gravimetrically [16] and the percentage of residual oil and hydrocarbon degradation percentage were calculated [17].

3. Results and Discussion
Bacteria in the petroleum hydrocarbon contaminated site have ability to utilize hydrocarbons as sole carbon source. This property of bacteria comes into expression when they inhabiting hydrocarbon rich environment [18]. Therefore, soil from petroleum oil contaminated sites could be a potential source for the isolation of hydrocarbon degrading bacteria. Hence, hydrocarbon contaminated soil sample was collected from motor vehicle workshops at Perambalur. Predominant morphologically different eleven bacteria were isolated from hydrocarbon contaminated soil sample by serial dilution on nutrient agar plate. The ability of the bacterial isolates to utilize and tolerate hydrocarbon was tested by growing them in BH broth containing crude oil as carbon source and measuring their growth. Among the eleven bacterial isolates four exhibited good growth and optical density was > 1.0 were selected for further study (fig.1).

3.1 Screening of hydrocarbon degradation
Four bacterial isolates HDB3, HDB5, HDB6 and HDB8 selected were screened for degradation of petroleum hydrocarbons by spectrophotometric method using DCPIP as indicator. The results showed that the percentage of degradation of the four isolates were 12.5%, 27.5%, 8.75% and 20% (Fig.2). The isolates HDB5 showed highest percentage of degradation 27.5% among the other four. Hence HDB5 was selected for further biodegradation studies. Buckova et al. [19] screened hydrocarbon degrading efficiency of bacterial isolates by DCPIP test and reported that 28 isolates able to degrade PAHs. Similarly Bidoia et al. [20] reported that the degrading potential of bacterial cultures occurs 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. Bacteria belonging to genera Burkholderia, Pseudomonas, and Serratia have ability to degrade different heavy molecular weight hydrocarbon Premium Motor Spirit (PMS), Dual Purpose Kerosene (DPK) and Low Pour Point Fuel Oil (LPFO) [21]. Different species of Bacillus, Burkholderia, Micrococcus, Proteus, Pseudomonas, have been reported by several authors to utilize hydrocarbon through the oxidation of DCPIP [22-25]. Ability of the isolates to degrade the hydrocarbon was confirmed by the colour change from blue to colourless.

3.2 Identification of the isolate
The hydrocarbon degrading bacterial isolate HDB5 was identified by gram staining, motility test, IMVIC test, oxidase test and catalase test. The results are presented as in the table 1. The isolate was confirmed to be Pseudomonas sp.

3.3 Hydrocarbon degradation by gravimetric analysis
The ability of Pseudomonas sp. to degrade petrol, diesel and engine oil were determined by gravimetric method. The results showed that the percentage of residual oil and biodegradation percentage of petrol, diesel and engine oil after 10, 20 and 30 days of incubation were 75%, 25%, 60%, 40% and 24%, 76% for petrol, 68%,32%,45%,55%,17% and 83% for diesel and 80%,20%,62.5%,37.5%,31% and 69% for engine oil (fig.3). The degrading efficiency of Pseudomonas sp.3 was 85.6% for petrol, 94% for diesel and 70.6% for engine oil [20]. Similarly Kumar and Manjunatha [21] observed that degradation of engine oil and diesel by isolate CSN-1 was 58.21% and 69.64% and 52.45% and 63.50% by isolate OK-6. P. aeruginosa and B. subtilis isolated from crude oil contaminated site exhibited maximum crude oil degrading ability 88.75% and 87.41% by gravimetric analysis [20]. It has been reported in several literatures that Pseudomonas sp. have potential to degrade many different PAHs [29, 33]. However in contrast it was reported that the mixed bacterial culture gave the maximum degradation percentage because there is no single strain of bacteria with the metabolic capacity to degrade all the components found within crude oil [34, 35].

4. Conclusion
In recent days various microbial genera isolated from petroleum contaminated soil suggests that they have pivotal role in the transformation of hydrocarbon [56]. When the bacteria previously exposed to hydrocarbons they exhibit higher biodegradation activity [37]. In view of this hydrocarbon utilizing bacteria Pseudomonas sp isolated from contaminated soil of motor vehicle workshop was screened for biodegradation of hydrocarbon by spectrophotometric DCPIP test exhibited 27.5% of degradation and it was further investigated for degradation of petrol, diesel and engine oil by gravimetric analysis revealed 76%, 83% and 69% of degradation after 30 days of incubation. From this study it can be concluded that under optimized conditions Pseudomonas sp could be an efficient and ecofriendly for degradation of hydrocarbon
Table 1: Identification of the Bacterial isolate

<table>
<thead>
<tr>
<th>Gram stain</th>
<th>Shape</th>
<th>Motility</th>
<th>Indole</th>
<th>MR</th>
<th>VP</th>
<th>Citrate</th>
<th>Oxidase</th>
<th>Catalase</th>
<th>Lactose fermentation</th>
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<tr>
<td>-ve</td>
<td>Rods</td>
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+ Positive, - negative

Fig 1: Screening of Hydrocarbon tolerant bacteria

Fig 2: Screening of Hydrocarbon degradation by DCPIP test

Fig 3: Hydrocarbon degradation by gravimetric method (B-Biodegradation %, R: Residual oil %).

References


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