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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 upto 10^{-5} and1ml of the suspension was transferred to 100ml of BH broth and incubated at 37 ºC for one week. After incubation the culture suspension was spreaded on sterile nutrient agar plate and incubated at 37 ºC 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 ºC 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 and30 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 [26]. Similarly Kumar and Manjunatha [27] 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 [28]. 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 [36]. 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

Gram stain	Shape	Motility	Indole	MR	VP	Citrate	Oxidase	Catalase	Lactose fermentation
-ve	Rods	Motile	-	-	-	+	+	+	-

+ Positive, - negative

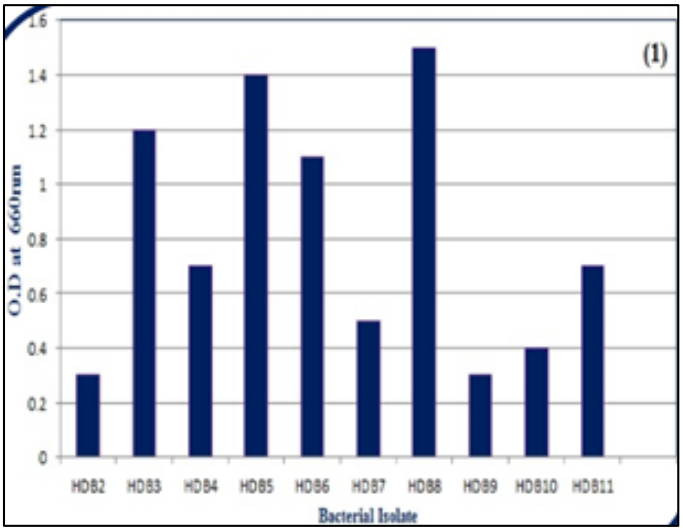

Fig 1: Screening of Hydrocarbon tolerant bacteria

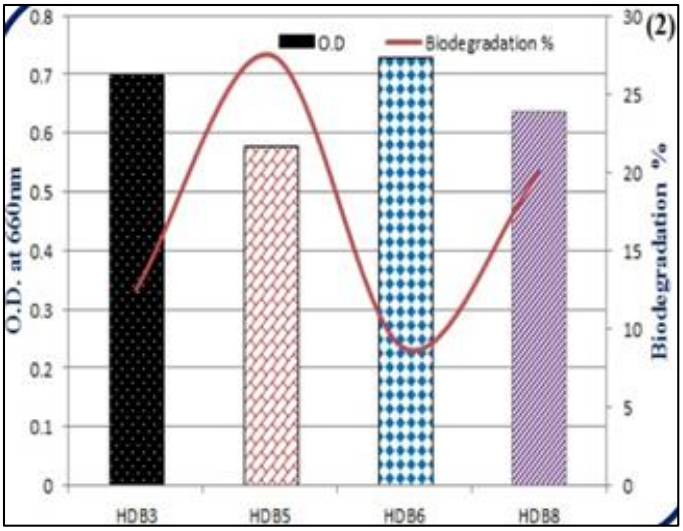

Fig 2: Screening of Hydrocarbon degradation by DCPIP test

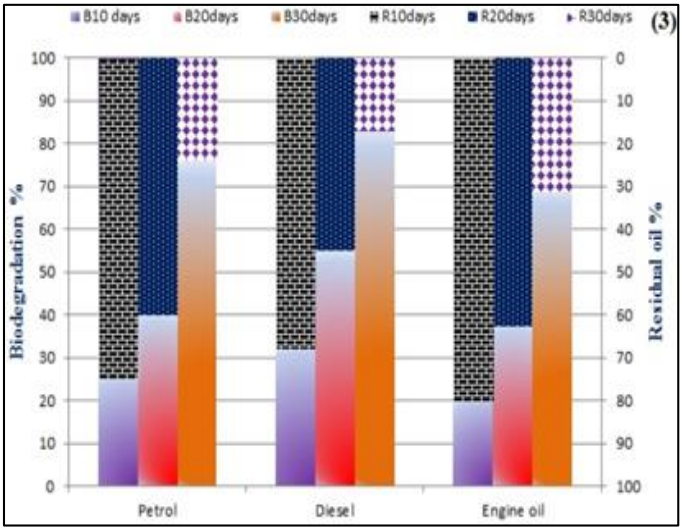

Fig 3: Hydrocarbon degradation by gravimetric method (B-Biodegradation %. R: Residual oil %).

References

1. Jain PK, Gupta VK, Gaur RK, Lowry M. Jaroli DP. Bioremediation of petroleum oil contaminated soil and water. Research journal of environmental toxicology. 2011; 5(1):1-26.
2. Brooijmans RJ, Pastink MI, Siezen RJ. Hydrocarbon-degrading bacteria: the oil-spill clean-up crew. Microbial Biotechnology. 2009; 2(6):587-594.
3. Das N, Chandran P. Microbial degradation of petroleum hydrocarbon contaminants: An overview. Biotechnology research international, 2011. Article ID 941810.
4. Ekhaise FO, Nkwelle J. Microbiological and physicochemical analyses of oil contaminated soil from major motor mechanic workshops in Benin City Metropolis, Edo State, Nigeria. Journal of Applied Sciences and Environmental Management. 2011; 15(4):597-600.
5. Udeani TKC, Obroh AA, Okwuosa CN, Achukwu PU, Azubike N. Isolation of bacteria from mechanic workshops' soil environment contaminated with used engine oil. African Journal of Biotechnology. 2009; 8(22):6301-6303.
6. Eze V, Onwuakor CE, Orok FE. Microbiological and physicochemical characteristics of soil contaminated with used petroleum products in Umuahia, Abia State, Nigeria. Journal of Applied & Environmental Microbiology. 2014; 2(6):281-286.
7. Sebiomo A, Bankole SA, Awosanya AO. Determination of the ability of microorganisms isolated from mechanic soil to utilize lubricating oil as carbon source. Afr. J Microbiol. Res. 2010; 4(21):2257-2264.
8. Youssef M, El-Taweel GE, El-Naggar AY, El-Hawary SE, El-Meleigy MA. Hydrocarbon degrading bacteria as indicator of petroleum pollution in Ismailia Canal, Egypt. World Applied Sciences Journal. 2010; 8(10):1226-1233.
9. Feng L, Wang W, Cheng J, Ren Y, Zhao G. Genome and proteome of long chain alkane degrading *Geobacillus thermodenitrificans* NG80-2 isolated from a deep-subsurface oil reservoir. Proc. Natl. Acad. Sci. USA. 2007; 104:5602-5607.
10. Liang Y, Van Nostrand J, Wang J, Zhang X, Zhou J, Li G. Microarray based functional gene analysis of soil microbial communities during ozonation and biodegradation of crude oil. Chemosphere. 2009; 75:193-199.
11. Haderlein A, Legros R, Ramsay BA. Pyrene mineralization capacity increased with compost maturity. Biodegradation. 2006; 17:293-302.
12. Hanafy AAEME, Anwar Y, Mohamed SA, Al-Garni SMS, Sabir JSM. Isolation and identification of bacterial consortia responsible for degrading oil spills from the coastal area of Yanbu, Saudi Arabia. Biotechnology & Biotechnological Equipment. 2016; 30(1):69-74.
13. Thenmozhi R, Nagasathya A, Thajuddin N. Studies on Biodegradation of Used Engine Oil by Consortium Cultures. Advances in Environmental Biology. 2011; 1051-58.
14. Marzan LW, Sultana T, Hasan MM, Mina SA, Islam MR. Characterization of furnace oil bioremediation potential

of hydrocarbonoclastic bacteria isolated from petroleum contaminated sites of the Sundarbans, Bangladesh. Journal of Genetic Engineering and Biotechnology. 2017; 15(1):103-113.
15. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergeys Manual of Determinative Bacteriology, 9th edn Baltimore: Williams & Wilkins, 1994.
16. Jeya KR, Veerapagu M, Sankaranarayanan A, Sathyapriya R. Isolation and screening of kerosene oil degrading bacteria from contaminated soil of motor mechanic workshop. International journal of Pharmacy and Biological Sciences. 2018; 8(2):683-688.
17. Daxini N, Mistry K. Bio-surfactant assistance in crude oil degradation by halophilic *Bacillus cereus* ND1. Indian Journal of Geo Marine Sciences. 2018; 47(8):1640-1647.
18. Gkorezis P, Daghio M, Franzetti A, Van Hamme JD, Sillen W. The Interaction between Plants and Bacteria in the Remediation of Petroleum Hydrocarbons: An Environmental Perspective. Frontiers in microbiology. 2016; 7:1836.
19. Buckova. M, Puskarova A, Chovanova K, Krakova L, Ferianc PA simple strategy for investigating the diversity and hydrocarbon degradation abilities of cultivable bacteria from contaminated soil. World Journal of Microbiology and Biotechnology. 2013; 29(6):1085-1098.
20. Bidoia E, Montagnolli R, Lopes P. Microbial biodegradation potential of hydrocarbons evaluated by colorimetric technique: A case study. Appl Microbiol Biotechnol. 2010; 7:1277-1288.
21. Balogun S, Ayangbenro A, Kareem S, Sojinu O. Screening for heavy molecular weight hydrocarbon utilizing bacteria from oil impacted, nonoil impacted soil and natural deposits. J Nat. Sci. Engr. Tech. 2013; 12:25-34.
22. Roy S, Hens D, Biswas D, Kumar R. Survey of petroleum degrading bacteria in coastal waters of Sunder ban Biosphere Reserve. World Journal of Microbiology and Biotechnology. 2002; 18:575-581.
23. Joshi RA, Pandey GB. Screening of petroleum degrading bacteria from cow dung, Research Journal of Agricultural Sciences. 2011; 2:69-71.
24. Patil TD, Pawar S, Kamble PN, Thakare SV. Bioremediation of complex hydrocarbons using microbial consortium isolated from diesel oil polluted soil. Der Chemica Sinica. 2012; 3:953-958.
25. Adegbola GM, Eniola KIT, Opasola OA. Isolation and identification of indigenous hydrocarbon tolerant bacteria from soil contaminated with used engine oil in Ogbomoso, Nigeria, Advances in Applied Science Research. 2014; 5(3):420-422.
26. Vignesh R, Arularasan A, Gandhiraj V, Deepika RC. Isolation identification and characterization of potential oil degrading bacteria from oil contaminated sites. International Research Journal of Engineering and Technology. 2016; 3(4):2503-2508.
27. Kumar PSV, Manjunatha BK. Isolation of Diesel, Engine Oil and Crude Oil Degrading Fungi from Oil Contaminated Soil for their Bioremediation Potential. J Agric. Food Nat. Resource. 2017; 1(1):65-70.
28. Chitra S, Hema S. Biodegradation of crude oil by gravimetric analysis. International journal of advanced technology in engineering science. 2014; 2(11):372-376.
29. Bharti P, Irafan M. *Pseudomonas aeruginosa* is present in crude oil contaminated sites of Barmer Region (India). Journal of bioremediation and biodegradation. 2011; 2(5):1-2.
30. Jayashree R, Nithya SE, Rajesh PP, Krishnaraju M. Biodegradation capability of bacterial species isolated from oil contaminated soil. J Academia Indust Res. 2012; 1(3):127-135.
31. Varjani Sunita J, Rana Dolly P, Bateja S, Upasani Vivek N. Original research article isolation and screening for hydrocarbon utilizing bacteria (HUB) from petroleum samples. Int. J. Curr. Microbiol. App. Sci. 2013; 2(4):48-60.
32. Sharma S, Pathak H. Pseudomonas in biodegradation. Int. J Pure Appl. Biosci. 2014; 2(2):213-222.
33. Vinothini C, Sudhakar S, Ravikumar. Biodegradation of petroleum and crude oil by *Pseudomonas putida* and *Bacillus cereus*. Int J Curr Microbiol App Sci. 2015; 4(1):318-329.
34. Bento FM, de Oliveira Camargo FA, Okeke BC, Frankenberger Jr WT. Diversity of bio-surfactant producing microorganisms isolated from soils contaminated with diesel oil. Microbiological research. 2005; 160(3):249-255.
35. Adebusoye SA, Ilori MO, Amund OO, Teniola OD, Olatope SO. Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. World journal of Microbiology and Biotechnology. 2007; 23(8):1149-1159.
36. Farinazleen MG, Raja NZ, Rahman A, Salleh AB, Mahiran B. Biodegradation of hydrocarbons in soil by microbial consortium. International Bio-deterioration and Bio-degradation. 2004; 54:61–67.
37. Al-Hadithi HT, Al-Razzaq EA, Fadhil GF. Bioremediation of polycyclic aromatic hydrocarbons by *Acinetobacter* species isolated from ecological sources. Journal of Environmental Biology. 2017; 38(5):785-789.