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Screening of diesel oil degrading bacteria from petroleum hydrocarbon contaminated soil

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Abstract

Four bacterial strains were isolated from diesel contaminated soil in TVS oil depot, Trichy, Tamil Nadu. From the soil sample four morphologically different bacterial species were isolated and the active strains were *Pseudomonas aeruginosa*, *P. putida*, *Arthobacter* sp and *Bacillus* sp. The diesel degrading efficiency of isolated organisms was tested in BH medium supplemented with diesel and DCPIP for 14 days. Among the four isolated species incubated and monitored in diesel supplemented medium for 14 days, the maximum diesel biodegradability was noticed in *P. putida* sp at fourth day itself and a TPC 64 x 10^5 CFU at a temperature of 30°C and pH 5. This study revealed a qualitative evaluation of potentials of hydrocarbonoclastic bacteria degradation of hydrocarbon. Thereby giving a measureable ability of these groups of bacterial possible use in hydrocarbon impacted soil remediation.

Keywords: *Pseudomonas putida*, petroleum hydrocarbon, DCPIP, Bioremediation.

Introduction

Petroleum based fuels are one of the most prevalent pollutants, particularly in developing countries (Joshi and Pandey, 2011). The widespread nature of petroleum products and their use is strongly associated with anthropogenic discharge of hydrocarbons into the environment (Bidoia *et al*., 2010). Environmental pollution arising from petroleum leakages in storage tanks, spillage during transportation of petroleum products, deliberate discharge of petroleum products and various industrial processes is hazardous to soil and water ecosystems (Geetha *et al*., 2013). This also results in huge disturbances of the abiotic and biotic components of the ecosystem (Okoh, 2006).

A major concern for petroleum hydrocarbon pollution is the presence of heavy compounds such as polycyclic aromatic hydrocarbons (PAHs), asphaltenes and many branched compounds with twenty or more carbon atoms (Bidoia *et al*., 2010).

Biological and non-biological approaches are being used for remediation of oil pollution. Bioremediation is one of the principle strategies for remediation, wherein the pollution can be removed by use of microorganism or by any biological process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition (Olu- Arotiowa *et al*., 2007).

Many species of microorganisms including bacteria, yeasts and fungi obtain both energy and tissue-building material from hydrocarbons. A wide range of studies have dealt with biotransformation, biodegradation and bioremediation of petroleum

hydrocarbons and interested in exploiting crude oil-degrading organisms for environmental cleanup has become central to petroleum microbiology. In the course of biological restoration of hydrocarbon contaminated soil, the main factors that affect the effect of remediation include the pH value, soil moisture, oxygen supply, the nutrient level, bacterial diversity and the temperature, among which the impact of petroleum hydrocarbon degrading bacteria on the effect is critical (Venosa and Xuequing, 2003; Chaillan *et al.*, 2006). The ability of many microorganisms in order to biodegradation of hydrocarbons has been studied by Liangli and Hungchen, (2009); Sarikhani *et al.* (2011); Ebrahimi *et al.* (2012) and Geetha *et al.* (2013). These methods are less expensive and do not introduce additional chemicals to the environment.

Hence in this investigation indigenous bacteria which degrade diesel and hydrocarbons are isolated from diesel contaminated site and screened for their hydrocarbon degradation efficiency. They were further characterized by morphological, cultural and biochemical techniques.

Materials and Methods

The diesel contaminated soil samples were aseptically collected from TVS oil depot, Trichy, stored in a sterilized container. The soil samples were placed in 4ºC and transported to the laboratory immediately and maintained at 4ºC until microbial analysis.

Diesel sample was collected from petrol bunk and the indicator 2, 6-dichlorophenol indophenol (DCPIP) used for the study was obtained from Lobachemie.

Bushnell-Hass (BH) medium (Atlas, 1995) was used as the enrichment media with 1% (v/v) diesel as the sole carbon source. 10 gm of the contaminated soil was added to 100 ml of BH medium and incubated at 30ºC at 170 rpm. After 5 days of incubation, loop full of inoculum from Bushnell-Hass medium was streaked onto the nutrient agar plate and incubated at 30ºC for 72 hours. Colonies with different morphological appearance were selected and purified in nutrient agar medium and were transferred to nutrient agar slants and stored for identification and further experimental studies. The cultures obtained were sub cultured monthly in nutrient agar medium.

The bacteria isolated were identified based on physical characterization and the biochemical tests outlined in Bergey s manual of determination bacteriology (Buchanan and Warwick, 1974). Morphologically distinct colonies were isolated and purified by replicating on the same solid medium to obtain pure cultures.

The identified ten bacterial isolates were tested for their ability of diesel degradation and to determine the most efficient degrader. Each of the four bacterial cultures was inoculated to 100 ml of nutrient broth medium and incubated to 37ºC for 24 hours at 150 rpm. The cultures were used as inoculum for the hydrocarbon degradation. 100ml of Bushnell-Hass (BH) medium was prepared with 1% diesel as sole carbon source. DCPIP at a concentration of 20 mg/l of the medium was used. The medium was sterilized at 121ºC for 15 minutes. 1 ml each of the inoculum was added to each set of experiment and incubated at 150 rpm for 14 days. Control was also maintained.

The initial and final growth was determined by pour plate method using nutrient agar. The plates were incubated at 37ºC for 48 hours. After the incubation period, colonies were counted and total microbial count/ml was calculated.

Diesel degradation was determined by the ability of microorganisms to utilize the diesel as sole carbon source which indicate the change in colour of DCPIP from blue to colourless. The indicator, when oxidized was blue and reduced was transparent. The reaction was observed visually till the end of incubation and also spectrophotometrically (600nm) at an interval of 4 days using Shimadzu model UV-3600 visible spectrophotometer according to Bharathi and Vasudevan (2001).

Results and Discussion

Microorganisms are diverse and are capable of utilizing contaminants as energy and carbon source to survive in natural environment (Singh *et al.*, 2010). Elimination of wide ranges of pollutants from the natural environment is required to enhance a sustainable development of the ecosystem with low ecological impacts (Selvakumar *et al*., 2014). Microorganisms play a major role in the removal of contaminants taking advantage of their versatile catabolic activity to degrade or convert such compound to harmless substances.

In the present study four diesel degrading bacterial species *Pseudomonas aeruginosa*, *P. putida*, *Arthobacter* sp and *Bacillus* sp were identified in the diesel contaminated sites (Table 1). Similarly Bhasheer *et al.* (2014) reported the hydrocarbon

degrading efficiency of some isolated bacteria from oil polluted sites, such as *Acinetobacter* sp, *Moraxella* sp, *Bacillus* sp, *Vibrio* sp and *Alcaligenes* sp. This agrees with the work of Youssef *et al*. (2010) who reported that the levels of hydrocarbon present in a contaminated site represent a nutrient rich environment where less-recalcitrant organic carbon may be limiting.

Table 1: Biochemical identification of bacterial isolates

S.no	Test	Strain-1	Strain -2	Strain -3	Strain -4
1	Oxidase	+	--	+	+
2	Catalase	+	+	+	+
3	Carbohydrate	+	+	--	+
4	DNA	--	--	--	--
5	Gelatin	--	--	+/--	+
6	Urea	--	--	+	--
7	Citrate	+	+	+	+
8	MrVp	+	--	+/--	+/--
9	Nitrate	--	+	--	+
10	Starch	+	+	--	+
11	Indole	--	--	--	+
12	Grams stain	+	+	--	--
13	Motility	+	+	+	+
14	Spore staining	+	NA	NA	NA
15	Strains identified	*Bacillus* sp.	*Arthobacter* sp.	*Pseudomonas putida*	*Pseudomonas aeruginoa*

Note: NA-Not Applicable; + Positive; - Negative

The degrading efficiency of isolated organisms was studied in BH medium supplemented with diesel and DCPIP. The experimental set up was kept for 14 days of incubation, in order to study the colour change of DCPIP, which is blue in colour and its oxidized form and colourless in its reduced form. This is due to reduction of the indicator by the oxidized product of hydrocarbon degradation which supports the facts that the isolates are potential hydrocarbon oxidizers (Selvakumar *et al*., 2014). Species of *Pseudomonas, Bacillus, Micrococcus* and *Proteus* isolated from hydrocarbon contaminated site have been found by several authors to utilize hydrocarbon through oxidation of DCPIP (Roy *et al.*, 2002; Joshi and Pandey, 2011; Patil *et al.*, 2013; Adegbola *et al.*, 2014). The oxidation of DCPIP supports the facts that the isolates were potential hydrocarbon degraders. Absorbance at a wavelength of 600 nm was monitored for the organisms because a peak in absorbance was observed at 600 nm as reported by Yoshida *et al*. (2001).

Among the four isolated species incubated and monitored in diesel supplemented medium for 14 days, the maximum diesel biodegradability was noticed in *P. putida* sp at fourth day itself and a TPC 64 x 10^5 CFU at a temperature of 30°C and pH 5. The degradation was also checked spectrophotometrically at an OD of 660nm; where in the OD reading was found to decrease in all the causes till the end of incubation period (Table 2). *Pseudomonas aeruginosa* and *Arthobacter sp* showed the diesel biodegradability at day 7 of incubation with a TPC of 61 x10^5 CFU and 55 x 10^5 CFU, respectively. While *Bacillus* sp showed diesel biodegradability at day 10^{th} of incubation of TPC 54x10^5 CFU. All these were carried at a temperature of 30^0C and pH 5 (Table 3).

Table 2: Biodegradation of diesel by isolated organisms

S. No	Day of incubation	OD at 660nm			
		Pseudomonas aeruginosa	***P. putida***	***Arthobacter* sp**	***Bacillus* sp**
1	4th day	0.424	0.454	0.395	0.305
2	7th day	0.402	0.426	0.356	0.294
3	11th day	0.373	0.408	0.321	0.282
4	14th day	0.311	0.386	0.295	0.260

Based on oil utilization capacity, *P. putida* is the most active hydrocarbon utilizer in crude oil. Previous observations have identified the *Pseudomonas* genus as the most efficient among hydrocarbon degrading microorganisms (Banat *et al*., 2000; Saadoun, 2002). Isolation and screening of microorganisms for their efficiency in utilization of hydrocarbons before field trials is important in bioremediation process and the development of efficient techniques is an important tool in recommending different approaches for bioremediation of hydrocarbon polluted areas (Varjani *et al.,* 2013).

Table.3. Bacterial growth determination during biodegradation of diesel

S. No	Name of the organism	TPC/ml		
		Initial	**Final**	**Final day of incubation**
1	*Pseudomonas aeruginosa*	18 x10^5 CFU	61 X10^5 CFU	7th day
2	*P. putida*	20 x10^5 CFU	64 X 10^5 CFU	4th day
3	*Arthobacter* sp.	15 x10^5 CFU	55 X 10^5 CFU	7th day
4	*Bacillus* sp.	12 x10^5 CFU	54 X10^5 CFU	10th day

This study revealed a qualitative evaluation of potentials of hydrocarbonoclastic bacteria degradation of hydrocarbon. Thereby giving a measureable ability of these groups of bacterial possible use in hydrocarbon impacted soil remediation

References

Adegbola G.M., Eniola K.I.T. and Opasola O.A. 2014. Isolation and identification of indigenous hydrocarbon tolerant bacteria from soil contaminated with used engine oil in Ogbomoso, Nigeria, *Advances in Applied Science Research*, **5**(3), 420-422.

Atlas, R.M. 1995. Bioremediation of petroleum pollutants. *Int. Biodegrad.Biodegrad.*, 35: 317 - 327.

Banat J.M., Makkar R.S. and Cameotra S.S. 2000. Potential commercial applications of microbial surfactants, *Applied Microbiology and Biotechnology*, **53**, 495-508.

Bharathi, S., Vasudevan, N. 2001. Utilization of petroleum hydrocarbons by *Pseudomonas fluorescens* isolated from petroleum contaminated soil. *Environ. Int.,* 26: 413 413.

Bhasheer, S.K., S.Umavathi*, D.Banupriya, M.Thangavel and Y.Thangam. 2014. Diversity of diesel degrading bacteria from a hydrocarbon contaminated soil. *Int.J.Curr.Microbiol.App.Sci.,* 3(11) 363-369.

Bidoia E.D., Montagnolli R.N. and Lopes P.R.M. 2010. Microbial biodegradation potential of hydrocarbons evaluated by colorimetric technique: a case study, In: Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Mendez-Vilas A. (Ed.), FORMATEX, Badajoz, Spain.

Buchanan JB and Warwick RM. 1974. An estimate of benthic macro faunal production in the offshore mud of the Northumberland coast. *Journal of the Marine Biological Association*, UK. 54: 197–222.

Chaillan, F., Chaineau, C.H., Point, V. 2006. Factors inhibiting bioremediation of soil contaminated with weathered oils and drill cuttings. *J. Environ. Poll.* 144(1): 255 265.

Ebrahimi, M., Fallah, R., Sarikhani, M.R., Taheri, M.T. 2012. Assessment of biodegradation efficiency of some isolated bacteria from oil contaminated sites in solid and liquid media containing oil-compounds. *Int. Res. J. Appl. Basic Sci.*, 3(1): 138 - 147.

Geetha S.J., Sanket J.J. and Shailesh K. 2013. Isolation and characterization of hydrocarbon degrading bacterial isolate from oil contaminated sites, *APCBEE Procedia*, **5**, 237 – 241.

Joshi R.A. and Pandey G.B**.** 2011. Screening of petroleum degrading bacteria from cow dung, *Research Journal of Agricultural Sciences*, **2**, 69-71.

Liangli, J., Hungchen, B. 2009. Surfactant mediated biodegradation of polycyclic aromatic hydrocarbons. *Materials.*, 2: 76 94.

Okoh A.I. (2006), Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants, *Biotechnology and Molecular Biology Review*, **1**, 38-50.

Olu-Arotiowa, O.A., Aremu, M.O., Alade, A.O. 2007. Ex-situ bioremediation of diesel polluted waste water in trophical hot climate. *Asia J. Info. Technol.*, 6(9): 961 963.

Patil T.D., Pawar S., Kamble P.N. and Thakare S.V. 2012. Bioremediation of complex hydrocarbons using microbial consortium isolated from diesel oil polluted soil, *Der Chemica Sinica*, **3**, 953-958.

Roy S., Hens D., Biswas D. and Kumar R. 2002. Survey of petroleumdegrading bacteria in coastal waters of Sunderban Biosphere Reserve, *World Journal of Microbiology and Biotechnology*, **18**, 575-581.

Saadoun I. 2002. Isolation and characterization of bacteria from crude petroleum oil contaminated soil and their potential to degrade diesel fuel, *Journal of Basic Microbiology*, **42**, 420-428.

Sarikhani, M.R., Ebrahimi, M., Fallah, A.R. 2011. Comparison of hydrocarbon-degradation by isolates of *Pseudomonas fluorescens* Chao, *P. Putida* and *Pantoea agglomerans* P5 in presence of gas oil, toluene and phenanthrene. 4th International Conference of Environmental Industrial and Applied Microbiology, 14-16 September, Malaga, Spain.

Selvakumar S., Sekar P., Rajakumar S. and Ayyasamy P.M. (2014), Rapid screening of crude oil degrading bacteria isolated from oil contaminated areas, *The Scitech Journal*, **1**, 24-27.

Singh C. and Lin J. 2010. Bioagumentation efficiency of diesel degradation by *Bacillus pumilus* JL and *Acinatobacter calcoacetics* LT in contaminated soils, *African Journal of Biotechnology*, **9**(41), 6881-6888.

Venosa, A.D., Xueqing, Z. 2003. Biodegradation of crude oil contaminated marine shorelines and freshwater wetlands. *J. Spill Sci.Technol. Bullet.* 8(2): 163 178.

Youssef M., El-Taweel G.E. and El-Naggar A.Y. 2010. Hydrocarbon degrading bacteria as indicator of petroleum pollution in Ismailia canal, Egypt, *World Applied Sciences Journal*, **8**(10), 1226-1233.

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