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RESEARCH ARTICLE

ISOLATION AND IDENTIFICATION OF POLYCYCLIC AROMATIC HYDROCARBON DEGRADING MICROBES AND IT'S RELATIONSHIP WITH LIGNINOLYTIC POTENTIAL

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ABSTRACT

Petroleum hydrocarbons are widespread in the environment and they have adverse effect on human beings. They are difficult to remove from environment by natural processes. In order to mitigate this problem, the efficiency of microorganisms has been evaluated for the degradation of poly cyclic aromatic hydrocarbons (PAHs). The soil samples were collected from three different places *viz., Kolli* hills, PAHs contaminated soil and garden soil. Bacterial and fungal strains were isolated from the collected soil samples and the physical parameters of soil were analysed. The ligninolytic activity of the isolated microorganisms were estimated and identified. Fifteen bacterial and seven fungal strains were isolated. Of them, seven bacteria and two fungi showed maximum ligninolytic activity.

INTRODUCTION

Polycyclic aromatic hydrocarbons are common environmental pollutants with toxic, genotoxic, mutagenic and carcinogenic properties (Mastrangela *et al.,* 1997). They mainly occur in petroleum industry activities (Blumer, 1976), oil spills because of pipeline breakages and tanks leakages. Storage and transportation accidents can be considered as the most frequent causes of hydrocarbon release, included PAHs in to soils (Bossert *et al.,* 1984). Chemical, physical as well as biological methods are used for PAHs degradation. Of these, biological methods are favored because of their good results and low costs (Wu *et al*., 2010). Bacteria and fungi play a vital role in hydrocarbon degradation due to their ability to utilize polycyclic hydrocarbons to satisfy their cell growth and energy needs. Bioremediation makes use of indigenous oil degrading microorganisms by enhancing and fertilizing them in their natural habitats. Microorganisms degrade these compounds by using enzymes in their metabolism and can be useful in cleaning up contaminated sites (Atlas, 1981; Atlas and Bartha, 1992; Steffan *et al*., 1997; Alexander, 1999). This work intends to screen the ligninolytic activity of some bacterial and fungal isolates in degradation of PAH compounds in the petroleum contaminated soil sample.

MATERIALS AND METHODS

Sampling site and determination of soil PAH concentration

Three different soil samples were collected from three different locations *Viz., kolli* hills, Namakkal district, PAHs contaminated soil from the site of refining process of Chennai Petroleum Corporation Limited, Manali and Garden soil from Puthanampatti village, Tiruchirappalli Dt. Tamilnadu. Soil was sampled from the 10 cm upper layer, sieved to about 3 mm and stored in closed polythene bags at 4^0C. The physical parameters were analysed by the methods suggested by Tandon (2005) and determination of soil texture (by Feel Method).

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Enrichment and isolation of bacteria

Crude oil degrading microorganisms were isolated by using the following enrichment procedure: 10 g of PAHs contaminated soil was transferred in to 100 ml of basal salt medium (BSM). The media enrichment with 1% (v/v) used crude oil was used as the sole carbon source to isolate crude oil-degrading bacteria and fungus. The flasks were shaken for 3 days at 180 rpm in a rotary shaker at 28°C. After 3days of incubation 10 ml of the supernatant was transferred in to 100 ml of fresh BSM. Then the culture was transferred to the fresh medium and it was repeated twice. At the third transfer, 0.1 ml sample of the recovered medium was streaked on to nutrient agar plates and the plates were incubated at 28°C at room temperature. The pure culture was streaked on to nutrient agar slants and they were incubated at 28°C. After the growth occurs the pure culture was kept at 4°C until further use (Rojas- Avelizapa *et al*., 1999).

Screening of ligninolytic activity in bacteria and fungus

The ligninolytic activity was tested using tannic acid medium (Thormann *et al.,* 2002). A 5 mm mycelia disc from a 5 day old PDA culture was placed in the centre of the plate and incubated in the dark at room temperature. After the incubation period, formation of a dark brown pigment surrounding the mycelia disc was observed and the same method was followed in bacteria to screen the ligninolytic activity.

Isolation of PAH degrading Microorganisms

Isolates were plated on BSM and sprayed with a 2% PAH stock solution in acetone. Presumptive PAH users were distinguished by formation of a clearing zone **(**Ensley *et al.,* 1983**)**. The organism producing high clearing zone was selected for further experiments.

Identification of bacteria

The pure culture of bacterial strain was identified using microscopic and biochemical methods (Cappuccino and Sherman, 2006).

Identification of fungi

The isolated pure cultures of fungal strains were identified, based on the fungal spores and conidia. Fungal strains were picked, and observed under microscope by staining with lacto phenol cotton blue (LPCB) (Nishida *et al.,* 1988;Kim *et al*., 2000; Samson *et al*., 2004). The isolates were identified through microscopic analysis of the mycelium with spore and were stored in PDA slants at 4˚C.

RESULTS AND DISCUSSION

Physical parameters of the PAH contaminated soil collected from Chennai Petroleum Corporation Limited, Manali, Kolli hill soil and Garden soil from Puthanampatti are presented in Figure 1. The mean values of pH, temperature and soil texture were 5.8, 29 and 10 (PAH contaminated soil); 5.4, 24 and 10 (Kolli hills) and 5.7, 28 and 9 (Garden soil) respectively. Fifteen bacterial and seven fungal strains were isolated from PAH contaminated soil, Kolli hills soil and garden soil (Figure 2). The results of the ligninolytic activity of the screened microbes are given in Figure 2. They are indicated as KB 1, KB 2, KB 4, GB 2, PAHB 2, PAHB 3, PAHB 5 and PAHB 6 (bacterial isolates) and KF 2, GF 1, PAHF 1, PAHF 2 and PAHF 4 (fungal strains) as and they did not show any zone of clearance. The other seven bacterial and two fungal strains showed zone of clearance. The results of biochemical characteristics of bacterial isolates have

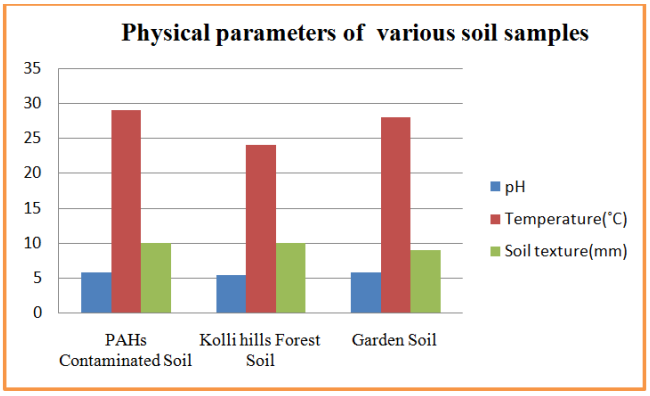

Figure 1. Physical parameters of various soil samples (PAHs contaminated soil, *Kolli* hills forest soil, garden soil)

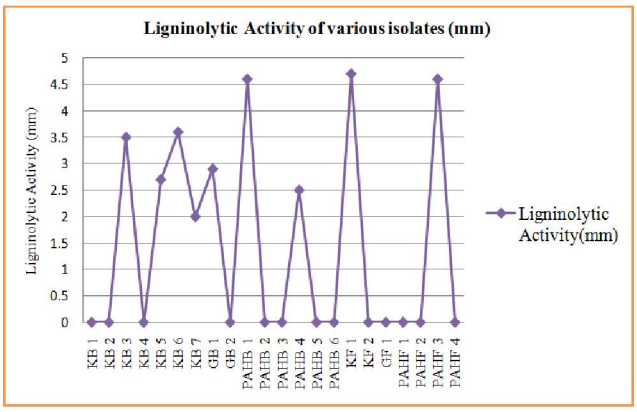

Figure 2. The ligninolytic activity of various isolates

been tabulated in Table 1 and they are indicated as KB 3, KB 5, KB 6, KB 7, GB 1 and PAHB 1 are identified as *Bacillus licheniformis, Acinetobacter* sp., *Bacillus cirulans, Klebsella pnemoniae, Bacillus* sp, and *Pseudomonas* sp. and Table 2 represents the fungal isolates KF1 and PAHF3 and were identified as *Trichoderma* sp. and *Aspergillus* sp *Trichoderma* using a combination of morphological characteristics as the identification based on morphological characteristics (Kim *et al*., 2000). Pure cultures were characterized for the various staining and biochemical activities and were compared with Bergey's manual done earlier by Udeani *et al*., 2009. The isolates showing maximum oil degradation abilities was gram positive, *Bacillus* sp. and catalase positive. Few studies (Annweiller *et al*., 2000; Ijah and Antai, 2003; Sorkhoh *et al*., 1993; Korda *et al.,* 1997; Rahman *et al.,* 2002; Sepahi *et al.,* 2008) have reported on the role of *Bacillus* sp. in hydrocarbon bioremediation; although there are several reports on bioremediation of pollutants by the action of *Bacillus* sp. occurring in extreme environments. Ijah and Antai, (2003) reported *Bacillus* sp. as being the predominant isolate of all the crude oil utilizing bacteria characterized from highly polluted soil samples (30 and 40% crude oil). It has been postulated that *Bacillus* sp. are more tolerant to high levels of hydrocarbons in soil due to their resistant endospores. There is growing evidence that isolates belonging to the *Bacillus sp.* could be effective in clearing oil spills (Ghazali *et al*., 2004).

Table 1. Biochemical characterizations of isolated microbes

S. No	Bio chemical parameters	KB 3	KB 5	KB 6	KB 7	GB 1	PAHB 1
1	Gram staining	G +ve	G -ve	G +ve	G -ve	G +ve	G –ve
2	Colony Morphology	Rough	muciod	Rough	Mucoid	Mucoid	Small moist
3	Motility	+	+	-	-	+	+
4	Oxidase	+	-	-	-	-	+
5	Catalase	-	+	-	+	+	+
6	Indole	-	-	-	-	-	-
7	Citrate	-	+	-	+	+	+
8	Urease	-	-	-	-	-	-
9	Unilin	-	-	-	-	-	-
10	Mannitol	-	-	-	-	-	-
11	Arabinose	+	+	+	-	+	-
12	Starch hydrolysis	+	-	+	-	+	-
13	Skim Milk	+	-	+	-	+	-
14	Arginine	+	+	+	+	+	+
	Identified Microbes	*Bacillus Licheniformis*	*Acinetobacter* sp.	*Bacillus cirulans*	*Klebsiella pneumoniae*	*Bacillus* sp.	*Pseudomonas* sp.

Table 2. Morphology Characterization of identified fungi

S.No.	Morphology Characterstics	KF1	PAHF 3
1	Conidia:		
	1) Shape	Globular	Ellipsodal
	2) Colour	Black	Pale green
2	Hyphae:		
	1) Type	Penicilliate	Tree branches
	Identified Fungi	*Aspergillus* sp.	*Trichoderma* sp.

Pseudomonas sp., is the most common bacterial hydrocarbon degrader (Rusansky *et al*., 1987; Kiyohara *et al*., 1992; Jonhson *et al*., 1996; Barathi and Vasudevan, 2001; Pokethitiyook *et al.*, 2003; Van Hamme *et al.*, 2003) widespread in nature and can degrade a wide range of xenobiotics. The results of the present study are in accordance with these reports and effectively prove that *Pseudomonas* sp., is one of the effective degrader of lignin and it showed 4.6mm of zone of clearance on BSM. Hydrocarbon biodegradation by fungal isolates were identified as *Aspergillus* sp*, Trichoderma* sp and *Mucor* sp. Some of these organisms have earlier been reported as hydrocarbon bio degraders by (April *et al.,* 2000; Oudot *et al.,* 1993).

Conclusion

The significance of ligninolytic enzymes lies in the fact that they can contribute in breaking down recalcitrant compounds in PAHs that may help in reduction of some environmental pollution problems. Based on these, higher biodegradation efficiency was exhibited by *Pseudomonas* sp*, Trichoderma* sp. and *Aspergillus* sp. proved that bacteria and fungi to be better hydrocarbon degraders. Thus, they can be effectively utilized for the degradation of oil polluted farm lands especially those located within the surrounding area of the isolation soil sites.

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