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Enzymatic bioremediation of polyaromatic hydrocarbons by fungal consortia enriched from petroleum contaminated soil and oil seeds

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Abstract

The present study focuses on fungal strains capable of secreting extracellular enzymes by utilizing hydrocarbons present in the contaminated soil. Fungal strains were enriched from petroleum hydrocarbons contaminated soil samples collected from Chennai city, India. The potential fungi were isolated and screened for their enzyme secretion such as lipase, laccase, peroxidase and protease and also evaluated fungal enzyme mediated PAHs degradation. Total, 21 potential PAHs degrading fungi were isolated from PAHs contaminated soil, which belongs to 9 genera such as *Aspergillus, Curvularia, Drechslera, Fusarium, Lasiodiplodia, Mucor, Penicillium, Rhizopus, Trichoderma,* and two oilseed-associated fungal genera such as *Colletotrichum* and *Lasiodiplodia were* used to test their efficacy in degradation of PAHs in polluted soil. Maximum lipase production was obtained with *P. chrysogenum, M. racemosus* and *L. theobromae* VBE1 under optimized cultural condition, which utilized PAHs in contaminated soil as sole carbon source. Fungal strains, *P. chrysogenum, M. racemosus* and *L. theobromae VBE1, as consortia,* used in the present study were capable of degrading branched alkane isoprenoids such as pristine (C17) and pyrene (C18) present in PAHs contaminated soil with high lipase production. The fungal consortia acts as potential candidate for bioremediation of PAHs contaminated environments.

Key words

Bioremediation, Fungal enzymes, *L. theobromae* VBE1, Lipase, PAHs-contaminated soil

Introduction

Polyaromatic hydrocarbons (PAHs), present in the environment due to anthropogenic activities, are carcinogenic and mutagenic in nature (Juhasz and Naidu, 2000; Ijah, 2003). On the other end, environmental concern has increased and potential techniques have been developed and adopted to overcome this issue. Bioremediation plays a vital role among various treatment techniques for PAHs removal. Biodegradation of hydrocarbons was achieved either by bacteria (Arulazhagan and Vasudevan 2011; Hamamura *et al.,* 2013), fungi (Hadibarata *et al.,* 2009; Cerniglia and Sutherland 2010) or algae (Munoz *et al.,* 2003; Chan *et al.,* 2006). Fungus is considered as an efficient candidate for potential degradation of polyaromatic hydrocarbons. Environmental pollution from petroleum and petrochemical derivatives is recognized as one of the most serious problem in developing countries due to their growing population (Ijah, 2003). Generally, PAHs contamination occurs by crude and refined oil which arises from tanker accidents, refinery effluents, municipal and industrial discharge from pipelines and offshore productions, waste oil from two wheeler and four wheeler service stations, which causes pollution (Uzoamaka *et al.,* 2009). However, filamentous fungus has ability to grow on wide spectrum of substrates by secreting extracellular hydrolytic enzymes, even capable of growing under ambient environment (Juhasz and Naidu, 2000). Enzymatic approach involved in biodegradation of undesirable toxic complex chemical into human welfare compounds by removing some functional groups either *in-vivo* or *in-vitro* process. Bioremediation involves either indigenous or exogenous microbial population which is known to be efficient degraders in contaminated site. Fungal bioremediation have been literally showing little attention in past

two decades. Fungi have advantages over bacteria because of their fungal hyphae and potential hydrolytic enzymes, which can penetrate and degrade the hydrocarbons contaminated soil (Balaji and Ebenezer, 2008; Messias *et al.,* 2009; Venkatesa gowda *et al.,* 2012).

Fungal enzymes especially, oxidoreductases, laccase and peroxidases have prominent application in removal of PAHs contaminants either in fresh, marine water or terrestrial. Previous studies on degradation of polycyclic aromatic hydrocarbons by fungal strains are: cold-adapted bacteria and yeast (Margesin *et al*., 2003), effects of surfactants (Chen *et al*., 2006), imperfective fungi *Penicillium sp*. (Leitao, 2009), presence of non-ionic surfactants (Chang *et al*., 2010), white-rot fungus *Anthracophyllum discolor* (Acevedo *et al*., 2011), potential of mushroom cultivation substrate (Li *et al*., 2012). However, lipases have been significantly less studied on bioremediation of PAHs (Haritash and Kaushik, 2009). Fungi isolated from oil spill environment can reduce oil pollution (Chaillan *et al.,* 2004; Das and Chandran 2011). Nevertheless, interest on fungi receives a considerable attention for bioremediation of hydrocarbon contaminated sites associated fungi for enzyme secretion (to remove hydrocarbons from the environment). The main objective of the present study was to isolate and identify indigenous fungal flora of PAHs contaminated soil and evaluate the biodegradation efficiency of potential isolates by enzyme production.

Materials and Methods

Culture and identification of fungal strains : Soil samples were collected from grounds of ten automobile garages located at different places in Chennai city (India) where spillage of diesel, kerosene, petrol, grease and motor oil had occurred over a period of 20 years. The soil samples were serial-diluted and plated (pour plate method) in Potato-Dextrose-Agar (PDA) containing streptomycin (25 µg ml^{-1}). Fungal colonies were allowed to grow at 25°C for 7 days and recorded the number of colony-forming units (CFU). Individual colonies of fungi were isolated and maintained on PDA slants.

The fungal strains were identified on the basis of spore morphology down to species level by standard mycological monographs (Nagraj 1993). Sporulation was induced in fungal cultures growing on PDA plates by exposure to daylight for 12hr day/night cycle at 25°C. Spores were collected for photo micrography by irrigating plates with sterile water and removing samples for examination. Altogether, 21 different morphological fungal isolates were obtained and identified (Table 1).

Cultivation of fungal isolates : Fungal inoculum was prepared by growing isolated fungi on PDA in Petri dishes (9-cm diameter) for 5 days until a rich mycelial mat covered the agar plate. Discs of agar plugs were taken from the growing edge of fungal-colonized

Table 1 : Growth of hydrocarbon degrading fungal strains from different contaminated sites

Name of fungal isolates	PAHs-contaminated sites (cfu g^{-1})*									
	AD	AN	GD	KB	MP	PC	PM	SP	TB	TN
Alternaria alternata	0.7	-	-	-	1.5	0.03	-	0.4	-	1.2
Aspergillus. fumigatus	-	1.2	1.4	1.2	1.0	0.09	0.8	0.7	0.27	0.6
A. ochraceus	-	0.7	1.0	-	0.3	0.04	1.0	1.0	2.3	0.09
A. niger	1.2	1.4	3.4	-	0.5	-	0.2	-	1.7	0.06
A. parasitics	0.12	0.13	2.0	-	-	-	0.04	0.4	0.7	1.6
A. tamarii	2.1	1.3	-	0.5	2.6	0.043	-	-	2.0	0.5
A. terreus	0.3	-	-	-	0.9	0.07	2.3	1.2	1.0	-
A. versicolor	-	2.0	0.09	-	-	-	-	0.3	1.2	0.5
Colletotrichum falcatum	1.6	0.09	-	0.4	-	-	1.2	-	-	-
Curvularia lunata	0.06	-	-	0.8	1.2	-	-	-	1.2	-
Drechslera halodes	1.8	1.1	0.04	0.6	0.8	-	-	1.3	-	-
Fusarium oxysporum	1.0	1.3	-	-	2.4	4.7	-	-	-	2.5
Lasiodiplodia theobromae	-	-	-	-	-	-	-	-	1.2	-
Mucor racemosus	-	0.9	6.3	0.2	-	0.08	0.01	-	0.2	0.3
Penicillium chrysogenum	1.9	2.8	5.3	1.9	-	-	1.5	-	0.8	3.0
P. citrinum	0.06	0.8	0.3	0.8	-	-	1.7	-	-	0.09
P. variable	1.2	0.05	-	-	1.0	-	-	2.5	2.7	-
P. variotii	1.2	1.0	-	-	‘-	-	-	2.2	-	-
Rhizopus stolonifer	0.2	-	-	0.5	1.0	-	1.4	-	-	-
Trichoderma harzianum	0.07	-	-	1.3	0.3	-	0.5	-	1.0	-
T. viride	-	-	-	1.8	2.3	-	1.2	1.5	-	-

*Soil samples were collected from the grounds of several automobile stations/garages located at different places within the city of Chennai (Tamil Nadu, India): Adayar (AD), Anna Nager (AN), Guindy (GD), Kelambakkam (KB), Mylapore (MP), Paris Corner (PC), Poonthamali (PM), Saidapet (SP), Thambaram (TB), and Thiyngananager (TN)

agar with the aid of a sterile cork borer of 7-mm diameter, and inoculated in medium. A 50 ml of Czapek-Dox medium was dispensed into 250 ml Erlenmeyer flask, and inoculated with 7-mm disc of mycelial-colonized agar. To evaluate fungal growth on PAHs, nutrient medium (50 ml) was prepared using CDM (sucrose omitted) containing 1 % (w/v) of the following carbon sources such as diesel, kerosene, petrol, grease, motor oil, and contaminated soil from garages. The flask was incubated at 25 ± 1°C for 7 days. After completion of growth, the fungal cultures were harvested, mycelium was removed by centrifugation (1,643 x *g*, 10 min/4°C), and the supernatant was recovered and used as enzyme source.

Zymogram methods were employed to detect the following enzyme activities in extracellular fluids (ECF): protease, laccase, non-specific peroxidase and lipase. Proteolytic activity was determined by loading 10 µl aliquots of ECF into 7-mm diameter in the wells scooped out of agar plates containing 1% (w/v) gelatin, and incubated at 28°C for 24 hr. The plates were then flooded with a solution of 1.5% $HgCl_2$ in 20% HCl and after 10 min flood solution was decanted, further washed with distilled water. The appearance of clear zone around the wells indicated proteolytic activity. Lipase activity was measured against tributyrin by zymogram method according to Venkatesagowda *et al*. (2012). The laccase activity was detected using fungal mycelium, placed inverted onto CDM-agar plates, incubated for 5 days at 28°C and plates were irrigated with a 10 ml solution of 1.7mM ABTS (2,2^1-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) followed by keeping the plates in dark for 5 min at 25°C. The appearance of clear bluish-green zones around the fungal colony was measured. Non-specific peroxidase activity was measured using the same technique as described for laccase, except that the plates were irrigated with a 10 ml solution of 1.7mM ABTS containing 2.5mM hydrogen peroxide. The diameters of the clearance zone in the plates were measured (mm) after incubation.

Extracellular enzyme assays by spectrophotometry : Different extracellular enzyme activity of the test fungi were detected using different assays such as protease activity (McDonald and Chen, 1965), lipase activity (Venkatesagowda *et al*., 2012), laccase activity (Mansur *et al*., 1997) and peroxidase activity (Thiyagarajan *et al*., 2008).

Optimization of cultural conditions for lipase production : The culture conditions for fungal strains were optimized to increase lipase production by culture carbon source, nitrogen source, lipid and surfactants at 1% concentration.

Biodegradation of PAHs by fungi

Enzymatic activity of fungal strains in the presence of contaminated soil : PAHs contaminants such as motor oil, petrol, diesel, kerosene, grease and PAHs contaminated soil (1%) as sole carbon source into 250 ml Erlenmeyer flasks containing CDA broth and autoclaved at 120 °C for 20 min. The test fungi were inoculated and incubated at 30 °C for 28 days. Each experiment was carried out in triplicate and lipase activity was measured. The fungal mycelium was removed by filtration and clear culture fluid was centrifuged at 10,000 rpm for 10 min. The clear supernatant was used to measure lipase activity of the test fungal strain. The experiment was also performed in autoclaved soil to test the efficiency of fungal consortia.

Gas chromatography analysis : Gas chromatography (GC) was used to determine degradation of hydrocarbons in the contaminated soil by fungal strain. The soil samples were collected from two wheeler service station in Thiyagaraya Nagar (TN) contaminated with motor oil, petrol, diesel, kerosene and grease. The fungal consortia with *P. chrysogenum, M. racemosus* and *L. theobromae* VBE1 was inoculated in the medium with contaminated soil and incubated at 25° C for 28 days. After incubation, extracted the whole flask and analyzed in GC. To the contaminated soil from garages (1.0 g), 50 ml of acetone was added. The hydrocarbons in the contaminated soil were extracted using acetone twice. The whole medium in the flasks were extracted with acetone (v/v) for hydrocarbon analysis. The extract was filtered through sodium sulphate and condensed to 1 ml with rotary evaporator unit. A 10 µl of extracted sample was injected into the injection port. The chromatograph consist of CHEMITO GC 8610 Flame Ionization Detector, carrier gas as nitrogen and hydrogen, oxygen for ignition purpose and a BPX-70 (50% cyanopropyl 50% methylsiloxane) column. Temperature regimen maintained during the operation was as follows: Injection port 250°C, detector port 260°C, oven starting temperature 160°C and increase by 7.5°C per minute to a final oven temperature of 240°C. A winchrom software package in the GC apparatus was used to analyze the data.

Results and Discussion

Petroleum based contamination has increased due to anthropogenic activities and not only affects the environment but also mycobiota associated with soil and water (Juhasz and Naidu, 2000; Ijah, 2003). Hence, fungi isolated from oil spill environment can reduce oil pollution. Nevertheless, interest in fungi has received a considerable attention for their bioremediation potential of polyaromatic contaminated soil associated fungi for enzyme secretion to remove pollutant from environment. PAHs contaminated soil samples were collected from 10 different places of Chennai, India. During isolation, in total 9 generic fungi namely *Mucor, Rhizopus, Aspergillus, Curvularia, Drechslera, Fusarium, Penicillium, Trichoderma* and *Lasiodiplodia* (Table 1) were isolated. The fungal population was high (CFU g^{-1} of soil) in the soil sample, obtained from Guindy PAHs contaminated soil samples in which *Mucor racemosus* (6.3), *Rhizopus stlonifer* (7.2), *Aspergillus niger* (3.4) and *Penicillium chrysogenum* (5.3) were dominant.

The fungi are potential candidates for removal of PAHs from biosphere by the action of fungal hyphae growth on toxic and undesirable substrates by their potential enzyme system (April *et al.,* 2000; Santos *et al.,* 2008). Hence, the present study mainly focused to screen PAHs associated fungi for different enzymes such as lipase, protease, laccase and peroxidases. Total 23 fungal strains were used in the study, out of which 21 belonged to PAHs contaminated soil associated fungi and two related to oil seed associated fungi, namely *L. theobromae* VBE1 (coconut kernal) and *Colletotrichum gleosporioidies* (pongamia oil seed). The strains were screened to determine their PAHs degradation efficiency by using PAHs contaminated soil solution as sole carbon source. The highest lipase production was observed in *P. chrysogenum* (112 U ml^{-1}) followed by *L. theobromae* VBE1 (100 U ml^{-1}). *C. gleosporioidies* showed highest lipase production in solid-state fermentation (Balaji and Ebenezer, 2008; Banu and Muthumary 2005), however failed to produce lipase enzyme during PAHs removal, due to toxic effect of PAHs in contaminated soil (Table 2). The highest laccase enzyme production was found in *P. chrysogenum* and *Aspergillus fumigatus* (79 U ml^{-1} and 73 U ml^{-1}) respectively (Table 2). Whereas, moderate peroxidase activity (52 U ml^{-1}) was noted in *Mucor racemose* and *Rhizopus stolonifer*. Similarly previous studies showed highest lipase production (108 U ml^{-1}) by *L. theobromae* and peroxidase enzyme production (516 U ml^{-1}) by *Coprinus* sp. (Venkatesagowda *et al*., 2012); Thiyagarajan *et al*., 2008). Hydrolytic enzyme profile of PAHs associated fungi determined by plate assay method is as follows: highest lipase activity occurred in *M. racemose*, *R. stolonifer*, *A. fumigatus*, *A. parasitics*, *P. citrinum*, *P. chrysogenum* and *L. theobromae* VBE1; Laccase activity in *A. fumigatus, P. chrysogenum* and *Trichoderma viride*, Peroxidase activity in *Aspergillus niger, A. fumigatus, P. chrysogenum* and protease activity in *M. racemose*, *L. theobromae* VBE1, respectively (Table 3). Initial screening for different hydrolytic enzyme activity showed prominent results. This was obtained with the highest lipase activity occurring in *P. chrysogenum* and *M. racemosus*, isolated from PAHs contaminated soils. *L. theobromae* VBE1, (coconut-kernel associated endophytic fungi) was identified as a good lipase producer and the consortia were further investigated for their efficiency in PAHs degradation. Based on the above results, three strains, *P. chrysogenum, M. racemosus* and *L. theobromae* VBE1 were able to utilize PAHs contaminated soil as substrate and were selected for further studies.

Natural resources contaminated with PAHs usually increase their mutagenic and carcinogenic effect in fresh-water, marine-water and terrestrial environment. This leads to undesirable condition for growth of even native microorganisms

Table 2 : Extracellular enzyme activity of hydrocarbons contaminated soils associated fungi

Fungal strain	Extracellular enzyme activity (U ml^{-1})			
	Lipase	Laccase	Peroxidase	Protease
Alternaria alternata	26±0.02	30±0.4	-	-
Aspergillus niger	77±0.01	-	39±0.06	-
A. fumigatus	86±0.8	73±0.3	52±0.04	-
A. ochraceus	79±0.06	-	24±0.2	-
A. parasitics	56±0.5	48±0.4	-	-
A. tamarii	62±0.09	-	-	-
A. terreus	35±0.06	-	-	-
A. versicolor	42±0.5	-	-	1.2±0.1
Colletitrichum falcatum	28±0.05	-	-	-
C. gleoidispoides	10±0.08	23±0.4	-	0.1±0.03
Curvularia laenta	18±0.6	-	-	0.50±0.003
Dreshctera halodes	37±0.7	-	-	-
Fusarium oxysporum	51±0.5	-	-	-
Lasiodiplodia theobrome	50±0.6	-	-	-
Mucor racemose	96± 0.3	46±0.7	52±0.2	3.2±0.03
Penicillium variotii	75±0.3	62±0.07	43±0.05	0.4±0.02
P. citrinum	84±0.04	-	-	-
P. variable	79±0.09	32±0.06	21±0.03	-
P. chrysogenum	112±0.5	79±0.6	47±0.01	-
Rhizopus stolonifer	92±0.7	-	43±0.8	2.5±04
Trichoderma harzianum	22±0.04	-	-	-
T. viride	10±0.02	23±0.05	-	-
L. theobrome VBE1	100±0.03	12±0.06	5±0.006	1.2±0.01

Values are mean of replicates ±SD

and also for bioaugmentation methods. To overcome this limitation, cultural condition has been optimized to increase lipolytic potential with special reference to extracellular lipases from *P. chrysogenum, M. racemosus* and *L. theobromae* VBE1 (Table 4). The influence of various environmental factors for secretion of this enzyme was studied using inducer PAHs soil solution as a sole carbon source. Hadibarata and Tachibana (2009) reported that *Trichoderma* S019 strain was potentially able to degrade n-eicosane when glucose was applied as a carbon source.

The raw materials needed for lipase production are crucial. The efficacy of enzyme synthesis depends on the chemical nature of inducer. Against this background, a few lipid sources have been explored for their efficacy in inducing enzyme production. Since lipases are lipolytic, substrate acts as an inducer which should be lipid in nature. Initially, different carbon sources tested for lipase production showed highest activity in *P. chrysogenum* (sucrose), *M. racemosus* (cellulose) and *L. theobromae* (sucrose) (Table 4). Various nitrogen sources were tested for lipase production in which yeast extract showed maximum lipase production in *P. chrysogenum, M. racemosus* and *L. theobromae* VBE1 (Table 4). Dharmsthiti and Kuhasuntisuk (1998) also reported that yeast extract, as an additional source enhanced lipase production from *Pseudomonas aeruginosa* LP602 potentially used in lipid-rich wastewater treatment. Garapati and Mishra (2012) also reported the importance of nutrients in biodegradation of hydrocarbon by fungal strain. During PAHs degradation, yeast extract plays a major role as additional substrate, which potently accelerated PAHs degradation by the halotolerant bacterial consortium (Arulazhagan and Vasudevan, 2011).

Effect of surfactant on PAHs degradation was investigated and maximum lipase production was achieved using Triton X 100 and showed *P. chrysogenum* (68 U ml^{-1}), *M. racemosus* (72 U ml^{-1}) and *L. theobromae* VBE1 (62 U ml^{-1}) of lipase activity was observed. Similarly, Chen *et al*. (2006) reported the impact of surfactants on PAHs degradation by white-rot fungi in soil-water system, Triton X-100 and SDS restrained the degradation of PAHs. Ali *et al.* (2009) reported highest lipase activity in *Metarhizium anisopliae*, when SDS and Tween 80 were added. Recently Gopinath *et al.* (2013) also detailed the importance of surfactant in lipase production and its various applications. But degradation of PAHs could be enhanced with increasing the concentrations of Tween80 and linear alkylbenzene sulphonates (LAS) to certain levels. However, Tween 80 and LAS with high concentrations were unable to promote PAHs degradation.

In this present study, PAHs contaminants and their degradation efficacy was investigated using *P. chrysogenum, M.*

Table 3 : Hydrolytic enzyme activity by the fungal strains from petroleum contaminated soil

Fungal strain	Hydrolytic zone (mm)			
	Lipase	Laccase	Peroxidase	Protease
Alternaria alternata	14±0.9	03±0.02	-	-
Aspergillus niger	17±0.01	-	19±0.08	-
A. fumigatus	21±0.08	19±0.09	19±0.3	-
A. ochraceus	19±0.03	-	02±0.5	-
A. parasitics	23±0.07	08±0.06	-	-
A. tamarii	14±0.05	-	-	-
A. terreus	16±0.08	-	-	-
A. versicolor	09±0.04	-	-	06±0.03
Curvularia laenta	17±0.02	-	-	06±0.05
Colletitrichum falcatum	10±0.06	-	-	-
C. gleoidispoides	-	-	-	01±0.004
Dreshctera halodes	17±0.07	-	-	-
Fusarium oxysporum	13±0.05	-	-	-
Lasiodiplodia theobrome	6±0.09	-	-	-
L. theobrome VBE1	23±0.007	09±0.02	01±0.005	12±0.03
Mucor racemose	25±0.04	12±0.6	12±0.02	10±0.05
Penicillium variotii	19±0.01	12±0.03	12±0.09	08±0.02
P. citrinum	21±0.03	-	-	-
P. variable	19±0.02	12±0.05	11±0.04	-
P. chrysogenum	22±0.08	17±0.04	18±0.02	-
Rhizopus stolonifer	21±0.07	-	14±0.03	09±0.2
Trichoderma harzianum	8±0.05	-	-	-
T. viride	20±0.04	20±0.05	-	-

Values are mean of replicates ±SD

racemosus and *L. theobromae* VBE1 lipase on motor oil, petrol, diesel, grease, kerosene and PAHs contaminated soil solution as source in presence and absence of autoclaved soil (Table 5). Whereas, PAHs contaminated soil from garages also serve as a carbon source to determine the removal of PAHs by test fungi. *P. chrysogenum* showed prominent utilization and degradation of motor oil (110 U ml^{-1}) and naturally PAHs contaminated soil solution (110 U ml^{-1}). Interestingly, different fungal behavior on enzyme production was observed in the presence of autoclaved soil and PAHs contaminated soil showed highest lipase activity (111 U ml^{-1}) followed by grease and motor oil (57 U ml^{-1}, 45 U ml^{-1}), respectively (Table 5).

PAHs degradation efficacy was determined using another native fungal strain, *M. racemose* that utilized PAHs in contaminated soil and motor oil as sole carbon source. Highest lipase activity of 95 U ml^{-1} and 94 U ml^{-1} was found at 28 days of incubation in CDB broth (Table 5). Whereas, PAHs contaminated soil showed highest lipase activity (97 U ml^{-1}) and autoclaved soil mixed with grease (96 U ml^{-1}) and motor oil (90 U ml^{-1} respectively at 28 days of incubation). Atagana (2009) reported that both ligninolytic and non-ligninolytic fungal strain was capable of degrading PAHs effectively even in the presence of heavy metals. Delira *et al.,* (2012) also reported *Trichoderma* strains as capable of tolerating and growing in crude oil contaminated culture media and naphthalene, phenanthrene and benzo(a)pyrene, but over doses retarted the growth of the fungal strain.

The PAHs removal efficacies were determined using coconut-kernel endophytic fungi, *L. theobromae* VBE1. The strain utilized different PAHs contaminants (motor oil, petrol, diesel, kerosene and naturally PAHs contaminated soil) as sole carbon source by bioaugmentation process. After 28 days of incubation, lipase activity decreased, that indicated bioavailability of PAHs was reduced and complete degradation was achieved (Table 5). As the incubation duration increasesd, there was an increase in removal of PAHs contaminant and a pronounced decline in lipase activity pointing to the situation where no more substrate is available for the enzyme to act (Table 5). Similar results were obtained for petroleum hydrocarbon-degrading fungi from *Detarium senegalense* seeds. The fungal strains obtained from this seeds were *Aspergillus flavus*, *A. niger*, *Pencillium sp.*, *Rhizopus sp.* and *Talaromyces sp*. *Aspergillus niger* strains showed high ability to degrade unspent engine oil. *Rhizopus sp.* potentially degraded kerosene and diesel; and *Talaromyces sp.* degraded spent engine oil (Adekunle and Adebambo, 2007).

PAHs contaminated soil inoculated using fungal consortia, contained *P. chrysogenum, M. racemosus* and *L. theobromae* VBE1, were incubated at 25° C for 28 days. After

Table 4. Optimization of fungal extra cellular lipase production in hydrocarbons contaminated soil amended medium

Experimental conditions	Variables	Lipase activity (U ml^{-1})		
		P. chrysogenum	*M. racemose*	*L. theobrome*
Carbon sources	Glucose	12±0.04	24±0.01	16±0.02
	Fructose	31±0.03	33±0.05	35±0.08
	Xylose	41±0.9	35±0.3	32±0.01
	Sucrose	85±1.1	40±1.05	62±0.5
	Maltose	43±0.06	42±0.7	24±0.01
	Mannitol	38±1.1	10±0.06	37±0.04
	Starch	52±0.8	27±0.05	39±0.09
	Cellulose	26 ±0.06	48±0.04	32±0.08
Nitrogen sources	Ammonium nitrate	23±0.02	15±0.03	18±0.03
	Ammonium sulfate	39±0.07	42±0.08	23±0.04
	Sodium nitrate	14±0.01	38±0.04	13±0.07
	Peptone	56±0.02	44±0.06	29±0.9
	Urea	43±0.09	40±0.08	36±0.5
	Yeast extract	68±0.4	56±0.3	55±0.06
Lipids	Tristearin	55±0.05	24±0.06	19±0.09
	Oleic acid	28±0.05	42±0.02	27±0.02
	Glycerol	53±0.4	58±0.5	24±0.5
	Cholesterol	59±0.3	11±0.2	39±0.1
Surfactants	Tween 20	35±0.7	42±0.05	24±0.09
	Tween 40	14±0.01	38±0.02	21±0.07
	Tween 60	45±0.02	25±0.08	32±0.05
	Tween 80	26±0.01	52±0.06	43±0.03
	Triton X-100	68±1.1	72±0.08	62±0.9
	Sodium dodecylsulfate	53±0.02	10±0.05	23±0.1
	Sodium deoxycholate	14±0.09	5±0.3	29.5±0.8

Table 5. PAHs degradation and extracellular lipase production by PAHs contaminated soils associated fungi

PAHs Contaminants	Lipase activity (U ml^{-1})											
	P. chrysogenum				*M. racemose*				*L. theobromae*			
	7	14	21	28	7	14	21	28	7	14	21	28
Absence of soil[1]												
Motor oil	16±04	37±0.8	88±0.2	110±0.6	14±0.6	29±0.8	59±0.3	94±0.4	34±0.06	66±0.2	88±0.5	96±0.4
Petrol	10±0.05	42±0.5	91±0.9	97±0.7	7±0.3	31±0.4	62±0.5	76±0.3	47±0.7	39±0.5	27±0.8	13±0.3
Diesel	8±0.01	27±0.07	34±0.02	41±0.05	13±0.8	42±0.8	61±0.8	82±0.7	27±0.4	13±0.2	9±0.03	0.6±0.2
Grease	23±0.4	45±0.6	78±0.8	105±0.2	65±0.5	54±0.3	47±0.6	42±0.9	68±0.5	76±0.8	83±0.9	62±0.5
Kerosene	5±0.02	18±0.7	26±0.5	55±0.4	13±0.1	33±0.4	57±0.1	74±0.4	53±0.08	8±0.01	2±0.4	0.1±0.01
Natural soil PAHs	31±0.3	52±0.9	92±0.08	110±0.5	42±0.5	67±0.3	81±0.9	95±0.4	100±0.3	82±0.02	78±0.6	67±0.3
Presence of soil[2]												
Motor oil	15±0.7	26±0.8	45±0.7	96±0.2	17±0.3	32±0.6	63±0.2	90±0.4	17±0.7	20±0.9	30±0.3	56±0.2
Petrol	8±0.5	19±0.2	38±0.4	66±0.3	12±0.1	43±0.4	67±0.8	82±0.3	14±0.3	30±0.1	38±0.4	42±0.7
Diesel	10±0.6	32±0.6	43±0.7	58±0.7	31±0.4	52±0.9	79±0.7	87±0.7	21±0.1	56±0.8	63±0.3	60±0.2
Grease	12±0.8	25±0.4	57±0.3	103±0.5	76±0.4	88±0.5	96±0.3	96±0.4	26±0.2	33±0.5	41±0.8	58±0.4
Kerosene	7±0.5	19±0.9	31±0.5	64±0.6	5±0.1	23±0.6	31±0.7	38±0.3	17±0.8	54±0.3	68±0.5	72±0.9
Natural soil PAHs	43±0.4	67±0.2	95±0.9	111±0.7	65±0.3	76±0.2	86±0.4	97±0.2	68±0.6	19±0.4	10±0.7	5±0.02

[1] The liquid CDB broth was artificially contaminated with PAHs. Whereas natural PAHs soil solution (1%) used as carbon source in CDB at 25°C for month interval; [2] The autoclaved soil with CDB and PAHs artificially contaminated, Whereas natural PAHs soil : CDB (1:1) used as carbon source at 25°C for month interval

incubation, the fungal enzyme catalyzed by the products of PAHs derivatives were extracted and analyzed by GC. The contaminated soil with the mixture of motor oil, petrol, diesel, kerosene and grease contained different hydrocarbons from C12-C24 and on 28th day, most of the compounds were completely degraded by fungal strains in relation to lipase production (Fig. 1). The extracellular enzyme filtrate were extracted and tested for their lipase activity by native gels using tributyrin as substrate. The highest lipase activity and clear zone was detected in fungal consortia growing in presence of petrol, grease and PAHs contaminated soil. The saturate profile of the contaminated soil showed successful and complete degradation of n- alkanes. The GC profile also confirmed that, the fungal strains were capable of degrading branched alkanes isoprenoids such as pristine (C17) and pyrene (C18) (April *et al.,* 2000 and Husaini *et al.,* 2008).

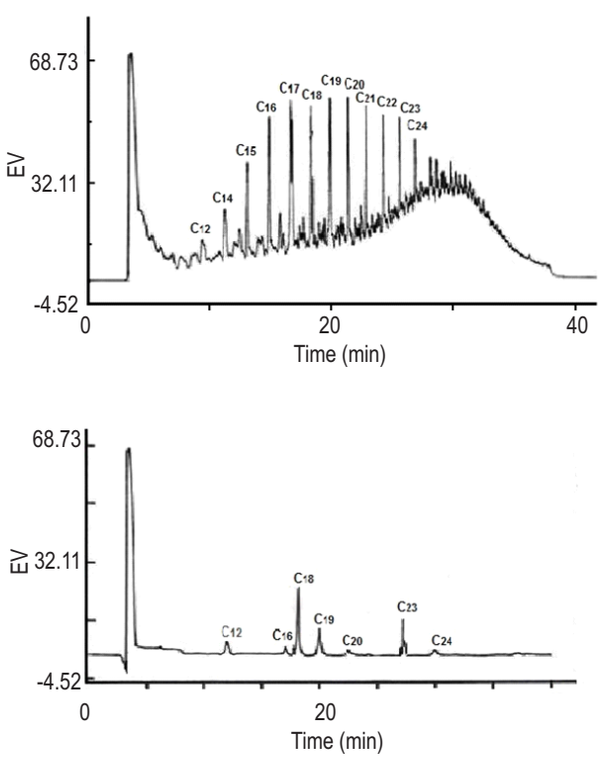

Fig. 1 : Gas chromatography profiles hydrocarbon degradation (C12 – C24) present in the contaminated soil by fungal strains after 28 days of incubation

The present study, revealed that PAHs contaminated soil-associated fungi and lipases from fungal consortia (*P. chrysogenum, M. racemosus* and *L. theobromae* VBE1) can be used in bioremediation of PAHs contaminated habitats such as motor oil, petrol, diesel, kerosene and grease. The present study also details about lipase production in relation with hydrocarbon biodegradation by the fungal strains. However, further studies are needed to compare lipase mediated degradation with non-lipase enzyme producing fungi isolated in this study.

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