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Lunfang Yao[ab], Ying Teng[a], Yongming Luo[c], Peter Christie[a], Wenting Ma[a], Fang Liu[b], Yonggui Wu[b], Yang Luo[a] & Zhengao Li[a]

[a] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China

[b] College of Resources and Environmental Engineering, Guizhou University, Guiyang, China

[c] Key Laboratory of Coastal Zone Environmental Processes, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, China

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Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) by *Trichoderma reesei* FS10-C and Effect of Bioaugmentation on an Aged PAH-Contaminated Soil

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[1]Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China
[2]College of Resources and Environmental Engineering, Guizhou University, Guiyang, China
[3]Key Laboratory of Coastal Zone Environmental Processes, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, China

ABSTRACT The co-metabolism of benzo[*a*]pyrene (B[a]P) and the capacity of the fungus *Trichoderma reesei* FS10-C to bioremediate an aged polycyclic aromatic hydrocarbon (PAH)-contaminated soil were investigated. The fungal isolate removed about 54% of B[a]P (20 mg L^{-1}) after 12 days of incubation with glucose (10 g L^{-1}) supplementation as a co-metabolic substrate. Bioaugmented microcosms showed a 25% decrease in total PAH concentrations in soil after 28 days, and the degradation percentages of 3-, 4-, and 5(+6)-ring PAHs were 36%, 35%, and 25%, respectively. In addition, bioaugmented microcosms exhibited higher dehydrogenase (DHA) and fluorescein diacetate hydrolysis (FDAH) activities and increased average well-color development (AWCD), Shannon-Weaver index (*H*), and Simpson index (*D*) significantly. Principal component analysis (PCA) also distinguished clear differentiation between treatments, indicating that bioaugmentation restored the microbiological function of the PAH-contaminated soil. The results suggest that bioaugmentation by *T. reesei* FS10-C might be a promising bioremediation strategy for aged PAH-contaminated soils.

KEYWORDS bioaugmentation, biodegradation, PAHs, soil contamination, *Trichoderma reesei* FS10-C

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are an important group of organic pollutants that are widely distributed in the environment. They originate mainly from the incomplete combustion of fossil fuels, volcanic eruptions, and forest fires and are released into the environment in the form of exhausts and solid residues (Samanta, Singh, and Jain 2002). They can induce a number of detrimental biological effects, such as mutagenic, carcinogenic, and teratogenic effects (Labana et al. 2007). Due to their hydrophobic nature, most PAHs are readily bound to particulates in soils; this renders them less available for

Address correspondence to Ying Teng, Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, No. 71 East Beijing Road, Nanjing 210008, China. E-mail: yteng@issas.ac.cn

biological uptake, so they can also accumulate in food chains (Boonchan, Britz, and Stanley 2000). Consequently, the remediation of PAH-contaminated soil sites has attracted worldwide attention.

Although PAHs may undergo chemical oxidation, photolysis, and volatilization, microbial transformation is an economic and environmentally friendly alternative and is a major environmental process affecting the fate of PAHs in both terrestrial and aquatic ecosystems (Johnsen et al. 2005). Several microorganisms (fungi, bacteria, and algae) have been used to degrade various PAHs and as bioremediation agents to remove PAHs from contaminated sites (Cerniglia and Sutherland 2010). Most bacteria but few fungi can utilize PAHs as a sole carbon and energy source (Cerniglia and Sutherland 2010; Boonchan, Britz, and Stanley 2000). Compared with bacteria, however, fungi exhibit higher resistance to PAHs and can rapidly colonize solid substrates. Fungi may therefore offer certain advantages over bacteria for bioremediation (Chulalaksananukul et al. 2006). Numerous previous studies have focused on lignolytic fungi, particularly *Phanerochaete* spp., *Pleurotus* spp., and *Trametes* spp. (Leonardi et al. 2007; Borràs et al. 2010; Cerniglia and Sutherland 2010; Pozdnyakova et al. 2011; Fulekar et al. 2013). These fungi are able to degrade some high-molecular-weight PAHs and detoxify them in polluted soils and sediments through secretion of extracellular lignin-degrading enzymes (Cerniglia and Sutherland 2010). Moreover, some nonlignolytic fungi such as *Cunninghamella* spp., *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. can also metabolize PAHs by cytochrome P450 monooxygenase– and epoxide hydrolase–catalyzed reactions to form *trans*-dihydrodiols. These reactions are highly stereoselective and other metabolites formed include phenols, quinines, and conjugates (Cerniglia and Sutherland 2001). PAH *trans*-dihydrodiols and phenols may be methylated or converted to sulfates, glucosides, glucuronides, or xylosides (Cerniglia 1997).

Trichoderma species belong to the group of filamentous fungi classified as Ascomycetes (Order Hypocreales), and they are genetically very diverse with a wide range of capabilities among different strains of agricultural and industrial significance (Harman et al. 2004; Tripathi et al. 2013). Some studies have shown that *Trichoderma* spp. possess the potential to tolerate and detoxify environmental contaminants, including heavy metals, pesticides, and some PAHs (Ravelet et al. 2000; Saraswathy and Hallberg 2002; Verdin, Sahraoui, and Durand 2004; Machín-Ramírez et al. 2010; Tripathi et al. 2013). However, little is known about the capability of *Trichoderma* spp. to degrade high-molecular-weight PAHs or as bioremediation agents for the remediation of aged PAH-contaminated sites.

The ultimate goal of any remediation process must be both to remove the contaminants from the polluted soil and also, most importantly, to restore the capacity of the soil function according to its potential (Epelde et al. 2009). The enzyme activities and the diversity of soil microbial communities may serve as good indices to evaluate soil quality (Labud, Garcia, and Hernandez 2007). The main objectives of the present study were to evaluate the ability of one fungal isolate, *Trichoderma ressei* FS10-C, to metabolize benzo[*a*]pyrene (B[a]P) in liquid culture, to investigate the efficiency of removal of PAHs from an aged PAH-contaminated soil bioaugmented with *T. ressei* FS10-C in the absence of indigenous soil microorganisms, and to analyze the changes in microbial activities during the bioremediation process.

MATERIALS AND METHODS

Reagents, Culture Medium, and Soil

B[a]P with a purity of 97–99% was purchased from Sigma-Aldrich (Shanghai, China), and a standard solution of 16 PAHs was supplied by Dr. Ehrenstorfer (Augsburg, Germany). All other solvents and chemicals used were of reagent grade or better. The composition of the mineral salt (MS) medium was (per liter of distilled water) (Wu et al. 2009): $(NH_4)_2SO_4$ 1000 mg, NaH_2PO_4 800 mg, K_2HPO_4 200 mg, $MgSO_4$ 200 mg, $CaCl_2$ 100 mg, $FeCl_3$ 5 mg, $MnSO_4$ 0.2 mg, $(NH4)_6Mo_7O_{24}$ 1 mg, $ZnSO_4$ 0.1 mg, $CuSO_4$ 0.02 mg, $CoCl_2$ 0.02 mg, and supplemented with 10 g L^{-1} glucose (Glu) as co-metabolic substrate. The initial pH of the medium was adjusted to 5.5 ± 0.1 using hydrochloric acid (0.5 M).

The soil used for the experiment was collected from the top 15 cm of the soil profile at a PAH-contaminated agricultural area at Wuxi City, Yangtze Delta, Jiangsu Province, eastern China. The soil had been contaminated for approximately 30 years previously, and the contaminants had therefore undergone a relatively long weathering process. Soil physicochemical properties were as follows: soil pH is 6.4 (in water), organic

matter content 23.4 g kg^{-1}, total nitrogen (ammonium-, nitrate-, nitrite-, and organic-N) 1.4 g kg^{-1}, total phosphorus 0.9 g kg^{-1}, and total potassium 12.3 g kg^{-1} on a dry weight basis. The concentration of the mixture of 16 individual PAHs was 9634 ± 193 μg kg^{-1} dry soil, with concentrations of the 3-, 4-, and 5 (+6)-ring PAHs of 443 ± 14, 3805 ± 76, and 5386 ± 269 μg kg^{-1}, respectively. According to the Canadian Environmental Quality Guidelines released by the Canadian Council of Ministers of the Environment (CCME 2004), this soil would not be suitable for agricultural use.

Fungus and Preparation of Inocula

T. reesei FS10-C was previously isolated in our laboratory from heavy metal–contaminated soil in which the main contaminants were Cu, Cd, Pb, and Zn at concentrations of 36, 0.4, 93, and 383 mg kg^{-1}, respectively. The soil organic matter content was 38 g kg^{-1}, alkali-hydrolyzable nitrogen 142.7 mg kg^{-1}, available phosphorus 9.7 mg kg^{-1}, and available potassium 63 mg kg^{-1}. However, we found that this strain can also degrade PAHs, and we therefore use it to remediate PAH-contaminated soil. Inoculum was prepared by growing the fungus on potato dextrose agar (PDA) plates (20 g dextrose, 18 g agar, 1 L of 20% potato extract) at 28°C for 72 h. A suspension of spores was prepared by washing a 72-h-old culture with sterile distilled water. Fragments of mycelium were removed from the spore suspension by filtration through sterile glass wool. The spore suspension density was estimated using a Malassez hemocytometer, and liquid culture medium was then inoculated with 10^5 spores ml^{-1}. Before inoculation of the soil microcosms, the spore suspension was transferred to a solution of sterile wheat bran–orange peel substrate for 72 h at 28°C in order to induce spore germination and hyphal elongation.

Experimental Design

B[a]P Depletion in Liquid Culture

Cultures for pure-culture assay were established as follows. Stock solution of B[a]P in acetone (1 ml) was added to 150-ml empty sterile Erlenmeyer flasks. After total evaporation of the organic solvent, sterile MS-Glu medium (20 ml per flask) was added to give a final B[a]P concentration of 20 mg L^{-1}. Inoculation was performed by adding a spore suspension to reach a spore concentration of 10^5 spores ml^{-1}. Erlenmeyer flasks without inoculum were also prepared to detect abiotic degradation of B[a]P. The Erlenmeyer flasks were incubated at 28°C in the dark on a rotary shaker at 150 rpm. Triplicates were set up to determine fungal biomass, enzyme activity, and residual B[a]P every 72 h.

Soil Microcosms

Each soil microcosm was established by placing 135 g soil in a glass Petri dish (diameter 15 cm). Bioaugmented microcosms (BA) were inoculated with 15 g fungal strain inoculum prepared as described above. Control microcosms (CK) were also prepared with 15 g sterilized inoculum. There were three replicates of each treatment. The moisture content of the microcosms was adjusted by weight to 60% of water holding capacity. All microcosms were incubated for 28 days at 28°C in the dark. Each soil sample was then divided into two parts. One was placed in a plastic bag at 4°C for subsequent analysis of microbial activities, and the other was freeze-dried and passed through a 60-mesh sieve prior to PAH analysis.

Analytical Methods

PAH Extraction and Analysis

The residual B[a]P in MS-Glu medium was totally extracted with 60 ml dichloromethane. The extracts were mixed in a vortex mixer for 2 min and on a rotary shaker at 150 rpm for 1 h, left to settle for 30 min, and subsequently a 1-ml aliquot was collected, dried by sparging with N_2, and redissolved in 5 ml *n*-hexane for gas chromatography–mass spectrometry (GC-MS) determination. Quality control checks showed that the average recovery of B[a]P was 96%. At the same time, PAHs adsorbed on and contained in the mycelium were extracted by digestion with 20 ml NaOH (5 M) over a period of 3 days. The slurry obtained was then extracted with 60 ml dichloromethane, and the B[a]P extraction method was the same as described above.

PAHs in bulk soil samples (2 g) were extracted using Soxhlet extraction with dichloromethane (Teng et al. 2010). The extracted compounds were determined with an Agilent 7890GC-5975MSD gas chromatography–mass spectrometry system (GC-MS) (Agilent

Technologies, Avondale, PA, USA) operating in electron impact and selective ion monitoring mode and with a DB-5 (30 m × 0.25 mm × 0.25 μm) fused-silica capillary column for chromatographic separation. High-purity helium (99.9999%) was used as the carrier gas and was maintained at a constant flow rate of 1 ml min^{-1}. The temperature program column oven was set to 50°C for 1 min and raised to 200°C at 25°C min^{-1}, then up to 280°C at 8°C min^{-1}, and then up to 283°C at 1°C min^{-1}, and finally up to 290°C at 2°C min^{-1}. Each extract (1 μl) was injected into the GC-MS in nonpulse and splitless mode with an injector temperature of 250°C. The GC-MS transfer line was set at 260°C, and the postrun temperature was 285°C for 2 min.

Fungal Growth and Enzyme Activities in MS-Glu Medium

Fungal growth in MS-Glu medium was determined by filtering the medium through quantitative filter paper, washing the mycelium with 200 ml of deionized water, and then drying to constant weight at 105°C.

Enzyme assays were conducted using the extracellular medium of liquid fungal culture as an enzyme source. Manganese peroxide (MnP) activity was assayed by monitoring the oxidation of vanillylacetone (Paszczyński, Huynh, and Crawford 1986). Laccase (Lac) activity was measured by monitoring the oxidation of 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Perez, Martinez, and de la Rubia 1996). Lignin peroxide (LiP) activity was determined by the veratryl alcohol oxidation assay (Tien and Kirk 1984).

Soil Enzyme Activities and Functional Diversity of the Soil Microbial Community

Soil dehydrogenase (DHA) and fluorescein diacetate hydrolysis (FDAH) activities were assessed by the method described by Teng et al (2010). With 1,3,5-triphenyltetrazolium chloride (TTC) as a substrate (hydrogen receptor), soil DHA can induce TTC to generate red triphenylformazan (TPF). The concentration of TPF was determined by spectrophotometry at 485 nm, and the results are expressed as μg TPF g^{-1} soil h^{-1}. Soil FDAH can induce fluorescein diacetate to generate yellow fluorescein by hydrolysis and dehydrogenation. The fluorescein concentration was measured using a spectrophotometer at 490 nm, and the results are expressed as μg fluorescein g^{-1} soil $(20\ min)^{-1}$.

Biolog Ecoplates (Biolog, Hayward, CA, USA) were used to investigate the soil microbial functional diversity. Reaction wells (96 per plate) were divided into three groups. Each group consisted of one control, four polymers, six amino acids, seven carbohydrates, two amines, nine carboxylic acids, and three phenols. Briefly, an aliquot of fresh soil (equal to 10 g dried soil) was placed in an autoclaved 250-ml flask with 100 ml of sterilized 0.85% (*w/v*) NaCl solution and shaken for 10 min. Tenfold serial dilutions were prepared, and the 10^{-3} dilution was used to inoculate the Biolog Ecoplates. The plates were incubated at 25°C, and color development in each well was recorded as optical density (OD) at 590 nm with a plate reader at regular 12-h intervals. The average well color development (AWCD) was calculated using the equation $AWCD = \sum OD_i/31$, where OD_i is the optical density value from each well (Teng et al. 2010). The Shannon diversity index (H) was calculated using the equation $H = -\sum p_i \ln p_i$, and the Simpson diversity index (D) was calculated using the equation $D = 1/\sum p_i^2$, where p_i is the ratio of the activity on each substrate (OD_i) to the sum of activities on all substrates ($\sum OD_i$) (Stephan, Meyer, and Schmid 2000). All values were used to calculate diversity indices according to the data when the cultivation period was 108 h.

Statistical Analysis

Statistical analysis was carried out using the SPSS version 13.0 for Windows software package (SPSS, Chicago, IL, USA). Data were processed statistically with analysis of variance (ANOVA) and least significant difference (LSD) multiple range tests for significant differences between treatments at $p < .05$.

RESULTS AND DISCUSSION

B[a]P Degradation in MS-Glu Medium

B[a]P degradation by *T. reesei* FS10-C was investigated in MS-Glu medium after 12 days of incubation and the cultures were supplemented with 10 mg L^{-1} glucose as co-metabolic substrate. B[a]P depletion and fungal growth profiles are shown in Figure 1, which clearly indicates that during 12 days of incubation strain FS10-C continuously degraded B[a]P, with a

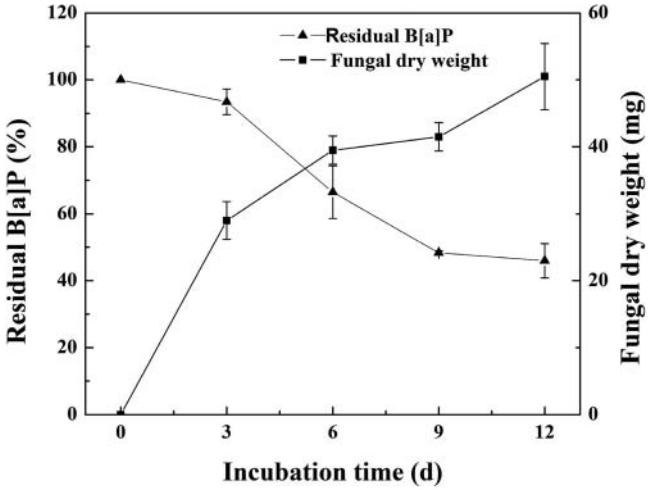

FIGURE 1 Residual B[a]P and fungal growth in liquid medium. Values are mean ± standard deviation of triplicate measurements.

final degradation efficiency reaching 54%. The degradation process corresponded to the fungal growth increment, and after 12 days the fungal biomass reached 50 mg. It should be noted that no extracellular oxidative enzymes (laccase [Lac], lignin peroxidase [LiP], or manganese peroxidase [MnP]) were detected in any cultures throughout the experiment.

Both fungi and bacteria metabolize a wide variety of PAHs. Most fungi cannot use PAHs as sole sources of carbon and energy, but they may co-metabolize PAHs to a wide variety of oxidized products and in some cases to CO_2 (Cerniglia and Sutherland 2010). Some deuteromycete fungi have been confirmed to tolerate and degrade PAHs by means of co-metabolism. Verdin, Sahraoui, and Durand (2004) reported that after 30 days of incubation, the degradation efficiency of B[a]P by *T. viride* strain reached 58%. Chulalaksananukul et al (2006) demonstrated that *Fusarium* sp. E033 was able to degrade 70% of the initial 100 mg L^{-1} B[a]P after 30 days of incubation, and Machín-Ramírez et al (2010) reported that after 5 days of incubation B[a]P (25 mg L^{-1}) was efficiently degraded by *Penicillium* sp. (84%) and *T. harzianum* (77%). However, as far as we know, the present study is the first to report PAH degradation by an isolate of *T. ressei*.

The degradation rates of PAHs by lignolytic fungi are related to the production of lignin-modifying enzymes (Cerniglia and Sutherland 2010). LiP and MnP produced by lignolytic fungi have been acknowledged to be especially important in the degradation of PAHs, and in addition the involvement of Lac secreted by many fungi in the degradation of PAHs is also possible (Wu et al. 2008; Cerniglia and Sutherland 2010; Wen et al. 2011). However, in the present study, no LiP, MnP, or Lac was detected in any of the cultures throughout the experiment. To our knowledge, *T. reesei* has never been described as a ligninolytic strain. The fact that *T. reesei* was able to degrade B[a]P at a high yield, even in the absence of LiP, MnP, and Lac, indicates that a pathway different from the commonly expected extracellular enzymatic system might be involved in PAH degradation. Our results also agree with several reports of degradation of PAHs in cultures of fungi such as *Trichoderma* spp. without detectable activities of LiP or MnP (Schützendübel et al. 1999; Potin, Veignie, and Rafin 2004; Verdin, Sahraoui, and Durand 2004). It is possible that *T. reesei* produces other oxidative and hydrolytic enzymes that we did not analyze but which may potentially have degraded B[a]P. Cerniglia and Sutherland (2010) reported that in species of *Aspergillus*, *Penicillium*, and *Cunninghamella*, the initial transformation of a PAH involved oxidation by a cytochrome P450 monooxygenase and O_2. A frequently investigated nonlignolytic zygomycete, *Cunninghamella elegans*, produced cytochrome P450 and metabolized PAHs with 2 to 5 aromatic rings (Cerniglia 1997; Wang et al. 2000).

PAH removal from Soils by *T. reesei* FS10-C

Concentrations and removal of PAHs in soil under the different treatments after 28 days are shown in Figure 2. Total PAH concentrations in soil in control microcosms and bioaugmented microcosms averaged 9258 ± 65 and 7181 ± 424 μg kg^{-1} dry soil, respectively, corresponding to 4% and 26% PAH removal, respectively. In general, the higher the molecular weight of the PAH molecule, the higher its environmental persistence and toxicity (Bamforth and Singleton 2005). Therefore, 3-, 4-, and 5(+6)-ring PAHs were grouped, and the removal efficiencies of different ring number PAHs were evaluated separately. Figure 2 shows the concentrations of 3-, 4-, and 5(+6)-ring PAHs in soil under the different treatments. In bioaugmented microcosms, the residual levels of 3-, 4-, and 5(+6)-ring PAHs were 284 ± 23, 2841 ± 203, and 4056 ± 198 μg kg^{-1} dry soil, respectively, representing 36%, 35%, and 25% removal. In control microcosms,

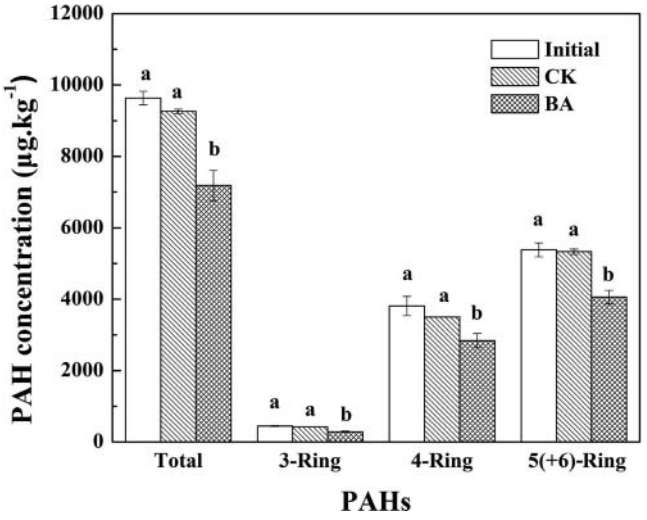

FIGURE 2 **Dissipation of total PAHs and 3-, 4-, and 5(+6)-ring PAHs in soil after 28 days of incubation in soil microcosms. Values are mean ± standard deviation of triplicate measurements. Mean values followed by the same letter are not significantly different among treatments by LSD test at the 5% level. Initial: original soil; CK: control microcosms; BA: bioaugmented microcosms.**

the residual levels of 3-, 4-, and 5(+6)-ring PAHs were 425 ± 5, 3502 ± 2, and 5332 ± 72 μg kg^{-1} dry soil, respectively, with only 4%, 8%, and 1% removal efficiency.

Soils represent one of the major sinks for organic pollutants due to the strong affinity of organic pollutants for naturally occurring organic matter, which is exacerbated by the long aging of contaminants in contaminated soils, and this phenomenon is especially important for high-molecular-weight PAHs (Potin, Veignie, and Rafin 2004; Zhu and Aitken 2010). Bioaugmentation (introducing specific competent microbial strains or communities) and biostimulation (supplementation with carbon sources or other nutrients) have been used as remediation agents to enhance the degradation rates and increase microbial activity during the bioremediation process (Juhasz, Stanley, and Britz 2000; Hamdi et al. 2007). In general, the bioremediation efficacy was more likely to rely on the selectivity and specialization of added microorganisms rather than nutrient load (Hamdi et al. 2007). In order to achieve higher remediation efficiency, both the biodegradation capability and the environmental adaptability of the selected microorganism must be established. Because of other factors such as die-off of laboratory-adapted strains, limited substrate availability, and the inability of the inocula to compete with the indigenous microbial populations or the absence of enzyme activity, some previous studies have shown opposite trends (Juhasz and Naidu 2000; Potin, Veignie, and Rafin 2004).

A number of studies have reported that degradation of PAHs was enhanced after bioaugmentation in PAH-contaminated soils. Silva et al. (2009) found that bioaugmentation with an isolate of *Aspergillus* sp. significantly increased the removal of benz[a]anthracene and B[a]P from soils. Teng et al. (2010) observed that bioaugmentation with *Paracoccus* sp. strain HPD-2 enhanced the degradation of high-molecular-weight PAHs and increased soil microbiological activity. Acevedo et al. (2011) observed a high removal capability for phenanthrene (62%), anthracene (73%), fluoranthene (54%), pyrene (60%), and B[a]P (75%) in autoclaved soil inoculated with *Anthracophyllum discolor* in the absence of indigenous microorganisms. The main objective of the present study was to evaluate the remediation ability of real PAH-contaminated soils by *T. reesei* FS10-C, so an autoclaved soil treatment was not included. After 28 days of incubation, the control microcosms showed low levels of PAH degradation, likely due to the role of indigenous soil microorganisms, and adding nutrients increased the low activity level of indigenous microbial populations, but this process could not be continued because the soil lacked specific competent microbial strains or communities (Johnson, Anderson, and McGrath 2005; Hamdi et al. 2007). Significant removal of total and individual PAHs was observed in the bioaugmented microcosms. Moreover, upon visual examination, *T. reesei* FS10-C showed relevant survival and growth, with the hyphae able to penetrate through the soil to the bottom of the dish. This suggests that this strain may have good potential as a bioaugmentation agent in the remediation of PAH-contaminated soils.

Soil Enzyme Activities and Functional Diversity of Soil Microbial Communities

Organic contaminants are generally considered to have adverse effects on soil microbial functional diversity, influencing soil fertility and plant growth and posing a serious threat to the sustainability of agricultural soils (Sun et al. 2012). Direct measurements of soil microbial community activity and diversity may

therefore provide additional information for evaluation of soil function.

Soil enzyme activity is one indicator of the general condition of the soil microbial population (Teng et al. 2010). Dehydrogenase activity, an intracellular process that occurs in every viable microbial cell, is used to indicate the overall microbial activity of the soil (Epelde et al. 2009). The hydrolysis of fluorescein diacetate has also been used to estimate microbial activity in soils (Sánchez-Monedero et al. 2008). Table 1 reveals the activities of dehydrogenase and fluorescein diacetate hydrolysis in different types of microcosms after 28 days of incubation. Higher dehydrogenase activities were observed in the control and bioaugmented microcosms (211.74 ± 21.08 and 211.05 ± 33.77 μg TPF g^{-1} soil h^{-1}, respectively), with significant enhancement ($p < .05$) compared with the initial soil. Fluorescein diacetate hydrolysis activities were also enhanced in both control and bioaugmented microcosms, reaching 130.33 ± 7.30 and 122.17 ± 16.58 μg fluorescein g^{-1} soil $(20\ min)^{-1}$, respectively, and fluorescein diacetate hydrolysis activity in both control and bioaugmented microcosms was significantly different ($p < .05$) from that in the control microcosms. This suggests that added substrates and some PAH metabolites are likely to stimulate soil microbial growth, which in turn increases the activities of microbial enzymes (Hamdi et al. 2007; Teng et al. 2010).

Soil microbial functional diversity can be determined through the utilization of community level physiological profiles (CLPPs), which reflect the potential of the cultivable portion of the heterotrophic microbial community to respond to carbon substrates (Epelde et al. 2009). Variation in AWCD of soil samples from the different treatments after incubation is presented in Figure 3. The bioaugmented microcosms showed the highest AWCD in soil microbial community carbon utilization profiles, and the control microcosms exhibited the second highest AWCD. Moreover, soil bacterial community metabolic profiles from both the bioaugmented and control microcosms showed significantly ($p < .05$) greater carbon utilization than those from the initial soil.

The Shannon-Weaver index is a measure of actual richness and evenness of the bacterial population, and the Simpson index is used to quantify the number of species as well as the relative abundance of each species (Simpson 1949; Garland 1996). Table 2 lists the diversity indices of the soil microbial communities after 108 h of cultivation in the different treatments after 28 days of bioremediation. The bioaugmented and biostimulated microcosms showed significant

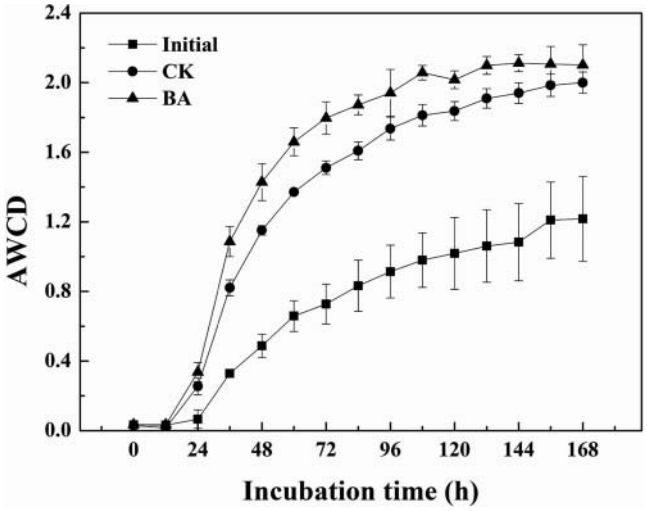

FIGURE 3 Variation in average well color development (AWCD) of soil samples from the different treatments after 28 days of incubation in soil microcosms. Values are mean ± standard deviation of triplicate measurements. Initial: original soil; CK: control microcosms; BA: bioaugmented microcosms.

TABLE 1 Changes in Soil Enzyme Activities after 28 Days of Incubation in Soil Microcosms

Treatment	Dehydrogenase activity (μg TPF g^{-1} soil h^{-1})	Fluorescein diacetate hydrolysis (μg fluorescein g^{-1} soil 20 min^{-1})
Initial	40.87 (± 2.04)[a]	5.74 (± 0.29)[a]
CK	211.74 (± 21.08)[b]	122.17 (± 16.58)[b]
BA	211.05 (± 33.77)[b]	130.33 (± 7.30)[b]

Note. Values are mean ± standard deviation of triplicate measurements. Mean values followed by the same letter are not significantly different by the LSD test at the 5% level. Initial: original soil; CK: control microcosms; BA: bioaugmented microcosms.

TABLE 2 Diversity Index of Soil Microbial Community after 28 Days of Incubation in Soil Microcosms

Treatment	AWCD	Shannon–Weaver index	Simpson index
Initial	0.98 ± 0.16[a]	2.97 ± 0.23[a]	17.30 ± 4.09[a]
CK	1.58 ± 0.20[b]	3.27 ± 0.02[b]	25.17 ± 0.64[b]
BA	1.81 ± 0.26[b]	3.34 ± 0.01[b]	27.24 ± 0.31[b]

Note. Values are mean ± standard deviation of triplicate measurements. Mean values followed by the same letter are not significantly different by the LSD test at the 5% level. Initial: original soil; CK: control microcosms; BA: bioaugmented microcosms.

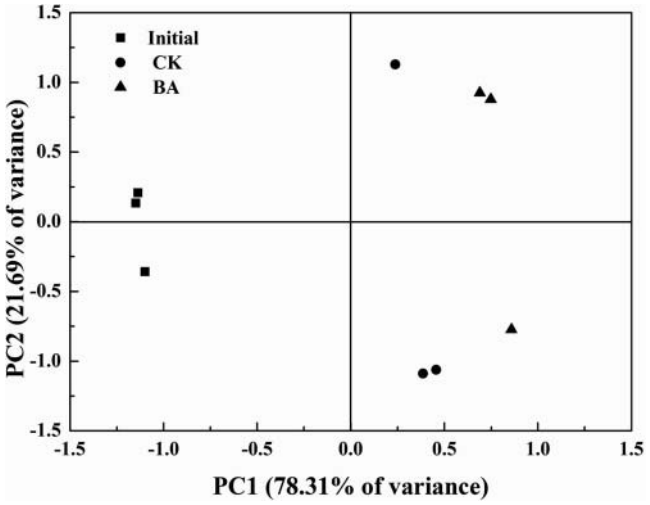

FIGURE 4 **Principal component analysis of carbon utilization profiles from PAH-contaminated soil after 28 days of incubation in soil microcosms. Values are mean ± standard deviation of triplicate measurements. Initial: original soil; CK: control microcosms; BA: bioaugmented microcosms.**

differences ($p < .05$) in the Shannon-Weaver index and Simpson index compared with the control microcosms.

Principal component analysis (PCA) was also conducted to further distinguish the extent of differentiation of the different treatments with regard to soil microbial community carbon utilization profiles (Figure 4). The first and second principal components (PC1 and PC2) explained 78% and 22% of the variance in the data. The bioaugmented microcosms were similar to the control microcosms, and both types were clearly differentiated from the initial soil. Principal component analysis of Biolog data found clear differentiation between treatments that was also observed for substrates that belonged to different classes.

CONCLUSIONS

T. ressei FS10-C, a deuteromycete fungus, showed the ability to degrade B[a]P in liquid medium supplemented with glucose as co-metabolic substrate. Bioaugmentation by *T. ressei* FS10-C used in this microcosm study enhanced PAH degradation in the soil significantly. In addition, bioaugmentation increased microbial activity and diversity in soil, suggesting that this strain of *T. ressei* can increase soil microbiological activity with some restoration of the microbial functioning of the PAH-contaminated soil. Bioaugmentation by *T. ressei* FS10-C is therefore a promising bioremediation strategy for aged PAH-contaminated soil. Elucidation of the potential applicability of this fungus for bioaugmentation needs to be further investigated using different soil types and contaminants under field conditions.

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