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Effect of bioaugmentation by *Paracoccus* sp. strain HPD-2 on the soil microbial community and removal of polycyclic aromatic hydrocarbons from an aged contaminated soil

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**A B S T R A C T**

A microcosm study was conducted to test the bioremediation potential of *Paracoccus* sp. strain HPD-2 on an aged PAH-contaminated soil. Bioaugmented microcosms showed a 23.2% decrease in soil total PAH concentrations after 28 days, with a decline in average concentration from 9942 to 7638 μg kg\(^{-1}\) dry soil. The percentage degradation of 3-, 4- and 5(+6)-ring PAHs was 35.1%, 20.7% and 24.3%, respectively. Higher counts of culturable PAH-degrading bacteria, microbial biomass and enzyme activities were observed in bioaugmented soil. The bioaugmented microcosms showed significant increases (p < 0.05) in the average well-color development (AWCD) obtained by the BIOLOG ecoplate assay and Shannon-Weaver index (H) compared to the controls. Principal component analysis of BIOLOG data clearly differentiated between the bioaugmented and control microcosms, implying that bioaugmentation restored the microbiological functioning of the PAH-contaminated soil. The results suggest that bioaugmentation by *Paracoccus* sp. strain HPD-2 may be a promising bioremediation strategy for aged PAH-contaminated soils.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants that are widely distributed in soils (Wilcke, 2007). Microbial remediation has become a promising approach to remediate PAH-contaminated soils. There are two commonly used types of treatment, namely bioaugmentation by inoculation with PAH-degrading strains and biostimulation by supplementation with carbon sources or other nutrients to stimulate indigenous microbial activity and to increase microbial activity during bioremediation (Hamdi et al., 2007; Mohan et al., 2009). Bioaugmentation (addition of a microbial consortium of selected species isolated from a contaminated soil plus nutrients) is often used in combination with biostimulation, especially when the native soil microbiota does not show the ability to degrade high-molecular-weight (HMW) PAHs efficiently. Bioaugmentation by introduced microorganisms with high degradation capability plays an important role in contaminated soils (Dejonghe et al., 2001; van Herwijnen et al., 2006; Jacques et al., 2008; Silva et al., 2009). In practice, the remediation effect of augmentation depends on both abiotic factors (e.g. soil pH, water and air, temperature, the bioavailability of carbon and energy sources and PAH rings) and biotic factors (e.g. soil biological complexity and the degradative capacity of introduced microbial strains) (Boopathy, 2000; Semple et al., 2006). More importantly, bioaugmentation requires different species of introduced PAH-degrading microorganisms, which can compete with the indigenous microbial community in PAH-contaminated soil, especially if they are to participate in the main carbon and energy flux processes and enhance PAH removal (Dejonghe et al., 2001).

During the last few decades a range of bacteria have been discovered that are capable of degrading PAHs, particularly low-molecular-weight (LMW) compounds such as naphthalene and phenanthrene. Such bacteria belong to the genera *Agerellum*, *Alcaligenes*, *Achromobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Corynebacterium*, *Cyclotrophicus*, *Flavobacterium*, *Micrococcus*, *Mycobacterium*, *Nocardioidea*, *Pseudomonas*, *Lutibacterium*, *Rhodococcus*, *Streptomyces*, *Sphingomonas*, *Stenotrophomonas*, *Sphingobium*, and *Paenibacillus* (Samanta et al., 2002). A few bacteria are known to degrade HMW PAHs such as fluoranthene, pyrene, and benzo(a)pyrene. These include members of the genera *Bacillus*, *Burkholderia*, *Cyclolasticus*, *Flavobacterium*, *Pseudomonas*, *Mycobacterium*, and *Stenotrophomonas* (Kanaly and Harayama, 2000). We recently isolated a bacterial strain identified as *Paracoccus* sp. strain HPD-2 from heavily PAH-contaminated soil.
This organism utilized fluoranthene, pyrene and benzo[a]pyrene as sole carbon and energy source for growth (Mao et al., 2008). However, little is known about the remediation effect of bioaugmentation by Paracoccus sp. strain HPD-2 on removal of polycyclic aromatic hydrocarbons and microbial activities in PAH-contaminated soil. A recent study found that total concentrations of 16 PAHs in some surface soils in the Yangtze River Delta region of east China reached about 10000 μg kg⁻¹ dry soil, with 3-ring, 4-ring and 5(+6)-ring PAH contents accounting for about 8%, 57% and 35% of the total PAH content. Bioremediation of PAH-contaminated agricultural soils has therefore become a major environmental concern in this region.

The ultimate goal of any remediation process must be not only to remove the contaminant(s) from the polluted soil but also, most importantly, to restore the capacity of the soil to function according to the Canadian Environmental Quality Guidelines released by the Canadian Environmental Quality Guidelines (2000). The measurement of microbiological parameters such as microbial biomass, enzyme activities and the diversity of soil microbial communities may serve as a good index of the impact of pollution on soil health (Labud et al., 2007).

In the present study soil microcosms were set up to study bioaugmentation by Paracoccus sp. strain HPD-2 in inoculum to evaluate the bioremediation potential of this strain and examine associated changes in microbial activities in an aged PAH-contaminated soil.

2. Methods

2.1. Soil

The soil used for the experiment was collected from the top 15 cm of the soil profile of PAH-contaminated agricultural land in the Yangtze Delta region of east China. The soil was contaminated approximately 30 years previously and the contaminants have therefore undergone a long weathering process. Gravel and plant root residues in the sampled soil were discarded and the soil was air-dried, sieved through a 2-mm mesh, and stored at 4°C in darkness. Physico-chemical analysis (Lu, 2000) shows that the soil is a silt loam with 11.1 g kg⁻¹ total organic carbon, a pH (in water) of 6.4, 1.0 g kg⁻¹ total nitrogen, 14.7 g kg⁻¹ total potassium, and 78.4 mg kg⁻¹ hydrolysable nitrogen on a dry weight basis. The concentration of 16 individual PAHs was 9942 ± 91 μg kg⁻¹ dry soil, with concentrations of the 3-, 4- and 5(+6)-ring PAHs of 823 ± 30, 5614 ± 119 and 3505 ± 181 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 because of the high concentration of PAHs, and especially 4- and 5(+6)-ring PAHs.

2.2. Bacterial strain and culture conditions

Paracoccus aminovorans strain HPD-2 was isolated from a historically PAH-contaminated soil collected from Wuxi, Jiangsu province, east China (Mao et al., 2008). The strain was screened for its ability to degrade the HMW PAHs fluoranthene, pyrene and benzo[a]pyrene. After incubation in MS medium containing B[a]P at 3.0 mg l⁻¹ for 5 days, 89.7% of the B[a]P was degraded by HPD-2. When this strain was grown in pyrene and fluoranthene at 50 mg l⁻¹ for 7 days, 47.2% and 84.5% of these were degraded respectively. Thus, this strain may have potential in improving HMW-PAH biodegradation. Strain HPD-2 was transferred onto a slant of nutrient agar medium. After 3 days of incubation at 28°C the slant was inoculated into two 500-ml Erlenmeyer flasks containing 100 ml of liquid medium composed of 3 g beef extract and 5 g peptone per liter of deionized water. The flasks were incubated for 48 h at 28°C on a rotary shaker at 200 rpm and produced cell suspensions of 2.0 × 10⁶ CFU l⁻¹ for bioaugmentation.

2.3. Inoculum preparation

Inoculum of strain HPD-2 was prepared using a solid matrix of organic material containing 563.6 g kg⁻¹ organic matter, 20.3 g kg⁻¹ total nitrogen, and 22.8 g kg⁻¹ total phosphorus on a dry weight basis, and with heavy metals (Cu, Zn, Pb, Cd) and 16 PAHs below their detection limits. Two hundred and forty grams of the organic material were adjusted to 50% moisture content with deionized water, mixed thoroughly, sterilized by autoclaving at 121°C for 1 h, then transformed into a granular powder when the temperature declined to ambient, inoculated with 12 ml of the cell suspension of strain HPD-2 based on 10% inoculum size, mixed thoroughly with a sterile glass rod, and cultivated at 30°C for 144 h by stirring every 24 h, thereby establishing an inoculum population of 1.3 × 10⁹ CFU g⁻¹ by solid-state fermentation. Inoculum prepared by this procedure was used for the soil microcosm experiment.

2.4. Soil microcosms

Each soil microcosm was established by placing one kilogram of non-sterile soil in a glass beaker. Bioaugmented soil microcosms were inoculated with 40 g bacterial strain inoculum (SA) prepared as described above. Biostimulated soil microcosms received 40 g sterilized bacterial strain inoculum (SS). Control microcosms were also prepared with no addition of viable or sterilized inoculum (CK). There were three replicates of each treatment. The moisture content of all the microcosms was adjusted to 60% of water holding capacity (WHC). All microcosms were covered with tin foil and incubated for 4 weeks at 28°C in darkness. After 28 days soil samples were collected from each soil microcosm. Each soil sample was divided into two parts, one of which was placed in a small 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 analysis for PAHs.

2.5. Extraction and analysis of soil PAHs

PAHs in bulk soil samples were extracted using Soxhlet extraction. In brief, 5 g of freeze-dried sample with filter paper was placed in a porous cellulose thimble (25 × 70 mm) and placed in a Soxhlet extractor. The extractor was then fitted to a 100 ml round bottom flask containing 60 ml dichloromethane and the extraction was performed for 24 h. All the extracts in the round bottom flasks were dried by rotary evaporation. The residues were dissolved in 2 ml of cyclohexane and 0.5 ml of the solute was transferred and purified with a silica gel column (8 × 220 mm) and washed with a mixture of hexane and dichloromethane (1:1). The first 1 ml of eluate was discarded because it contained non-polar saturated hydrocarbons and was less retained than PAHs by silica gel. The second 2-ml aliquot of eluate was collected, dried by sparging with N₂ and then re-dissolved in 1 ml acetonitrile for HPLC determination.

Determination of 16 EPA PAHs was carried out according to the method of Ni et al. (2008). Briefly, analysis was conducted on a Shimadzu Class-VP HPLC system (Shimadzu, Japan), with a fluorescence detector (RF-10AXL). A reversed phase column C18 (VP-ODS 150 × 4.6 mm I.D., particle size 5 μm), using a mobile phase of water and acetonitrile mixture (1:9, v/v) at a constant solvent flow rate of 0.5 ml min⁻¹, was used to separate the 16 PAHs. The
excitation and emission wavelengths for individual PAHs were set separately.

An external standard mixture was used for quantification of the 16 PAHs. The detection limit of the HPLC method for the 16 PAHs was in the range of 0.12–1.57 μg kg⁻¹. Method blanks (solvent) and spiked blanks (standards of EPA610 PAH mixture, LA 96245, Supelco, USA spiked into soil) were extracted and analyzed by the methods described above. The recoveries and the relative standard deviations of this method for 16 PAHs were in the ranges of 74–110% and 0.53–3.57%, respectively. Results of blanks extracted under the same conditions were below detection limits and sample results without recovery ratio correction are presented.

The percentage of PAH removal (%) was given by the formula: 
removal% = 100 × [(M₀ – Mₙ)/M₀], where M₀ was the concentration of PAHs in each treatment and Mₙ was the initial PAH concentration present in soil.

2.6. Enumeration of PAH-degrading soil bacteria

After incubation, PAH-degrading soil bacteria were counted using a miniaturized most probable number (MPN) method in 96-well microplates with five replicates per dilution (Wrenn and Venosa, 1996). Briefly, phenanthrene, anthracene, fluorene, and dibenzothiophene were added as the sole carbon sources to support the proliferation of aromatics-degrading bacteria. Serially diluted samples were inoculated into the wells and the microplates were incubated at room temperature for 3 weeks. Wells turning yellow or brown owing to the accumulation of partial oxidation products of aromatic substrates were treated as positive. Published MPN tables were used to determine the MPN value.

2.7. Soil microbial biomass C and N

Soil microbial biomass C and N were determined by the fumigation–extraction method (Brookes et al., 1985; Vance et al., 1987). Chloroform fumigation was carried out with ethanol-free CHCl₃ for 24 h at 25 °C in the dark, the CHCl₃ was removed and the soil samples extracted by shaking with 50 ml 0.5 mmol l⁻¹ H₂SO₄ for 30 min on a rotary shaker. The suspensions were then filtered through Whatman No. 42 filter paper. Triplicate sub-samples of unfumigated control soils were stored at 4 °C during fumigation and were extracted in the same way and at the same time as the fumigated samples. Organic C was measured by an automated TOC Analyzer (Shimadzu, TOC-500, Japan). Biomass C (Bc) was calculated from: Bc = 2.22 × Ec, where Ec = [(organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil)]. The factor 2.22 is a proportionality constant, accounting for the observation that about 45% of biomass C is extracted after fumigation. Soil microbial biomass N was estimated from the relationship: biomass N = EN/kEN, where EN is total N extracted from soil minus total N extracted from non-fumigated soil and kEN = 0.54 (Brookes et al., 1985).

2.8. Soil enzyme activities

Soil dehydrogenase activity (DHA) was assessed by a modification of the method described by Singh and Singh (2005). Weighed five-gram sub-samples of soil were placed in 50 ml polypropylene centrifuge tubes and mixed with 5 ml 0.5% 1,3,5-triphenyltetrazo- lium chloride (TTC) solution. Tubes were incubated for 6 h at 30 °C in the dark. After incubation, triphenylformazan (TPF) formed by reduction of TTC was extracted with three batches of 100 ml methanol. Tubes were shaken in an orbital shaker at 300 rpm for 1 h, centrifuged (1744 × g, 5 min), and the supernatant was filtered with filter paper. Blanks without the addition of TTC were carried out in the same manner. The concentration of TPF was determined by spectrophotometry at 485 nm and the results were expressed as g TPF g⁻¹ soil.

Fluorescein diacetate hydrolysis (FDAH) was determined according to the modified method of Adam and Duncan (2001). Briefly, 5 g soil sub-samples were mixed with 15 ml phosphate buffer (pH 7.6) in 50 ml Erlenmeyer flasks and 0.2 ml of a solution of fluorescein diacetate (1000 μg ml⁻¹) in acetone was added to each sample. Flasks were incubated for 20 min at 30 °C on a rotary shaker at 200 rpm. After incubation, fluorescein was extracted immediately from soil by adding 15 ml of 2:1 chloroform:methanol solution, then transferring to a 50 ml centrifuge tube and centrifuging (1744 × g, 3 min) before filtering. Blanks were performed with soil:phosphate buffer suspensions without the addition of FDA and their absorbance readings were subtracted from the above values. Fluorescein concentration was measured using a spectrophotometer at 490 nm. Results were expressed as g fluorescein g⁻¹ soil.

2.9. Physiological profiles of soil microbial community

Soil microbial community level physiological profiles were performed as described by Yao et al. (2003). Briefly, 10 g of fresh soil was added to 100 ml of distilled water in a 250 ml flask and shaken for 10 min. Ten-fold serial dilutions were made and the 10⁻² dilution was used to inoculate the BIOLOG® ECOPlates (BIOLOG, Hayward, CA). 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. Microbial activity in each microplate, expressed as average well-color development (AWCD) was determined as follows: AWCD = ∑ODᵢ/31, where ODᵢ is the optical density value from each well. The Shannon–Weaver index (H) was calculated using an OD of 0.25 as threshold for positive response (Garland, 1997). The Shannon index was calculated using Eq. H = –∑piLnpi, where pi is the ratio of the activity on each substrate (ODᵢ) to the sum of activities on all substrates (∑ODᵢ).

2.10. Statistical analysis

Statistical analysis was carried out using the SPSS 13.0 for Windows software package. Data were analyzed by one-way analysis of variance. Mean values were compared by least significant difference (LSD) at the 5% level using SPSS software.

3. Results

3.1. Soil PAH removal

Concentrations of total PAHs in soil under different treatments after 28 days of bioremediation are presented in Fig. 1. By the end of the experiment soil total PAH concentrations in microcosms receiving bioaugmentation (SA), microcosms receiving biostimulation (SS) and control microcosms (CK) averaged 7638 ± 286, 9307 ± 224, and 9601 ± 91 μg kg⁻¹ dry soil, respectively. Compared with the initial soil PAH concentrations, significant differences (p < 0.05) were observed in treatments SA and treatment SS in which 23.2 and 6.4% of total PAHs were degraded.

Fig. 1 also shows the concentrations of 3-ring, 4-ring, and 5(+6)-ring PAHs in soil under the different treatments. In treatment SA the residual levels of 3-, 4- and 5(+6)-ring PAHs were 534 ± 103, 4451 ± 207 and 2652 ± 74 μg kg⁻¹ dry soil, representing 35.1, 20.7 and 24.3% removal from the initial soil values. Compared with the control, significant degradation (p < 0.05) was observed in 3-, 4- and 5(+6)-ring PAHs in the bioaugmented microcosms. In treatment SS the residual levels of 3-, 4- and 5(+6)-ring PAHs were
792 ± 29, 5259 ± 172 and 3256 ± 167 µg kg⁻¹ dry soil, with only 3.8, 6.3 and 7.1% degradation in the biostimulated microcosms.

### 3.2. Soil PAH-degrading bacteria and microbial activities

Soil PAH-degrading bacterial counts in the different types of microcosm after 28 days of bioremediation are presented in Table 1. Compared with the controls, significantly higher counts (p < 0.05) of PAH-degrading bacteria were observed in bioaugmented microcosms. Furthermore, bacterial counts in bioaugmented microcosms (61.3 ± 3.8 × 10³ MPN g⁻¹ dry soil on average) were 16.6 times higher than in biostimulated microcosms (3.7 ± 0.2 × 10³ MPN g⁻¹ dry soil) and the difference between bioaugmented and biostimulated microcosms was significant (p < 0.05).

After 28 days of incubation both bioaugmented and biostimulated microcosms showed higher soil microbial biomass C and N (p < 0.05) compared with the control (Table 1). Soil microbial biomass C and N in bioaugmented microcosms were 347.1 ± 22.4 µg C g⁻¹ dry soil and 64.2 ± 5.6 µg N g⁻¹ dry soil, increases of 101% and 102% compared with control soil. Soil microbial biomass C and N in biostimulated microcosms were 261.2 ± 19.7 µg C g⁻¹ dry soil and 48.3 ± 6.9 µg N g⁻¹ dry soil, increases of 51.5% and 51.8%. Furthermore, both microbial biomass C and N were significantly higher (p < 0.05) in bioaugmented soil than in biostimulated soil.

### Table 1

Changes in PAH-degrading bacterial counts, microbial biomass and soil enzyme activities after 28 days of bioremediation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PAH-degrading bacteria (×10³ MPN g⁻¹ dry soil)</th>
<th>Microbial biomass carbon (µg C g⁻¹ dry soil)</th>
<th>Microbial biomass nitrogen (µg N g⁻¹ dry soil)</th>
<th>Dehydrogenase activity (µg TPF g⁻¹ dry soil)</th>
<th>Fluorescein diacetate hydrolysis (µg fluorescein g⁻¹ dry soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>1.3 ± 0.3b</td>
<td>172.4 ± 5.3c</td>
<td>31.8 ± 3.5c</td>
<td>231.5 ± 7.2c</td>
<td>114.7 ± 8.2b</td>
</tr>
<tr>
<td>SS</td>
<td>3.7 ± 0.2b</td>
<td>261.2 ± 19.7b</td>
<td>48.3 ± 6.9b</td>
<td>311.7 ± 8.1b</td>
<td>227.1 ± 6.2a</td>
</tr>
<tr>
<td>SA</td>
<td>61.3 ± 3.8a</td>
<td>347.1 ± 22.4a</td>
<td>64.2 ± 5.6a</td>
<td>356.2 ± 7.4a</td>
<td>238.5 ± 6.5a</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations of triplicate measurements. Mean values with the same letter are not significantly different among treatments by the LSD test at the 5% level. CK: control microcosms; SS: biostimulated microcosms; and SA: bioaugmented microcosms.
Higher dehydrogenase activities (DHA) were observed in the soil after 28 days of incubation, with significant enhancement (p < 0.05) in both bioaugmented and biostimulated microcosms (356.2 ± 7.4 and 311.7 ± 8.1 μg TPF g⁻¹ dry soil, respectively) (Table 1). Soil DHA activities in bioaugmented and biostimulated microcosms were 1.54-fold and 1.35-fold higher than those measured in the control (CK). Both bioaugmented (238.5 ± 6.5 μg fluorescein g⁻¹ dry soil) and biostimulated microcosms (227.1 ± 6.2 μg fluorescein g⁻¹ dry soil) also showed significantly higher (p < 0.05) soil fluorescein diacetate hydrolysis (FDAH) activity after 28 days of incubation compared to the control (14.7 ± 8.2 μg fluorescein g⁻¹ dry soil) (Table 1), but there was no significant difference (p > 0.05) between bioaugmented and biostimulated microcosms.

3.3. Physiological profiles of soil bacterial community

Variation of average well-color development (AWCD) for soil samples from the different treatments after 28-day bioremediation period is shown in Fig. 2. The bioaugmented microcosms (SA) showed the highest AWCD in soil bacterial community carbon utilization profiles, the biostimulated microcosms exhibited the second highest AWCD. Moreover, soil bacterial community metabolic profiles from both bioaugmented and biostimulated microcosms showed significantly greater carbon utilization than those from the control (CK) (p < 0.05).

Fig. 3 presents variations in Shannon–Weaver index (H) of soil microbial communities in the different treatments after 28 days of bioremediation. The Shannon–Weaver index was higher in bioaugmented and biostimulated microcosms compared to the controls. The bioaugmented microcosms showed significant differences (p < 0.05) in the Shannon–Weaver index compared to the biostimulated and control microcosms.

Principal Component Analysis (PCA) was also conducted to further distinguish the extent of differentiation of different treatments with regard to soil bacterial community carbon utilization profiles (Fig. 4). The first and second principal components (PC1 and PC2) explained 30.8% and 21.6% of the variance in the data. The bioaugmented microcosms were closer to the biostimulated microcosms, which were differentiated from the control microcosms. The carbon sources significantly correlated with PC1 and PC2 (r > 0.70) under the different treatments are shown in Table 2. Substrates such as D-galactonic acid-γ-lactone, L-arginine, Tween 80, 4-hydroxybenzoic acid, α-cyclodextrin, N-acetyl-D-glucosamine, glycogen, β-cellulose, α-D-lactose, β,β′-glycerol phosphate, D-malic acid, putrescine, β-methyl-β-D-glucoside, D-galacturonic acid, L-asparagine, D-mannite, L-serine, γ-hydroxybutyric acid, and glucose-1-phosphate were intensively metabolized by soil microbial communities in bioaugmented microcosms. Soil microbial communities from both the biostimulated microcosms mainly used β-methyl-D-glucoside, α-galactonic acid-γ-lactone, L-arginine, pyruvic acid methyl ester, D-xylene, β-galacturonic acid, L-asparagine, Tween 40, L-erythritol, 2-hydroxybenzoic acid, L-phenylalanine, Tween 80, D-mannitol, 4-hydroxybenzoic acid and L-serine.

4. Discussion

Bioaugmentation has been proposed as a strategy to enhance bioremediation of contaminated soils. There are three kinds of approach in bioaugmentation, namely (1) inoculation with a functional strain or microbial consortium as a valuable approach to broadening the biodegradation potential of soil (Dejonghe et al., 2001), (2) not to focus on the inoculant but rather on genetic information that can be transferred from an introduced donor strain to the well-established and competitive indigenous bacterial populations of the soil, and (3) the introduction of an unspecified group of bacteria such as those present in soil. Bento et al. (2005) found that bioaugmentation of Long Beach soil had the greatest degradation effect on the light (72.7%) and heavy (75.2%) fractions of TPH. Jacques et al. (2008) observed that a microbial consortium degraded an average of 96–99.99% of different concentrations of anthracene,
phenanthrene and pyrene in the soil in 70 days. Silva et al. (2009) also reported that bioaugmentation with an isolate of Aspergillus significantly increased the removal of benz[a]anthracene and benz[a]pyrene from soils. In the present study, after 28 days of incubation significant removal of total and individual PAHs was observed in the bioaugmented microcosms, with percentage degradation of 3-, 4- and 5(+6)-ring PAHs reaching 35.1%, 20.7% and 24.3%, respectively, indicating a pivotal role for Paracoccus sp. strain HPD-2. Moreover, higher degradation of both 4- and 5(+6)-ring PAHs was observed. This suggests that this bacterial strain may have good potential as a bioaugmentation agent in the remediation of PAH-contaminated soils. However, some other published studies have reported that bioaugmentation did not result in improved PAH removal rate or extent (Vanbroekhoven et al., 2004). Kästner et al. (1998) suggested that the bioaugmentation of PAH-contaminated soils has always been associated with the addition, origin and type of foreign strains of PAH degraders. In a previous study conducted by Tchelet et al. (1999) a strain (PS1) originally isolated from sediments was used in soil columns and sewage sludge for bioaugmentation experiments. The survival and activity of the bacteria in the soil column were successful but the strain was not able to maintain itself in the bioreactor and thus no degradation was observed. Our work further confirms this using Paracoccus sp. strain HPD-2 initially isolated from PAH-contaminated soil. It can clearly be seen that the survival of augmenting strains and the extent of their stimulatory effects on indigenous microbial activities in soil are often the major bottleneck in bioaugmentation processes.

In general, bioaugmentation of PAH-contaminated soils depends on the presence of autochthonous degrading bacteria and inoculation with selected microorganisms with the desired catalytic capabilities (Margesin and Schinner, 1997). PAH-degrading microorganisms are ubiquitous in contaminated soils. Bioaugmentation has shown an important role in bioremediation by promoting degradative microorganisms in soil contaminated with such compounds. The remediation effect of bioaugmentation depends on the specific metabolic capacity of the strain used or the low PAH concentration remaining to support the PAH autochthonous populations in soil. In the present study a significant increase in counts of PAH-degrading bacteria was observed in bioaugmented microcosms after 28 days of incubation and this may have resulted in substantial degradation of 3-, 4- and 5(+6)-ring PAHs in the soil studied. These results are similar to Hamdi et al. (2007) who showed higher proportions of PAH-degrading bacteria in amended soil treatments due to the persistence of spiked PAHs in TOC-enriched soils. Soil microbial biomass is a sensitive indicator for evaluating changes in microbial activity in polluted soils (Moreno et al., 2007). Moreover, we also found that both microbial biomass C and N were significantly higher in bioaugmented soil than in biostimulated soil and this may be attributable to the contribution of the degradative microbial population formed to the soil microbial biomass.

Soil enzyme activity is one way of describing the general condition of soil microbial populations (Margesin and Schinner, 1997; Margesin et al., 2000). Soil dehydrogenase activity (DHA), an intracellular process that typically occurs in all intact and viable microbial cells, can be used to determine overall microbiological activity of soil. Thus, its measurement is usually related to the presence of viable microorganisms and their oxidative capability (Andreoni et al., 2004). Fluorescein diacetate hydrolase (FDAH) has often been used as a sensor and functional indicator of soil health (Adam and Duncan, 2001). As the fluorogenic substrate is taken up by active cells and then transformed by a large array of hydrolytic enzymes, the enzyme has been considered a measure of soil microbial activity (Killham and Staddon, 2002). In the present study soil dehydrogenase activity (DHA) and fluorescein diacetate hydrolysis (FDAH) activities increased significantly in the bioaugmented microcosms. PAHs or their metabolites were likely used as substrates to increasing the soil microbial biomass which in turn increased the activities of the enzymes. Some studies have found that naphthalene and phenanthrene can also stimulate dehydrogenase (Margesin et al., 2000). Changes in enzyme activities in the bioaugmented microcosms also confirm that bioaugmentation with Paracoccus sp. strain HPD-2 showed the highest microbiological activity.

When microorganisms are added to speed up the degradation of organic contaminants in soil, the biological process efficiency depends on the evolution of bacterial communities (Andreoni et al., 2004). 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

<table>
<thead>
<tr>
<th>Control microcosms</th>
<th>Biostimulated microcosms</th>
<th>Bioaugmented microcosms</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td>β-Methyl-α-glucoside</td>
<td>PC1</td>
</tr>
<tr>
<td>Galactonic-γ-lactone</td>
<td>β-Galactonic-γ-lactone</td>
<td>α-Cyclohexanonic acid</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>α-Arginine</td>
<td>Itaconic acid</td>
</tr>
<tr>
<td>4-Hydroxybenzoic acid</td>
<td>Pyruvic acid methyl ester</td>
<td>Glucose-1-phosphate</td>
</tr>
<tr>
<td>α-Cycloextrin</td>
<td>α-Xylose</td>
<td>α-Butanone acid</td>
</tr>
<tr>
<td>Itaconic acid</td>
<td>α-Galacturonic acid</td>
<td>α-α-Lactose</td>
</tr>
<tr>
<td>α-Galacturonate</td>
<td>α-L-Asparagine</td>
<td>α-α-Glycerol phosphate</td>
</tr>
<tr>
<td>L-α-Lactose</td>
<td>α-Erythritol</td>
<td>α-Malic Acid</td>
</tr>
<tr>
<td>Tween 40</td>
<td>PC2</td>
<td>Tween 80</td>
</tr>
<tr>
<td>2-Hydroxybenzoic acid</td>
<td>α-Phenylalanine</td>
<td>α-L-Asparagine</td>
</tr>
<tr>
<td>α-Malic acid</td>
<td>2-Hydroxybenzoic acid</td>
<td>α-Mannitol</td>
</tr>
<tr>
<td>Tween 80</td>
<td>α-Mannitol</td>
<td>4-Hydroxybenzoic acid</td>
</tr>
<tr>
<td>α-Mannitol</td>
<td>α-L-Serine</td>
<td>α-Serine</td>
</tr>
<tr>
<td>2-Hydroxybenzoic acid</td>
<td>γ-Hydroxbyturic acid</td>
<td>Glucose-1-phosphate</td>
</tr>
</tbody>
</table>
5. Conclusions

Bioaugmentation by *Paracoccus* sp. strain HPD-2 used in this microcosm study enhanced PAH degradation in the soil, in particular HMW PAHs such as 4- and 5(+6)-ring PAHs were degraded to some extent. Bioaugmentation can increase PAH-degrading bacterial counts and microbial activities in soils, suggesting that this strain of *Paracoccus* sp. can increase soil microbiological activity, with some resorption of the microbial functioning of the PAH-contaminated soil. Therefore, bioaugmentation by *Paracoccus* sp. strain HPD-2 is a promising bioremediation strategy for aged PAH-contaminated soil. Elucidation of the potential applicability of this bacterium for bioaugmentation will require further studies different soil types and contaminants under field conditions.

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References


