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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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ABSTRACT

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.

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 *Agmenellum*, *Aeromonas*, *Alcaligenes*, *Acinetobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Corynebacterium*, *Cyclotrophicus*, *Flavobacterium*, *Micrococcus*, *Moraxella*, *Mycobacterium*, *Nocardioides*, *Pseudomonas*, *Lutibacterium*, *Rhodococcus*, *Streptomyces*, *Sphingomonas*, *Stenotrophomonas*, *Vibrio*, 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*, *Cycloclasticus*, *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.

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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 its potential (Epelde et al., 2009). Soil microorganisms are very sensitive to any ecosystem function shifts because their activity and diversity are rapidly altered by perturbation (Margesin et al., 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 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 was 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 2.0×10^8 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 1.3×10^9 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 N_2 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 mm), 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