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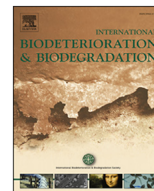

Short communication

Effect of a bacterial consortium on the degradation of polycyclic aromatic hydrocarbons and bacterial community composition in Chinese soils

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ABSTRACT

The metabolism of polycyclic aromatic hydrocarbons (PAHs) in polluted soil is limited by low contaminant bioaccessibility and low degradation by bacteria. A bacterial consortium that degraded 76 per cent of pyrene within 10 days was enriched from a contaminated soil. Hydroxypropyl-β-cyclodextrin (HPCD) enhanced the rate of degradation by the consortium. The bacterial consortium was used to bioremediate PAH-contaminated soil (pyrene and benzo[α]pyrene) in the presence or absence of HPCD. Enhanced PAH mineralization was observed after 10 weeks, particularly in the presence of HPCD, which increased the bioavailability of PAHs. Illumina sequencing was performed to analyze the changes in the bacterial communities. The dominant genus was *Burkholderia* in all treated soils. Importantly, the inoculated bacteria cannot compete with native bacteria and will therefore not invade the community. The significantly stimulated genera were *Burkholderia*, *Nitratireductor*, *Nevskia* and *Sulfuritalea*. These results suggest that the combination of bacteria with HPCD can enhance the bioremediation of PAH-contaminated soil.

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1. Introduction

Among organic soil contaminants, polycyclic aromatic hydrocarbons (PAHs) are one of the most prevalent groups with known or potential toxic, mutagenic, or carcinogenic properties (Ren et al., 2015). PAHs are a substantial threat to ecological function and soil biodiversity (Badejo et al., 2013). Due to their low solubility and bioavailability, PAHs are generally recalcitrant and resistant to microbial attack, particularly high-molecular-weight PAHs (HMW-PAHs), which have four or more fused benzene rings. Because of recalcitrant, HMW-PAHs are persist long time in the environment (Zeng et al., 2010b).

Biodegradation is the most efficient pathway to remove PAHs and restore the ecosystem (Simarro et al., 2013). The potential degradation of PAHs by native microorganisms in polluted soil has been studied extensively (Yrjala et al., 2010). However, less than 1 per cent of the total population of soil microorganisms is adapted to degrade PAHs, which prolongs PAHs removal (Lors et al., 2012). Thus, bioaugmentation via the addition of allochthonous degrading microorganisms is required. Individual strains that can degrade PAHs containing four or more rings have been isolated; these strains primarily belong to the genera *Sphingomonas*, *Mycobacterium* (Wang et al., 2012), *Rhodococcus*, and *Arthrobacter oxydans* (Peng et al., 2012). Microorganisms that can degrade PAHs, particularly those with four or more rings, remain scarce and have yielded unsatisfactory microbial degradation results. Thus, PAH-degrading strains or a bacterial consortium that can be used for PAH removal remain to be discovered. Single microbial strains have been widely used in the bioremediation of PAH-contaminated soils in the laboratory and in situ (Dave et al., 2014b). Microbial consortia appear to be more effective than pure cultures in the biodegradation of PAHs (Dave et al., 2014b). Despite numerous studies of the biodegradation of PAHs in soils using microbial consortia, little information exists on the changes in microbial communities that occur in unpolluted soils exposed to PAHs and bioaugmentation. The changes in the soil microbial community from bioremediation introductions are fundamental to their ecological impact. Microbiological properties, such as soil enzymatic properties and community structure, can provide useful insights into the impact of

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pollution on soil health and bioremediation (Teng et al., 2010b).

The extent and rate of bioremediation of PAHs are often limited by the generally low aqueous concentrations of PAHs and their sorption to sediments. Biodegradation is dependent on bioavailability (Sun et al., 2012). Bioavailability can be increased by the addition of solubility-enhancing agents, such as cyclodextrins (CDs), which facilitate the release of PAHs from the solid phase and increase microbial accessibility to PAHs (Sun et al., 2012, 2013; Dave et al., 2014b). CDs are environmentally acceptable organic molecules that can enhance the biodegradation of PAHs in soil (Stroud et al., 2009). Among CDs, β-cyclodextrin has been widely used due to its cost effectiveness but has low solubility in water. A modified β-cyclodextrin, HPCD, exhibits improved water solubility and lower toxicity, which can be utilize to extract PAHs that are potentially available for biodegradation (Juhasz et al., 2014). HPCD is biodegradable in soil and water and has low toxicity to microorganisms and very high solubility (Reinhardt et al., 2009). However, the effects of HPCD addition with a specific PAH-degrading bacterial consortium on the bioremediation of PAH-contaminated soils are not well understood.

Despite extensive study of microbial populations in polluted soil or sediments, the PAH-degradation potential of a consortium in the presence of HPCD and the changes in the microbial community that occur in unpolluted soils exposed to PAHs are not well understood. The aims of the present study were to enrich a bacterial consortium using pyrene as the sole carbon source, to study the bioremediation potential of the consortium and to investigate the effect of the bacterial consortium in the presence of HPCD on the community structure in soil using Illumina sequencing.

2. Materials and methods

2.1. Soil

Soil samples were collected from the upper 15 cm of the soil profile in the city of Yingtan in Jiangxi Province. The soil was air dried and sieved through a 2-mm mesh to remove stones and roots. The particle size distribution identified the soil as a red clay soil. The organic matter content was 0.66per cent, and the pH was 4.51. The cation exchange capacity (CEC) was 9.06 cmol kg^{-1}. Nutrient levels were 40.8 mg kg^{-1} of available N, 1.33 mg/kg of available P, and 47.0 mg/kg of total K.

The soil samples were treated with pyrene and benzo[α]pyrene. Stock solutions of pyrene and benzo[α]pyrene were prepared in methanol, and 10 ml of the stock solutions was added to air-dried soil samples and stirred thoroughly. The soil samples were left at room temperature overnight to allow the methanol to evaporate and then aged in sealed Erlenmeyer flasks in darkness for 60 days at room temperature. The nominal concentrations of pyrene and benzo[α]pyrene were 200.0 μg g^{-1} and 100 μg g^{-1}, respectively. Measurements were taken before and immediately after the inoculation experiment to measure the concentrations. The treatments were conducted in triplicate.

2.2. Chemicals

HPCD, pyrene and benzo[α]pyrene for soil spiking were obtained from Aladdin (>99.9 per cent, Aladdin Co. Ltd., China). Tenax TA (60–80 mesh) was purchased from Beijing KangLin science and technology company, China. Other HPLC-grade solvents included *n*-hexane (Sinopharm Chemical Reagent Co. Ltd., China).

2.3. The PAH-degrading consortium

A bacterial consortium was enriched from an oil-polluted soil. A sample (5 g) was added to 150 ml of sterilized minimal medium supplemented with pyrene (60 μg ml^{-1}) in a 500-ml Erlenmeyer flask (Zeng et al., 2010a). The consortium was incubated aerobically at 30 °C in the dark and shaken at 200 rpm, and then transferred to fresh medium (20-fold dilution) every fortnight. After seven transfers, the bacterial consortium was used to metabolize pyrene in the presence or absence of HPCD. Samples were collected from the flask at set intervals and extracted as described by Guo et al. (2016) for degradation tests.

The bacterial consortium was cultured in fresh medium containing 3 g l^{-1} of yeast extract for bioremediation. The flasks were cultured in the dark for 24 h at 30 °C and shaken at 200 rpm. The consortium cultures were centrifuged at 4000 rpm for 10 min to harvest the cells and then re-suspended in fresh autoclaved distilled water (repeated twice) for bioaugmentation studies.

2.4. Soil microcosms

The following treatments were utilized in this study: control (CK) containing 200.0 μg g of pyrene and 100.0 μg g^{-1} of benzo[α]pyrene and no HPCD or bacterial consortium with soil moisture maintained at 60 per cent of field capacity; bioaugmentation (CB) inoculated with a cell suspension of the bacterial consortium to obtain an initial bacterial population of 2×10^6 cells g^{-1} of soil; HPCD-assisted bioaugmentation (CM) containing HPCD but omitting bacterial inoculation; and HPCD- and bacterial-assisted bioaugmentation (CMB), which combined HPCD addition and inoculation with the bacterial consortium. After adjusting the soil moisture, all of the treatments were incubated at (25 ± 0.5)°C for 10 weeks in a dark room. The flasks were opened once a week for 15 min to ensure aerobic conditions.

2.5. Bioavailability prediction method validation

Tenax TA was used according to the method of Sun et al.(2013). Briefly, 1 g samples of soil were transferred to separating funnels that each contained 100 ml of deionized water. After the addition of Tenax TA beads (1 g), the funnels were shaken on a rotary shaker. After 6 h of consecutive extraction, the Tenax beads were refreshed, rinsed three times with 20 ml of deionized water, and ultrasound-extracted with 2×20 ml of hexane/acetone (3:1, v/v) for 1 h. All of the extracts were passed over a purification column (1 g of Florida silica and 1 g of anhydrous sodium sulfate) and concentrated to near dryness. The residues were dissolved in 2 ml of methanol for HPLC analysis. All of the treatments were conducted in triplicate.

2.6. Extraction of soil PAHs

PAHs in bulk soil samples were extracted according to the literature (Dave et al., 2014a) with some modifications. Each air-dried soil sample (1 g) was extracted with a 20-ml volume of dichloromethane in an ultrasonic extractor (KQ-500E, Kun Shan Ultrasonic Instruments Co., Ltd, China) for 2 h. The extracts were dried, and the residual material was dissolved in 1 ml of hexane.

2.7. PAHs quantification

The extracted PAHs were quantified by HPLC (Agilent 1100) (Agilent, USA) using a reversed phase C_{18} column (ZORBAX 4.6×200 mm 5 μm, Agilent, USA) and isocratic elution with 90% acetonitrile: 10% water with flow rate 1 ml/min. The UV detector wavelength was 240 nm for pyrene and 295 nm for benzo[α]pyrene. The external standard method was selected for quantification. The mean recovery values for pyrene and benzo[α]pyrene were in the ranges of 75–110 per cent and 85–97 per cent, respectively. The

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