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Degradation of polycyclic aromatic hydrocarbons in soil mesocosms by microbial/plant bioaugmentation: Performance and mechanism

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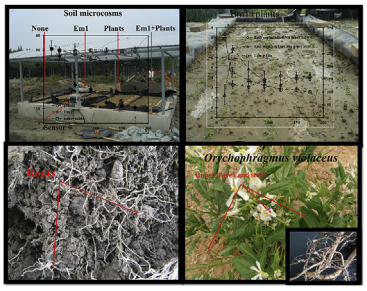
HIGHLIGHTS

- Four bioremediation strategies were employed and the degradation of PAHs was examined in soil mesocosms.
- The elimination of PAHs was greatly enhanced by strain Em1 combined with *O. violaceus*.
- Quantitative PCR indicated that copy numbers of linA and RHD-like gene in the mesocosm with plant were higher.
- Transcript copy numbers of RHD-like gene and 16S rRNA gene of strain Em1 in mesocosm with plant were higher.

GRAPHICAL ABSTRACT



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ABSTRACT

In order to study the degradation of polycyclic aromatic hydrocarbons (PAHs) in an aged and highly contaminated soil, four bioremediation strategies (indigenous microorganisms, microbial bioaugmentation with a PAH-degrading and bioemulsifier-producing strain, *Rhodococcus ruber* Em1, plant bioaugmentation with *Orychophragmus violaceus* and their combination) were compared and the enhanced degradation mechanism was investigated in soil mesocosms. Degradation rates over a period of 175 days showed that Em1 combined with *Orychophragmus violaceus* promoted a significant enhancement of PAHs degradation. In inoculated microcosms with *Rhodococcus ruber*Em1, mineralization reached a lower level in the absence than in the presence of plants. Elimination of PAHs was significantly enhanced (increased by 54.45%) in the bioaugmented mesocosms. Quantitative PCR indicated that copy numbers of linA and *RHD*-like gene (encoding PAH-ring hydroxylating dioxygenase) in the mesocosm with plant were three and five times higher than those in the mesocosm without plant, respectively. Transcript copy numbers of *RHD*-like gene and 16S rRNA gene of strain Em1 in mesocosm with plant were two and four times higher than those in the mesocosm without plant, respectively. Taken together, the results of this study show that plants or *Rhodococcus ruber* Em1 enhance total PAHs removal, moreover their effects are necessarily cumulative by combined *strains* and plants.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are amongst the most widespread organic contaminants in soils, water and wastewater (Puglisi et al., 2007), which can cause serious damage to ecosystems and persist in soil for a long time (Chaîneau et al., 2005). Microbiological decontamination of soil contaminated with PAHs is an efficient, economic and versatile alternative to degrade of PAHs-based pollutants (Balachandran et al., 2012; Sherafatmand &Ng, 2015), However, the bioremediation efficiency of PAHs may inhibited by the low bioavailability and toxicity, non-uniform spatial distribution of microorganisms and pollutants and low capability of microbial metabolism, etc (Li et al., 2017). In this realm, additional in-suit strategies should be integrated into bioremediation process by indigenous microbes to enhance its efficiency in removing organic pollutants in soil environment.

Phytoremediation, i.e., the use of plants to remove contaminants, is considered a promising approach to eliminated organic pollutants such as PAHs from the soil (Seklemova et al., 2001). Laboratory or greenhouse experiments show that plants can promote PAH degradation in soils by stimulating microbial metabolism in the rhizosphere (Frutos et al., 2012). Dissipation of PAHs in the rhizosphere has been correlated with a decrease in toxicity (Peng et al., 2011). However, it is still unclear how roots exert their positive effect. In addition, the effect of byproducts resulting from the biodegradation of PAHs and their possible uptake by plants is rarely studied in phytoremediation experiments.

Addition of surfactant which can increase the solubilization and desorption is a promising means to enhance the bioavailability of PAHs. Low biodegradability of high molecular weight (HMW) PAHs with 4 and more rings is due to their low aqueous solubility and high sorption to soil particles (Couto et al., 2010) thus preventing their elimination from the contaminated soil. Previous studies indicated that dirhamnolipid and monorhamnolipid improved the biodegradation of PAHs in aqueous systems (Peng et al., 2011). Biosurfactants from *Acinetobacter* sp. increased the apparent solubility and biodegradation of PAHs (Haritash and Kaushik, 2009). They are biodegradable, less toxic and cheap in comparison to chemical surfactants (Rosenberg &Ron, 1997; Zhang &Young, 1997). A bioemulsifier-producing bacterial strain capable of degrading some alkanes and PAH compounds was isolated from petroleum-contaminated soil and identified as *Rhodococcus ruber* Em1 (Huang et al., 2007; Li et al., 2003). It had been successfully used for the treatment of refinery wastewater and PAHs-contaminating soil (Huang et al., 2007; Margesin et al., 2000). However, little is known about the effect of bioremediation by combined *Rhodococcus ruber* Em1 and plants on the removal of PAHs and microbial activities in PAH-contaminated soil, which needs to be studied as the soil microorganisms are very sensitive to any ecosystem function shifts because their activity and diversity are rapidly altered by perturbation (Margesin et al., 2000). Moreover, the efficiency of bioaugmentation by *Rhodococcus ruber* Em1 combining with *O. violancus* for the removal of PAHs and microbial activities in PAH-contaminated soil should also be investigated, since soil microorganisms are very sensitive to ecosystem function shifts and their activity and diversity are rapidly altered by perturbation.

In this study, bioaugmentation with strain Em1 and/or *O. violancus* was conducted in different mesocosms in an aged and highly PAH-contaminated under field conditions. Removal performance of PAHs in different soil mesocosms was first studies, and then real-time PCR and transcript-based PCR were employed to detect the numbers of strain Em1 and degradative genes with experiment process, in order to reveal the existence and function of strain Em1 and to assess the potential combination of strain Em1 and *O. violancus* in the bioremediation of highly contaminated soil. This study will be beneficial for the utilization of bioremediation by combined *Rhodococcus ruber* Em1 and plants on the removal of PAHs and microbial activities in PAH-contaminated soil.

2. Materials and methods

2.1. Contaminated soil

Contaminated soil was obtained from the abandoned site of Beijing Coking Plant, Beijing, China, which had been continuously poisoned by coking chemicals for more than 50 years. A high content of PAHs especially HMW-PAHs were present, indicating a heavy pollution by these compounds according to US EPA Guidelines. After sifting (2 mm) to remove the cinders and stones, the contaminated soil was blended thoroughly. Some principal properties (including TOC, total nitrogen and total phosphorus et al.) of the soil were measured and summarized in Table S1 of the supporting information.

2.2. Plant and bacterial strain

Orychophragmus violaceus, a biennial herb belonging to *Crucuferae* family, is widely distributed in China in various environments, such as (i.e., plains, mountains and roadsides). *O. violanceus* is commonly used for forage, health care and gardening (Luo et al., 1998), and is highly regarded for its great economic and ornamental usages. Various topics have been studied on *O. violancus*, including phytoremediation for heavy metal (Ma et al., 2011). In recent years, this plant has also been used in many cities in China as a ground cover plant in gardens, streets and understory in a large scale.

Rhodococcus ruber Em1 was used in this study. Strain Em1 could degrade n-alkanes and PAHs, and produce bioemulsifier resulting in decrease of the surface tension of distilled water from 72 mN/m to 30 mN/m (Zhi-Pei and Shuang_Jiang, 2004). Strain Em1 was grown in LB on a rotating shaker (160 rpm) at 30 °C for 24 h. Then, the cells were harvested by centrifugation at 6000 × *g* for 30 min, washed twice with sterile distilled water and resuspended in sterile distilled water to $OD_{600} = 1.0$, as the inoculum for remediation experiments. *O. violaceus* seeds were coated with cells of strain Em1 by submerging them in cell suspension prepared above for 2 h, and then implanted into soil. Growth of *O. violaceus* was monitored by the cumulated length of leaves and biomass increase.

2.3. Set up of ex situ remediation mesocosms

Pilot scale *ex situ* remediation mesocosms were conducted under outdoor conditions as detailed in Table 1. All soil mesocosms were periodically tilled to improve aeration and to promote soil homogeneity for biological degradation. In order to follow the PAH degradation, five soil samples (10 g for each) were taken once every two weeks from each mesocosm for a period of 175 days. The samples (10 cm beneath the surface) were collected from the center of the field and four places 30 cm to the corner, and then they were mixed thoroughly as one sample. Rhizosphere soil samples were collected after the whole experimental period - shaking off the soil from the roots.

The environmental temperature at the experimental site was noted daily both during day andnight, and it varied between a minimum of 17 °C and a maximum of 35 °C during the study.

2.4. PAH analysis

PAHs in the soil samples were extracted by ultrasonic extraction method as recommended by US EPA method 3550C. One gram of

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