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The influence of root exudates of maize and soybean on polycyclic aromatic hydrocarbons degradation and soil bacterial community structure

Meixia Guo[a,b], Zongqiang Gong[a,*], Renhui Miao[c], Dan Su[d], Xiaojun Li[a], Chunyun Jia[a], Jie Zhuang[a]

[a] Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China
[c] International Joint Research Laboratory for Global Change Ecology, College of Life Sciences, Henan University, Kaifeng, Henan 475004, PR China
[d] School of Environmental Sciences, Liaoning University, Shenyang 110036, PR China

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ABSTRACT

In order to determine the influence of plant root exudates on degradation of polycyclic aromatic hydrocarbons (PAHs) and the change of bacterial community structure in soils, we added different total organic carbon (TOC) concentrations of maize (*Zea mays* L.) and soybean (*Glycine max* L.) root exudates in basal salts medium (BSM), pyrene-spiked soil, and original PAH-contaminated agricultural soil, respectively. PAHs degradation in the above liquid and soil microcosms inoculated with *Mycobacterium* strain was studied. The changes of bacterial community structure in agricultural soil were assessed using 16S rDNA based polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE). Results demonstrated that the addition of maize and soybean root exudates led to a significant enhancement in the degradation of PAHs at the early stage. With possible depletion of root exudates, the effect declined. The removals of total PAH in the aged agricultural soil with maize root exudates (69.2%–78.4%) were higher than those with soybean root exudates (66.8%–74.5%). Shannon diversity indexes (H') for soil bacterial diversity in the former were lower than those in the latter. There was an increase of H' values for soils with root exudates compared with those for control soil. Our results indicate that the *Mycobacterium*-root exudate interactions accelerated the removal of PAHs by increasing the biodegradation of pollutants and the diversity of soil bacterial community structure in PAH-contaminated agricultural soil.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of hazardous and ubiquitous pollutants that can persist in environments for long time due to their hydrophobicity and chemical stability (Sun et al., 2010; Cvancarova et al., 2013). Human exposure to PAHs may occur through multiple ways, such as air, soil, food, water, and occupational exposure (D'Orazio et al., 2013). So far, more and more attention has been paid to effective remediation of PAH-contaminated soils (Haritash and Kaushik, 2009).

Phytoremediation, as a cost effective and environment-friendly method for the environmental decontamination, is defined as the use of plant and associated microorganisms to remove, or degrade contaminants in soils (Toyama et al., 2011; Phillips et al., 2012). Many studies have shown the suitability of phytoremediation for decontaminating PAH-contaminated soils (D'Orazio et al., 2013; Tejeda-Agredano et al., 2013; Liu et al., 2014; Storey et al., 2014). The PAH dissipation in the plant rhizosphere can be significantly improved compared to that in unplanted soils (Fan et al., 2008; Liu et al., 2013). In fact, the positive effect of plants on PAH removal is mainly attributed to the enhancement of general microbial activity and the increase of PAH solubility via root exudates (Cébron et al., 2011; Louvel et al., 2011; Thomas and Cebron, 2016). Plant roots are also able to increase microbial biomass and diversity by releasing nutrients, exudates and oxygen into soils (D'Orazio et al.,

* Corresponding author.
E-mail address: zgong@iae.ac.cn (Z. Gong).

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2013; Muratova et al., 2015). However, the contrasting tendencies that plants or exudates have either no effect or even an inhibitory effect on PAH degradation have been reported (Rezek et al., 2008; Phillips et al., 2012). The role of plant in PAH removal is still not clearly understood (Louvel et al., 2011). To our knowledge, several crop species have been investigated in PAHs rhizoremediation assays, such as maize, rice and wheat (Xu et al., 2006; He et al., 2015; Shahsavari et al., 2015), but there is no study on soybean. Soybean is a typical leguminous crop in the world. Legumes influence overall rhizosphere microbial community structure, and may affect its function in response to PAH-stress (Kawasaki et al., 2012). However, the influence of soybean root exudates on PAH degradation and soil bacterial community structure in contaminated agricultural soils is unknown. Till now no study was carried out on the effect of the concentrations of root exudates on PAHs degradation and bacterial community structure in aged PAH-contaminated agricultural soils.

To better control the rhizoremediation processes, it is necessary to determine the influence of root exudates on the diversity and activity of PAH-degrading bacteria in soil (Cébron et al., 2011). Therefore, the aims of the present study are to investigate the effects of total organic carbon (TOC) concentrations of root exudates from maize and soybean on the biodegradation of PAHs in different microcosms including both aqueous and soil systems, also on the change of bacterial community structure during the inoculation time. To attain these objectives, we studied the catabolism of pyrene in basal salts medium (BSM), spiked soil, and $\sum$12PAHs in an original contaminated agricultural soil by *Mycobacterium* strain and indigenous microorganisms. The molecular tool of polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) was applied to assess the bacterial community in the aged soils with the amendment of maize or soybean root exudates.

2. Materials and methods

2.1. Soils

Two agricultural soils were used in this study. One PAH-uncontaminated soil sample, ES, was collected from Shenyang Ecological Experimental Station (Shenyang, China). The organic matter content of this soil was 2.64%, and the percentages of clay, silt, and sand were 16%, 30%, and 54%, respectively. The other original PAH-contaminated soil, WI, was collected from a wastewater-irrigated area in a suburb of Shenyang, China. The organic matter content of this soil was 2.4%, and the percentages of clay, silt, and sand were 61.4%, 28.5%, and 10.1%, respectively. The total ($\sum$12PAHs) and individual PAH concentrations in the WI soil are listed in Table S1. Both soils were collected from the surface layer (0–20 cm) of the sites. They were air dried, sieved through a 2-mm mesh to remove small rocks and plant material, homogenized, and kept in darkness at 4 °C until analysis.

To prepare the pyrene-spiked soil, referred as SP, 30 mg pyrene (HPLC, 98%, Sigma-Aldrich, USA) was dissolved in 300 mL acetone solution and which was uniformly added to a portion (0.6 kg) of the ES soil. After volatilization of acetone in fume hood for 48 h at room temperature (20–25 °C), this portion of soil was then mixed thoroughly with a large portion (5.4 kg) of ES soil to achieve the final pyrene level of 5 mg kg^{-1}.

2.2. Microorganism and inoculum preparation

The PAH-degrading bacterium used in this study was *Mycobacterium gilvum* (Genbank accession number DQ512892), which was described previously (Guo et al., 2016). The strain was incubated in fresh BSM and placed in a shaker at 28 °C for 5 d. The number of *Mycobacterium* sp. in BSM for following inoculation was determined by most probable number assay. BSM constituents were described elsewhere (Toyama et al., 2009).

2.3. Collection of root exudates

Maize (*Zea mays* L.) and soybean (*Glycine max* L.) seeds were purchased from Shenyang Agriculture University (Shengyang, China). Before planting, maize and soybean seeds were sterilized according to the method described by Toyama et al. (2011). Then the seeds were germinated on sterile Hoagland's nutrient medium (Louvel et al., 2011). Each seeding was aseptically transferred to a flask containing 300 mL of sterile Hoagland's nutrient medium and maintained in a growth chamber (7000 Lx, 16/8 h,) at 28 °C. After one-month growth, root exudates were collected, rinsed twice, and transferred to containers filled with 1 L sterile distilled water for 6 h of exudation (Louvel et al., 2011). Then the root exudates were collected, filter sterilized at 0.45 μm (Nalgene filter units) and measured for the TOC concentrations using a Shimadzu TOC-5050A TOC Analyzer, which were 4082 and 1548 mg L^{-1} for maize and soybean root exudates, respectively.

2.4. Experimental design for PAH degradation

2.4.1. Pyrene degradation in BSM under the effects of root exudates

First, Mycobacterium sp. was grown in 20 mL pyrene-BSM (10 mg L^{-1}) with three TOC concentrations of maize or soybean root exudates (16, 32 and 64 TOC mg L^{-1}, respectively) in 50 mL Erlenmeyer flasks. The amounts of *Mycobacterium* sp. in individual flasks were equal and were about 10^5–10^6 cells mL^{-1} BSM. In all, five treatments were performed for each type of root exudates: (i) control-B: BSM without root exudates and *Mycobacterium* strain; (ii) M-B: BSM with Mycobacterium strain but no root exudates; (iii) MR1-B: BSM with root exudate (concentration 1, 16 mg TOC L^{-1}) and *Mycobacterium* strain; (iv) MR2-B: BSM with root exudate (concentration 2, 32 mg TOC L^{-1}) and *Mycobacterium* strain; (v) MR3-B: BSM with root exudate (concentration 3, 64 mg TOC L^{-1}) and *Mycobacterium* strain. Triplicate flasks for each treatment were incubated at 28 °C on a rotary shaker at 120 rpm and sampled on 1, 2, 4, 6 and 9 days for pyrene analysis.

2.4.2. PAH degradation in soil microcosms

The *Mycobacterium* inoculum suspension was diluted with BSM and added to 150 g of pyrene-spiked soil or aged PAH-contaminated agricultural soil to a final concentration of approximate 10^5 cells g^{-1} soil. Maize and soybean root exudates were diluted with sterilized water to 200, 400 and 800 mg TOC L^{-1}, respectively. Then 30 mL of diluted root exudates was added to 150 g of soils and homogenized to a final added TOC concentration of 40, 80 or 160 mg kg^{-1} soil. In all, six treatments were performed for each type of root exudates and each soil: (i) Control-SP and Control-WI: SP and WI soils without root exudates and *Mycobacterium* strain, respectively; (ii) R-SP and R-WI: SP and WI soils with root exudates (80 mg TOC kg^{-1}) but no *Mycobacterium* strain, respectively; (iii) M-SP and M-WI: SP and WI soils with *Mycobacterium* strain but no root exudates, respectively; (iv) MR1-SP and MR1-WI: SP and WI soils with root exudates (40 mg TOC kg^{-1}) and *Mycobacterium* strain, respectively; (v) MR2-SP and MR2-WI: SP and WI soils with root exudates (80 mg TOC kg^{-1}) and *Mycobacterium* strain, respectively, and (vi) MR3-SP and MR3-WI: SP and WI soils with root exudates (160 mg TOC kg^{-1}) and *Mycobacterium* strain, respectively. For each microcosm, the moisture was adjusted to 55% soil water holding capacity by weighing the microcosm. The flasks were hermetically sealed and incubated in darkness at 28 °C for 3 (5 for SP soil), 10, 20 and 30 days. The incubated soils