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# Interactions between *Pteris vittata* L. genotypes and a polycyclic aromatic hydrocarbon (PAH)-degrading bacterium (*Alcaligenes* sp.) in arsenic uptake and PAH-dissipation☆

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ABSTRACT

The effects of two *Pteris vittata* L. accessions and a polycyclic aromatic hydrocarbon (PAH)-degrading bacterium (*Alcaligenes* sp.) on arsenic (As) uptake and phenanthrene dissipation were studied. The *Alcaligenes* sp. survived in the rhizosphere and improved soil As bioavailability with co-exposure. However, bacterial inoculation altered *Pteris vittata* L. stress tolerance, and substantially affected the As distribution in the rhizosphere of the two *P. vittata* accessions. Bacterial inoculation was beneficial to protect the Guangxi accession against the toxic effects, and significantly increased plant As and phenanthrene removal ratios by 27.8% and 2.89%, respectively. In contrast, As removal was reduced by 29.8% in the Hunan accession, when compared with corresponding non-inoculated treatments. We conclude that plant genotype selection is critically important for successful microorganism-assisted phytoremediation of soil co-contaminated with As and PAHs, and appropriate genotype selection may enhance remediation efficiency.



## 1. Introduction

Co-contamination with arsenic (As) and polycyclic aromatic hydrocarbons (PAHs) is gaining great attention, since these two contaminants are frequently found in anthropogenic industrial contaminated sites, and even in urban residential areas (Sun et al., 2014; Elgh-Dalgren et al., 2009; Kay et al., 2008). The combination of As and PAHs could strongly potentiate the environmental risks in a synergistic manner (Li et al., 2010). Consequently, remediation of As and PAHs co-contamination is of particular interest for environmental protection and public health.

*Pteris vitatta* L. has previously been identified as a promising material for the As and PAH phytoremediation because of its As hyperaccumulation capacity, as well its high tolerance to co-exposure to As and PAHs (Sun et al., 2011). However, use of *P. vitatta* to remediate As and PAH co-contamination is limited by the low efficiency of PAH degradation. The convergence of phytoremediation and microbial bioremediation is recognized as one of the most effective strategies for soil remediation, and is particularly promising for addressing contamination with organic compounds such as PAHs (Gan et al., 2009). Microorganism-assisted phytoremediation, involving interactions between plant roots, the soil, and microorganisms, has the advantages of stimulating microbial activities, improving soil conditions and supplying root exudates. Together, these effects lead to enhanced degradation of organic pollutants through direct metabolism or co-metabolism (Gerhardt et al., 2009). A variety of PAH-degrading microorganisms have been isolated from contaminated soils to date. The successful use of such microbial isolates, together with plants, in the remediation of PAH-contaminated soils has been well-documented (Lu et al., 2010; Gao et al., 2006). However, it is unclear whether a combination of *P. vitatta* and PAH-degrading microorganisms might be advantageous for addressing As and PAH co-contamination than the use of these strategies individually. With confirmation and optimization, such an approach could have enormous implications for remediation of co-contamination. Recently, preliminary testing

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of this hypothesis was carried out by Feng et al. (2014), and provided some insight into the potential use of the technology.

A successful co-contamination remediation strategy is dependent on having suitable plant-microorganism pairs. There is consensus that the plant genotype is a predominant factor in obtaining the benefits from microorganisms (Nwoke et al., 2009). Interactions between the plant genotype and microorganism might cause large differences in plant growth through, for example, influencing root morphology and microbial activity. Such interactions may lead to positive or negative effects upon plant nutrient utilization and metabolism (Andreote et al., 2010). Genotypic differences in As tolerance and hyperaccumulation have been reported in different *P. vittata* species (Wu et al., 2009). Because the interactions between plants and microorganisms are complex, the first step for determining the suitability of their co-application is screening for responsive and appropriate plant genotypes. This screening process could help to optimize the phytoremediation and biodegradation benefits of plant-microorganism coupling.

The selection of PAH-degrading microorganisms that have intrinsic tolerance to As and PAH co-contamination should also be considered. In general, an introduced PAH-degrading bacterial strain may achieve an acceptable removal rate of PAH in case it has the capacity to colonize the soil and survive. However, high metal concentrations can impose an additional stress on the microbial population, and inhibit its biodegradation of organic pollutants. Consequently, selection of PAH-degrading bacterial strains with As resistance might be a way to overcome this limitation.

This study aimed to determine the effectiveness of microorganism-assisted phytoremediation for soil co-contaminated with As and PAHs, using co-application of *P. vittata* with PAH-degrading bacteria. Two As-hyperaccumulating genotypes, Guangxi and Hunan, were used. Natural variability in As-accumulation capacity exists between these two fern accessions, which is attributed to differential environmental adaptations. We evaluated combinations of *P. vittata* genotypes and PAH-degrading bacteria on As uptake and PAH-dissipation from soil. Our results may provide practical insight into the suitability of this remediation strategy for remediating As and PAH co-contamination.

## 2. Materials and methods

### 2.1. Plant propagation and soil preparation

Spores were collected from fertile fronds of *P. vitatta* grown at two As-contaminated sites in Guangxi and Hunan province, China. Spores were germinated and propagated in As- and PAH-free seed base. After two months, 6–7 fronds from similarly-sized plants each were transplanted to pots for experiments.

Sandy loam (9.0% clay, 36.4% silt, 54.7% sand) soil was collected from a 20-cm layer of uncontaminated agricultural land in Beijing, China. The soil had the following properties: pH 8.11, 32 cmol/kg CEC, 2.49% organic matter, 0.17% nitrogen (N), 0.10% phosphorus (P), 8.82 mg/kg As, and undetectable levels of PAHs. The soil was air dried and sieved through 2-mm mesh, and was then autoclaved three times at 120 °C for 30 min over a 3–4 d period, with at least a 24-h interval between autoclavings to eliminate indigenous microbial populations. The sterility of the soil was confirmed by plating soil suspensions onto LB and PDA medium. Phenanthrene was selected as representative PAH as it is widely distributed throughout the environment. As was added in the form of $Na_2HAsO_4{\cdot}7H_2O$. As and phenanthrene dissolved in sterilized deionized water and in acetone, respectively, were spiked into the soil to achieve final concentrations of 89.66 mg/kg As and 50.01 mg/kg phenanthrene. To ensure homogeneous As and phenanthrene distributions in the soil, a spiking/rehydration process was applied to the dry, spiked soil (Reid et al., 1998). The soil was mixed thoroughly and then equilibrated in the dark in a sterile room for 4 months before starting the experiment.

### 2.2. Bacterial incubation

The PAH-degrading bacterial strain used in the experiment was isolated from soil of a coking plant in Beijing, China. The soil was contaminated with up to 228 mg/kg As and 34 mg/kg PAH. The strain was isolated by enrichment culture using phenanthrene as the sole source of carbon, and was identified as *Alcaligenes* sp. by Peking University. The bacterial strain was gram negative, motile, and formed short rods. After 3 d incubation at 25 °C, colonies appeared non-pigmented and translucent, with smooth surfaces and sizes ranging from 1 to 3 mm in diameter. The phenanthrene dissipation rate of the *Alcaligenes* sp. was tested over three weeks, and 60% phenanthrene could be mineralized as the sole carbon source. The strain was cultured with mineral medium (composition as described in Lu et al., 2011) in the presence of 50 mg/L phenanthrene using a shaking incubator at 25 °C and 200 rpm. To form the inoculum, bacterial cells were harvested at log phase by centrifugation at 6500 rpm, and then washed twice with 0.9% NaCl, and resuspended in 5 mL sterilized deionized water. The innoculum was transferred to the soil with an initial bacterial concentration of approximately $10^6$ CFU $g^{-1}$ dry soil. As resistance of the *Alcaligenes* sp., defined as the lowest As concentration that completely inhibited the growth of strain, was determined using mineral medium (with 50 mg/L phenanthrene as carbon source) amended with different concentrations of arsenate. The strain showed the minimum inhibitory concentration of 4000 mg/L for arsenate.

### 2.3. Experimental design

Sterilized rhizopots (designed by Gonzaga et al., 2006) containing 1.5 kg of spiked soil was used to grow the plants. Plastic frames covered with 45-μm nylon mesh cloth were used to separate the rhizosphere (0.3 kg) from the bulk of the soil (1.2 kg) in each rhizopot. The plants were transplanted to the central compartment within nylon cloth such that the roots were confined. The experiment consisted of six treatments: control (CK), bacterial inoculation (B), Guangxi accession cultivation (G), Hunan accession cultivation (H), bacterial inoculation-Guangxi accession cultivation (BG), and bacterial inoculation-Hunan accession cultivation (BH). There were four replicates for each treatment. The rhizopots were placed randomly in a growth chamber with an average temperature of 22–25 °C and 75–80% humidity. Soil moisture was maintained at 70% of field capacity by weight. Soil solution samples to monitor As and iron (Fe) concentrations, and pH, were collected 1 d before harvest using a soil moisture sampler installed in the root compartment. The plants were harvested after 60 d of growth.

### 2.4. Bacterial quantification

The number of PAH-degrading bacteria in the soil was determined by conventional plating techniques, using the mineral medium described for bacterial culture, which contained 50 mg/L phenanthrene. Three replicates for each dilution were plated and incubated at 25 °C, and colonies were counted after 5 d. The number of bacteria was expressed as colony-forming units (CFU) $g^{-1}$ dry soil.

### 2.5. Chemical analysis

Plant and soil samples were digested using a modified EPA Method 3050B. The improved sequential extraction procedure was