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Arsenic transformation and plant growth promotion characteristics of As-resistant endophytic bacteria from As-hyperaccumulator *Pteris vittata*

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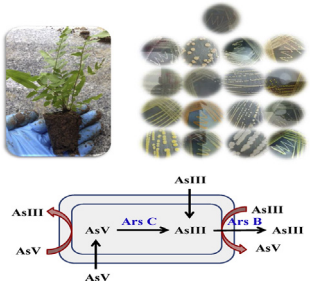
HIGHLIGHTS

- 43 As-resistant endophytic bacteria were identified from *Pteris vittata*.
- All endophytes showed plant growth promotion characteristics.
- The roots isolates showed higher tolerance to AsV.
- The leaflet and stem endophytes showed higher tolerance to AsIII.
- Bacterial As resistance was positively correlated to their ability in AsV reduction.

GRAPHICAL ABSTRACT



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ABSTRACT

The ability of As-resistant endophytic bacteria in As transformation and plant growth promotion was determined. The endophytes were isolated from As-hyperaccumulator *Pteris vittata* (PV) after growing for 60 d in a soil containing 200 mg kg^{-1} arsenate (AsV). They were isolated in presence of 10 mM AsV from PV roots, stems, and leaflets, representing 4 phyla and 17 genera. All endophytes showed at least one plant growth promoting characteristics including IAA synthesis, siderophore production and P solubilization. The root endophytes had higher P solubilization ability than the leaflet (60.0 vs. 18.3 mg L^{-1}). In presence of 10 mM AsV, 6 endophytes had greater growth than the control, suggesting As-stimulated growth. Furthermore, root endophytes were more resistant to AsV while the leaflet endophytes were more tolerant to arsenite (AsIII), which corresponded to the dominant As species in PV tissues. Bacterial As resistance was positively correlated to their ability in AsV reduction but not AsIII oxidation. The roles of those endophytes in promoting plant growth and As resistance in *P. vittata* warrant further investigation.

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1. Introduction

Arsenic (As), a toxic metalloid, is ubiquitous in the environment, which results from both natural and anthropogenic sources (Abernathy et al., 2003). Due to its toxicity and carcinogenicity, As contamination in soils poses a potential risk to public health (Zhao

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et al., 2010). Therefore, it is necessary to develop remediation strategies to reduce its effects on ecosystems and humans.

Phytoremediation is a promising technology to remove As from soils, which is cost-effective and environment-friendly compared to traditional remediation methods. Arsenic hyperaccumulator *Pteris vittata* (PV) has a potential to be used for phytoremediation in As-contaminated soils, which accumulates up to 2.3% As in the fronds (Ma et al., 2001). However, most hyperaccumulators are unsuitable for phytoremediation due to their low biomass and low As uptake (Lebeau et al., 2008). So plant-associated microbes have attracted attention for their roles in promoting plant growth and enhancing metal resistance in contaminated soils (Glick, 2010).

Endophytic bacteria (endophytes) colonize the internal plant tissues with little negative effects on their host (Schulz and Boyle, 2006). They help to promote plant growth under adverse conditions and have potential to improve phytoremediation (Ryan et al., 2008). Their beneficial effects on plant growth have been attributed to their ability in indole acetic acid (IAA) synthesis, siderophore production, and phosphate solubilization (Rajkumar et al., 2009). While IAA helps plant growth, siderophores solubilize Fe from soils to enhance plant Fe uptake. Many endophytic bacteria from hyperaccumulators have shown plant growth promotion (PGP) characteristics. For example, Luo et al. (2011) isolated 30 endophytes from Cd-hyperaccumulator *Solanum nigrum* from a mine tailing, with 70% strains producing IAA at 1.60–122 mg L^{-1} and 8 strains solubilizing phosphate at 22–400 mg L^{-1}. Visioli et al. (2014) isolated 5 endophytes from Ni-hyperaccumulator *Noccaea caerulescens*, showing ability of siderophore production. However, endophytes isolated from PV grown in clean soils by Zhu et al. (2014) showed limited PGP characteristics. It is possible that PGP ability of a plant is related to its metal resistance. Therefore it is necessary to isolate and characterize As-resistant endophytic bacteria from PV grown in As-contaminated soils.

Besides PGP traits, endophytes exhibit high resistance to metal stress, which helps to alleviate metal phytotoxicity and improve plant growth (Sessitsch et al., 2013). Since hyperaccumulators have high metal concentrations in their biomass, endophytes probably have developed mechanisms to adapt to high metal stress (Idris et al., 2004). For instance, Long et al. (2011) found endophyte from *Sedum alfredii* tolerated Zn and Cd while endophytes from hyperaccumulators *Thlaspi goesingense*, *Alyssum bertolonii*, and *S. nigrum* showed high tolerance to Ni and Cd (Barzanti et al., 2007; Idris et al., 2004; Luo et al., 2011).

However, only one study focused on endophytes from PV growing in a clean soil (Zhu et al., 2014). It is known that microbial composition and diversity are affected by soil As contamination. Furthermore, endophytes from hyperaccumulators growing in contaminated soils are probably more tolerant to metals than those from clean soils. Zhu et al. (2014) found that the ability of PV endophytes to reduce AsV and oxidize AsIII was related to their As tolerance. Nevertheless, the study had limited number of PV endophytes. In addition, rhizosphere bacteria of PV have been investigated for their As resistance and As transformation, some rhizobacteria showed a dual function of AsIII oxidation and AsV reduction (Ghosh et al., 2011; Huang et al., 2010; Wang et al., 2012). However, there is limited information on endophytes associated with PV and their roles in As tolerance and transformation.

The objectives of this study were to: (1) isolate and characterize As-resistant endophytic bacteria from PV grown in a soil containing 200 mg kg^{-1} AsV, and (2) explore the relationship between bacterial ability in As resistance and As transformation. The results from these investigations are important for better understanding As tolerance and transformation mechanisms in As-resistant endophytes and help to improve phytoremediation of As-contaminated soils.

2. Materials and methods

2.1. Plant cultivation in As-spiked soils

To obtain endophytic bacteria with high As-resistance, PV plants were grown in a soil containing 200 mg kg^{-1} AsV for 60 d (Xu et al., 2014). The plants were propagated using spores from University of Florida, Gainesville, USA. The spores were sown onto a moist soil (40% soil, 40% peat moss, and 20% quartz sand) in seedling boxes with a transparent cover to maintain moisture. After spores were germinated and developed to sporelings with 2–3 fronds, they were transplanted into plastic pots with 10 cm diameter. They were cultivated until they had 5–6 fronds and ~10 cm in height before transplant.

Surface soil (0–15 cm) was collected from Nanjing, China. The soil properties were as follows: pH 7.02, 0.98% OC, 6.79 mg kg^{-1}As, 673 mg kg^{-1} Mn, and 27.5 g kg^{-1} Fe (Xu et al., 2014). The soil was air-dried, sieved through a 2-mm sieve and mixed thoroughly with solution containing $Na_2HAsO_4{\cdot}7H_2O$ to reach 200 mg kg^{-1} As (Sigma–Aldrich, ≥98%). Basal fertilizers (120 mg kg^{-1} N as NH_4NO_3, and 30 mg kg^{-1} P and 75.5 mg kg^{-1} K as K_2HPO_4) and 1% organic matter were mixed in the soil. After aging for two weeks at ~50% field capacity, PV plants were transplanted into one per pot with 3 replicates. The plants grew in a growth chamber with 25 °C average temperature, 70% humidity, 16 h photoperiod and light density of 350 μmol m^{-2} s^{-1}. The plants were watered daily as required and grew for 60 d before being harvested.

2.2. Isolation of As-resistant endophytic bacteria

Three PV plants were collected and washed thoroughly with Milli-Q water, and then separated into the roots, stems and leaflets, which are referred to as R, S and L for their location. The endophytic bacteria were isolated according to Zhu et al. (2014). The tissues were surface-sterilized by sequential immersion in 70% ethanol and 2% sodium hypochlorite solution for 30 min, and then rinsed thoroughly with sterile distilled water. To validate surface disinfection, 100 μL of final washing water was plated onto Luria–Bertani (LB) agar media, followed by incubation at 28 °C for 3 d and the plates showed no microbe.

The surface-sterilized plant tissues were macerated in a sterile mortar containing 10 mL sterile phosphate buffered saline (pH 7.2), and ground with a sterile pestle. Meanwhile, sterile quartz sand was added to improve cell wall disruption. Tissue extracts (100 μL) and their serial dilutions were plated onto modified LB agar media (Ghosh et al., 2011) supplemented with 10 mM As ($Na_2HAsO_4{\cdot}7H_2O$). After incubation at 28 °C for 7 d, colonies with different morphologies were picked out and purified by re-streaking onto the modified LB agar media. The isolate colony was ensured to reach morphologically homogenous. All purified bacteria were stored in 15% glycerol at −80 °C until analyses.

2.3. Identification of As-resistant endophytic bacteria

The bacterial isolates were cultured overnight in LB broth and then extracted for genomic DNA by an EZNA® Bacterial DNA Kit (Omega Bio–Tek, USA). The quantity and quality of the extracted DNA were determined by a Nanodrop ND–2000 Spectrophotometer (NanoDrop Co., USA). Further identification was carried out through PCR amplification of 16S rRNA following Zhu et al. (2014). The amplification primers were 27F (5′–GAGTTTGATCACTGGCTCAG–3′) and 1492R (5′–TACGGCTACCTTGTTACGACTT–3′) (Zhu et al., 2014). PCR products were examined electrophoretically in a 1.5% (w/v) agarose gel.

The amplified products of ~1400 bp 16S rRNA were sequenced