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Arsenic uptake, arsenite efflux and plant growth in hyperaccumulator *Pteris vittata*: Role of arsenic-resistant bacteria

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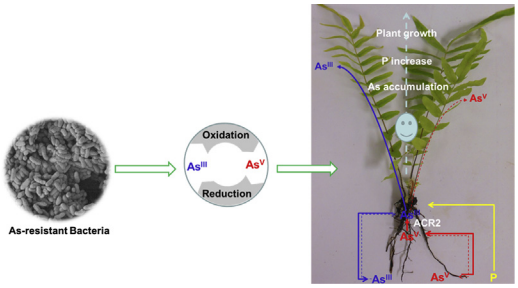
HIGHLIGHTS

- As-resistant endophytic and rhizospheric bacteria were investigated.
- All bacteria were AsV reducers except one strong AsIII oxidizer.
- *Pteris vittata* (PV) sporophytes took up more AsV than AsIII.
- Bacteria significantly enhanced As uptake and plant growth of PV.
- Large amount of AsIII effluxed by PV roots in sterile-media with 37.5 mg kg^{-1} AsV.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacteria-mediated arsenic (As) transformation and their impacts on As and P uptake and plant growth in As-hyperaccumulator *Pteris vittata* (PV) were investigated under sterile condition. All As-resistant bacteria (9 endophytic and 6 rhizospheric) were As-reducers except one As-oxidizer. After growing two months in media with 37.5 mg kg^{-1} AsV, As concentrations in the fronds and roots were 3655–5389 (89–91% AsIII) and 971–1467 mg kg^{-1} (41–73% AsIII), corresponding to 22–52% decrease in the As in the media. Bacterial inoculation enhanced As and P uptake by up to 47 and 69%, and PV growth by 20–74%, which may be related to elevated As and P in plants ($r = 0.88–0.97$, $p < 0.05$). Though AsV was supplied, 95% of the As in the bacteria-free media was AsIII, suggesting efficient efflux of AsIII by PV roots (120 μg g^{-1} root fw). This was supported by the fact that no AsV was detected in media inoculated with As-reducers while 95% of AsV was detected with As-oxidizer. Our data showed that, under As-stress, PV reduced As toxicity by efficient AsIII efflux into media and AsIII translocation to the fronds, and bacteria benefited PV growth probably via enhanced As and P uptake.

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1. Introduction

Arsenic (As) has received increasing concern due to its toxic and carcinogenic impacts on human health (Abernathy et al., 2003). Both natural and anthropogenic activities lead to As pollution in the environment (Zhu et al., 2014a). Consumption of crops growing in polluted soil and water increases As intake by humans (Ohno et al., 2007). Thus, it is important to reduce plant As uptake, thereby reducing its impact on humans.

Pteris vittata (PV) is the first known As-hyperaccumulator, which can accumulate up to 2.3% As in the fronds and has potential to remediate As-contaminated soils (Lessl et al., 2014; Ma et al., 2001).

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Arsenic has been shown to enhance PV growth as much as 46% (Xu et al., 2014). Since arsenate (AsV) and P are chemical analogs, they are both transported by P transporters in plants including PV (DiTusa et al., 2015; Poynton et al., 2004; Wang et al., 2002). Although several studies have focused on AsIII uptake mechanisms in PV (Mathews et al., 2011; Wang et al., 2011), it is still unclear how AsIII enters PV roots.

Compared to non-hyperaccumulators such as *Oryza sativa* and *Arabidopsis thaliana*, PV accumulates more AsV than AsIII (Dai et al., 2013; Meharg and Jardine, 2003; Zhao et al., 2002). Once being taken up, AsV is reduced to AsIII in plant roots and most AsIII is sequestered as AsIII-thiols or pumped out of the roots in non-hyperaccumulators (Danh et al., 2014), but it is not the case for PV, which accumulates AsIII as the predominant species in the fronds (Danh et al., 2014). So it is possible that non-hyperaccumulators detoxify As by rapid reduction of AsV to AsIII and efflux of AsIII to the external medium, whereas little efflux has been observed in PV roots (Huang et al., 2011; Su et al., 2008; Xu et al., 2007). However, microbial transformation of As in the medium probably makes it difficult to determine the amount of AsIII effluxed by the roots.

Bacteria-mediated As transformation including oxidation and reduction influences As uptake and speciation in rice plants (Jia et al., 2014; Zhang et al., 2015). Similar observations were found for rhizospheric bacteria from PV (Ghosh et al., 2011; Lampis et al., 2015; Wang et al., 2012). These studies, however, focused only on bacterial solubilization of As minerals. Similar study for endophytic bacteria from PV tissues also confirmed their roles in As transformation (Xu et al., 2016; Zhu et al., 2014b). Yet, no research has investigated the roles of bacteria on both As transformation in growth media and plant As uptake by PV. Therefore, the objective of this study was to investigate the impact of As transformation by bacteria and their effects on As uptake and growth by PV plants.

2. Materials and methods

2.1. Bacterial isolates and bacteria-free PV

Nine endophytic bacteria from PV roots (R5, R17 and R19), stems (S1, S2 and S7) and fronds (L2, L8 and L11) and six rhizospheric bacteria (PG5, PG6, PG9, PG10, PG12 and PG16) were isolated previously (Ghosh et al., 2011; Zhu et al., 2014b). Bacterium YHB6-1 capable of AsIII oxidation was isolated from PV rhizosphere (NCBI accession NO. KP986958.1). All bacteria were As-resistant as they were isolated in the presence of 75 mg L^{-1} As (Ghosh et al., 2011; Zhu et al., 2014b).

To remove the impact of bacteria on As transformation, sterile PV were used. The ferns were germinated under sterile condition from spores (Mathews et al., 2010). Briefly, PV spores collected from Florida, USA were soaked in water for 30 min, then in 70% ethanol for 30 s, and followed by disinfection with 10% NaClO for 30 min. The disinfected spores were soaked in autoclaved-water for 30 min and washed 3–5 times to remove chemicals. Successful disinfection was verified following Luo et al. (2011). After incubating the spores on Luria–Bertani (LB) agar medium at 30 °C for 4 d, no bacterial growth was observed. The spores were germinated in Petri dish with sterile 1/2 Murashige and Skoog (MS) agar medium (0.8% at pH 5.7). When PV gametophytes were formed in 2 weeks, they were transplanted into fresh medium in autoclaved containers. Six-month old sporophytes with 3–5 fronds (~2.5 g) were used for this study. To verify the sterile status of MS medium during the six months, photos of 1.5-month old gametophytes, and 3- and 6-month old sporophytes of PV were taken, no bacterial growth on the agar medium was observed (Fig. 1).

2.2. As transformation by bacteria

All 16 isolates were incubated in a medium containing 37.5 mg L^{-1} AsIII and 37.5 mg L^{-1} AsV in modified LB medium with 1.08% (m/v) sucrose shaking at 180 r min^{-1} at 30 °C for 24 h. Their growth was evaluated at 600 nm using an UV spectrophotometer (UV–2550, Shimadzu, Japan). The supernatant of bacterial suspension was collected and analyzed for total As and As speciation (Xu et al., 2014).

2.3. Bacterial impacts on As uptake and PV growth

Based on As tolerance and transformation traits, three endophytic bacteria (R19, S7 and L2) and two rhizospheric bacteria (PG6 and YHB6-1) were tested for their impacts on plant growth and As accumulation in PV. They were first grown in modified LB medium for 12 h at 30 °C and 180 r min^{-1}. After washing 3 times by PBS and resuspension, 200 μL of bacterial suspensions at $OD_{600} = 1.5$ were added to the roots of six-month old PV sporophytes. The ferns grew on 1/2 MS agar medium containing 37.5 mg kg^{-1} AsV in a bacteria-free growth chamber. After 2 months of growth, PV sporophytes were harvested and separated into the fronds and roots.

Before chemical analysis, PV roots were placed under running distilled water, rinsed with P buffer (1 mM Na_2HPO_4, 10 mM MES and 0.5 mM $Ca(NO_3)_2$ at pH 5.7) and washed again with distilled water to remove As adsorbed on the root surface (Mathews et al., 2010).

2.4. Chemical and statistical analysis

For As analysis in PV, plant tissues stored at −80 °C for 12 h were freeze-dried (FreeZone 6 plus, Labconco, USA) and ground using liquid nitrogen. For total As in all samples, EPA Method 3050B for the Hot Block Digestion System was used (Environmental Express, USA) (Xu et al., 2014). For As speciation, PV tissues were extracted with methanol:water (1:1, v/v) under ultrasonication for 2 h three times (Zhang et al., 2002).

For As speciation in the growth medium, 0.5 g dry weight sample was mixed with 30 mL solution consisting of 1 M phosphoric acid and 0.5 M ascorbic acid and then maintained at 105 °C for 10 min in the Hot Block Digestion System (Giral et al., 2010). Total As was determined by inductively coupled plasma mass spectrometry (ICP-MS, NexION 300, PerkinElmer, USA), and As speciation was determined by high performance liquid chromatography (HPLC; Waters 2695, USA) coupled with ICP-MS.

All experiments were performed in triplicates and values were expressed as mean ± standard deviation. Analysis of variance and Tukey's multiple comparison tests were done using GraphPad Prism 6.0, and least significant difference (LSD) was determined at $\alpha = 0.05$.

3. Results and discussion

3.1. All As-resistant bacteria were AsV reducers except one oxidizer

Evaluation of bacterial ability in As transformation helps elucidate their impact on As bioavailability in the growth media and plant uptake in PV. In this study, we determined bacterial growth and As transformation in the media containing 37.5 mg L^{-1} AsIII and 37.5 mg L^{-1} AsV under sterile condition. Two groups of As-resistant bacteria were included: rhizospheric and endophytic bacterial isolates. Though all bacteria were isolated in the presence of 75 mg L^{-1} AsV, their growth rates were impacted differently by As during 24 h of inoculation. While the presence of As slightly reduced the growth of rhizospheric bacteria (87–98% of the