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Arsenic-enrichment enhanced root exudates and altered rhizosphere microbial communities and activities in hyperaccumulator *Pteris vittata*

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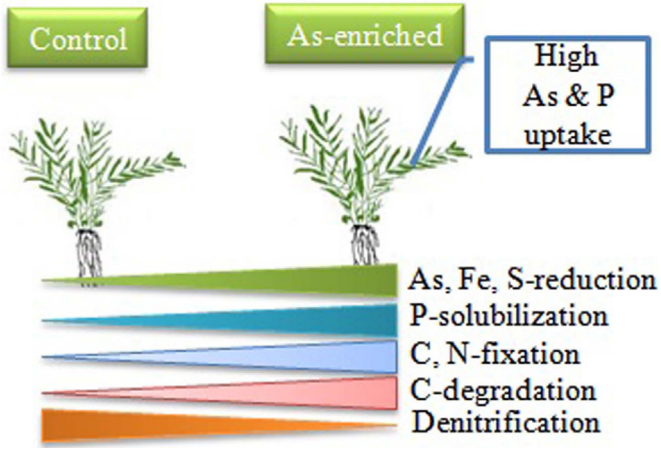
HIGHLIGHTS

- As-enrichment enhanced root exudates and increased rhizosphere soil pH of *P. vittata*.
- As-enrichment enhanced As and P mobilization, uptake and growth of *P. vittata*.
- As-enrichment enhanced Fe- and S-reducing gene abundance of *P. vittata* rhizosphere.
- C and N fixation in *P. vittata* rhizosphere increased due to As-enrichment.
- As-enrichment reduced microbial community in the rhizosphere of *P. vittata*.

GRAPHICAL ABSTRACT



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ABSTRACT

Phytoremediation of arsenic (As)-contaminated soil by hyperaccumulator *Pteris vittata* is promising. A better understanding of the rhizosphere microbial dynamics that regulate As availability and plant growth is important to optimize the phytoremediation process. In this study, Illumina sequencing of 16S rRNA genes was applied to assess the rhizosphere microbial community structure of *P. vittata*. Microbial functionality was monitored by soil enzyme activities and MPN-PCR targeting genes of interest. Arsenic (100 mg kg^{-1} AsV) addition to soil significantly increased DOC, root exudates, As and P uptake and the frond biomass of *P. vittata*. Moreover, As-enrichment significantly increased soil enzyme activities involved in N, P and S cycling and the gene abundance of As transforming bacteria, Fe- and S-reducing bacteria and N and C fixing bacteria in the rhizosphere of *P. vittata*. Together, the results revealed that the combined selective pressure of As and rhizosphere resulted in stimulation of microbial community,

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which most likely has a role in reductive dissolution of Fe and S, As and P mobilization, C degradation and fixation, and N fixation. These changes appeared to have a role in mitigation of As toxicity and to promote growth and the As uptake ability of *P. vittata* under As-enriched conditions.

1. Introduction

Arsenic-contamination in soil is a pressing current public health issue globally. This is because the incorporation of this toxic metalloid into the food chain poses long term risks to human health and a reduction in crop yield in As-contaminated areas [1,2]. Remediation of As-contaminated soil by means of phytoextraction (the use of metal/metalloid accumulating plants to remove contaminants from the environment and concentrate them in the above-ground plant tissue) is gaining popularity because compared to other techniques, it is eco-friendly and cost-effective [3]. Phytoremediation of As-contaminated soil by a hyperaccumulator *Pteris vittata* is promising and has received considerable attention in recent years [4–6]. It has been reported that the frond of *P. vittata* can accumulate 100-fold As from As-contaminated soil [4]. In the past few years, many studies have investigated the phytoremediation efficiency and As uptake mechanism of *P. vittata* as well as the factors regulating the growth of the plant for maximum removal of As from As-contaminated soils [7–9]. However, the potential influence of rhizosphere microbial communities and activities on As uptake and accumulation by *P. vittata* has had little attention.

Apart from As transformation (oxidation, reduction and methylation) processes, microorganisms play a pivotal role in dissolution of As-minerals in soil, which in turn make As labile and available to plants [10]. Xiong et al. [11], using a functional gene microarray (GeoChip 3.0), revealed that rhizobacteria playing a role in As, S, P, C and N transformation likely influence the As uptake and hyper-accumulation processes of *P. vittata*; however, the potential rhizobacteria were not identified. Notably, past studies investigating the effects of As stress on the rhizosphere microbiome of *P. vittata* have been largely based on culturing techniques [12–15], which are now known to select only a small subset of the actual soil population [6]. In earlier studies [12–15] and in a recent study [16], As-resistant, −reducing and −oxidizing bacteria in the rhizosphere of *P. vittata* were identified and characterized to shed light on As transformation in a *P. vittata* rhizosphere; however, the rhizosphere microbial community potentially influencing nutrient (mostly C, N, P, S, Fe) cycling that may control As-uptake ability and plant growth under As-stressed conditions have not been studied. In this study, using a high throughput sequencing technique, we explored the complex rhizosphere microbial community up to the lowest taxonomic level (species) because taxonomy compositions at lower levels can differ significantly [17], and we additionally linked the identified species to their probable role in the rhizosphere of *P. vittata*. In addition, the gene abundance of bacteria involved in As transformation, Fe and S reduction, C and N fixation and denitrification were assessed to understand the microbial expression profile in the rhizosphere of *P. vittata* on soil As contamination.

Soil microbes are the source and sink of plant nutrients and are instrumental in nutrient cycling [18]. Soil enzyme activities are sensitive indications of microbially-driven nutrient cycling and soil pollution by heavy metals/metalloids [18,19]. Although As-toxicity inhibits As-sensitive bacteria and their activities essential for soil functions, As-resistant bacteria proliferate by using the debris of killed sensitive microorganisms as an energy source and thus stimulate microbial processes that inactivate As toxicity [18,19]. The changes in the rhizosphere microbiota of *P. vittata* due to As-contamination are expected to interfere with nutrient cycling with possible beneficial effects on the plant–soil association [11,18]. In the present work, activities of soil enzymes indicative of C-cycling (β-glucosidase), N-cycling (protease, urease), P-cycling (phosphodiesterase) and S-cycling (arylsulfatase) were evaluated [18–20]. It has been well documented that root exudates not only sustain high levels of microbial activity but also can be regarded as important in altering rhizospheric chemical compositions that facilitate As uptake by *P. vittata* [5]. In this study, the differences in root exudates of *P. vittata* grown in control and As-spiked soil were evaluated.

The hypothesis of our study posits that the root exudate characteristics of *P. vittata* as influenced by As-contamination alter rhizosphere microbial communities and activities that promote As-hyperaccumulating ability and plant growth under As-enriched conditions. The objectives of the study were to (1) characterize root exudates of *P. vittata*, (2) determine physicochemical changes in the rhizosphere of *P. vittata*, (3) study the rhizosphere microbial community and abundance, and (4) study soil enzyme activities involved in C, N, P and S cycling in the rhizosphere of *P. vittata* under control and As-enriched conditions.

2. Materials and methods

2.1. Experimental design and sampling

P. vittata, a Chinese Brake fern, was propagated following the protocol described by Apuan et al. [21] and was maintained in a greenhouse. Uniform plants of similar height and with the same number of fronds (4–5 fronds) were chosen for the pot experiment. For the pot experiment, surface soil (0–15 cm) was collected from an abandoned site in the Hsuechia experimental site (23°12′4.02″ N and 120°10′50.7″ E), Taiwan. The soil was sandy loam in texture with 11.0% clay, 14.0% silt and 75.0% sand, pH (H_2O) 6.4, cation exchange capacity 11 cmol kg^{-1}, total organic carbon 1.2%, available (Olsen) phosphorus 6.3 ± 0.8 mg kg^{-1}, total As content 12.0 ± 0.6 mg kg^{-1}. The soil was air-dried and sieved through a 2 mm mesh. One half of the soil was mixed thoroughly with 100 mg kg^{-1} As(V) ($Na_2HAsO_4{\cdot}7H_2O$) and left for 3 weeks, whereas the other half served as the control soil. Basal fertilizers (120 mg kg^{-1} N as NH_4NO_3, 30 mg kg^{-1} P and 80 mg kg^{-1} K as K_2HPO_4) were added to all the soils and mixed thoroughly. Control and As-enriched soil (3.0 kg) were placed into plastic pots (18 cm diameter × 22 cm height) lined with a polythene sheet. The *P. vittata* plants were transplanted individually into the plastic pots and allowed to grow for 60 days in a growth chamber (28/16 °C day/night temperature with a relative humidity of 70%, 10/14 h light/dark photoperiod and a light density of 300 μmol/m^2s^{-1} in the daytime). The plants were irrigated with distilled water throughout the growth period to keep the soil at ~80% of its field capacity.

The rhizosphere (immediately surrounding the root) soil and soil pore water were collected at 0, 30 and 60 days after transplantation (DAT), whereas root exudates were collected 60 DAT. Each rhizosphere soil sample was divided into two subsamples i.e., for chemical and microbial analysis. The rhizosphere pore water sampling was done using a rhizome soil moisture sampler (RSMS, Soil Moisture Equipment Corp., USA) used at the rooting zone of the