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Arsenic contamination influences microbial community structure and putative arsenic metabolism gene abundance in iron plaque on paddy rice root

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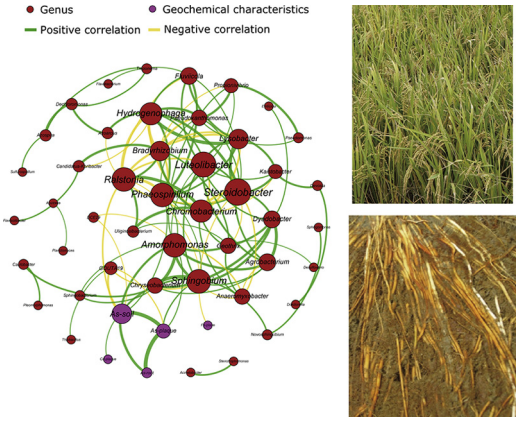
HIGHLIGHTS

- As contamination affects rice-root Fe-plaque microbiota community structure.
- *ars*C and *ars*B are the most abundant in As-contaminated soil-derived plaque.
- As contamination affects the arsenic metabolism gene abundance.

GRAPHICAL ABSTRACT



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ABSTRACT

Iron (Fe) plaque on rice roots contains a unique microbiota that connects the root and rhizosphere environments. However, the factors controlling the microbial community structure and function in Fe plaque are unknown. We performed Illumina sequencing of 16S rRNA gene amplicons and of total community DNA to compare the microbial community structure and metabolic potential of Fe plaques derived from arsenic (As)- and non-contaminated sites. *Geobacter* and *Hydrogenophaga* were identified as the genera that differed significantly in abundance between As-contaminated and control samples ($P < 0.05$). Significant differences were found between contaminated and control samples in the relative abundances of predicted As functional genes of the microbial community in Fe plaque, in which the relative abundances of the *arsC* (encoding As(V) reductase) and *arsB* genes (encoding As(III) efflux membrane protein) in Fe plaque from contaminated sites (YH and TP samples) were significantly higher than those from the control samples ($P < 0.05$). In addition, the As concentration in Fe plaque contributed significantly to the relative abundance of genes related to As metabolism and correlated most strongly with the abundance of *arrB* genes (encoding respiratory arsenate reductase, FeS subunit). These results suggest that As contamination influences the community structure and metabolic potential of Fe plaque-associated microorganisms and may help in understanding the environmental behavior of As at the interface of Fe plaque.

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1. Introduction

Arsenic (As) is the most ubiquitous environmental toxic element and causes health problems worldwide (Zhu et al., 2014). As contamination in paddy soil has attracted much attention because As(V) is easily reduced to the more mobile As(III) and taken up by plant roots during the flooding stage of rice production (Meharg and Rahman, 2003). The microbe-mediated transformations of As species in soils usually involve oxidation (mediated by As(III) oxidase, which is encoded by *aioAB* genes), reduction (As(V) reductase, *arrAB* and *arsC* genes), and methylation (As(III) methyltransferase, *arsM* genes) (Stolz et al., 2006). Recent studies on paddy soil have shown that As uptake by rice is influenced by microbe-mediated As redox changes in the rhizosphere (Jia et al., 2014).

Rice roots are often coated with Fe(III) (oxyhydr)oxide precipitates, referred to as Fe plaque (Zhang et al., 1999). The structural identity of Fe plaque is characterized as a mixture of crystalline and amorphous Fe(III) (oxyhydr)oxides, mainly in the form of ferrihydrite and goethite (Chen et al., 1980). The chemical and physical properties of Fe plaque are similar to those of Fe(III) (oxyhydr)oxides in soil, which are generally considered to have high adsorption capacity for inorganic anions (Chen et al., 2005; Zhang et al., 1999). Fe plaque can sequester significant amounts of phosphate and metals, including As, Zn, Cu, and Pb (Liu et al., 2008; Liu et al., 2007; Zhou and Shi, 2007). This sequestering can prevent the excessive uptake of toxic metals by plants grown in waterlogged soil (Liu et al., 2008; Liu et al., 2007; Liu et al., 2006). Because of the high As adsorption capacity of Fe(III) (oxyhydr)oxides coated on Fe plaque, the As concentrations in Fe plaque are much higher than that in rhizosphere soil (Liu et al., 2006). It has been reported that As distribution and speciation in the rhizosphere are influenced by Fe plaques and affect As accumulation by rice plants (Yamaguchi et al., 2014).

The formation of Fe plaque on rice roots is thought to be facilitated by the release of oxygen and oxidants into the rhizosphere (Chen et al., 2005). However, recent studies on microbial communities in root Fe plaque on wetland plants (*Sagittaria australis* and *Leersia oryzoides*) demonstrated high abundances of Fe(II)-oxidizing bacteria (FeOB) (*Thiobacillus ferrooxidans* and *Sideroxydans paludicola*) associated with rhizosphere Fe(II) oxidation (Emerson et al., 1999; Neubauer et al., 2007), which are facilitated by high Fe(II) availability and microoxic conditions in the immediate vicinity of plant roots (Neubauer et al., 2007). Studies using scanning or transmission electron microscopy have shown the presence of bacterial cells in the Fe matrix surrounding plant roots (St-Cyr et al., 1993; Trolldenier, 1988), and bacterial cell numbers were positively correlated with the total amount of Fe present in the Fe plaque on *Sagittaria australis* and *Leersia oryzoides* (Emerson et al., 1999).

The biosphere of Fe plaque should be different from that of rhizosphere soil because of differences in the concentration of As, root secretions, and oxygen. We previously demonstrated that the microbial community structure of Fe plaque is distinct from those of bulk and rhizosphere soils. *Acidobacteriales*, *Myxococcales*, and *Desulfuromonales* predominated in rhizosphere and bulk soil. However, the microbiota of Fe plaque was enriched with *Pseudomonadales*, *Burkholderiales*, *Sphingomonadales*, and *Rhizobiales* (Hu et al., 2015). A wide distribution and high diversity of microbial As metabolism genes have been identified in various paddy soils (Xiao et al., 2016; Zhang et al., 2015). Soil pH; electron conductivity; total C, N, As, and Fe; C/N ratio; SO_4^{2-}-S; NO_3^--N; and NH_4^+-N were identified as the key factors affecting the diversity of the microbial community involved in As biotransformation in paddy soils (Zhang et al., 2015). However, the distribution, diversity and abundance of genes responsible for As metabolism have not been characterized in Fe plaque. More importantly, the geochemical factors controlling the distribution of As metabolism genes in Fe plaque should be investigated. In the present study, Fe plaque samples were collected from As-polluted and non-contaminated paddy soils. The goals of this study were to determine whether As contamination influences the microbial community structure of Fe plaque and to explore the impact of As contamination on the metabolic potential of the Fe plaque microbial communities by metagenomic analysis.

2. Material and methods

2.1. Sampling site and geochemical characterization

Soil and plant samples were collected in July 2014 in rice paddy fields in Yanhong Town (designated as YH) and Tiepu Town (TP), all of which surround the Lianhuashan tungsten mine located in Shantou City, Guangdong Province, China. Sampling sites were selected in two adjacent towns to reduce geographical effects on soil microbial communities. To minimize the effect of plant growth stage on the composition and quantity of Fe plaque, we collected mature rice plants 7–10 d before harvest in July. Based on the results of our previous study, paddy soils in YH and TP were seriously polluted by metals/ metalloids, especially with As and Cd (Liu et al., 2010; Liu et al., 2015). Forty-seven and 30 paddy fields in YH and TP, respectively, were selected as contaminated sites. Furthermore, six non-contaminated fields (three near TP and three near YH) were selected as control samples. At each site, soil and rice samples were collected in the field with a random approach, and three replicates were collected to form a composition sample at one location. To avoid redox changes during transport and to facilitate Fe plaque extraction, rice root samples were transported on ice and stored at 4 °C prior to processing within 1 d. The rice roots were washed with distilled water at least three times to remove the soil particles adhering to the root surface. The Fe plaque was extracted from the root materials using a dithionite-citrate-bicarbonate (DCB) solution, as previously described (Chen et al., 2008), and the DCB-extract solution was centrifuged at 16,000 ×*g* for 10 min to pellet any microorganisms present in the plaque. Then, after centrifugation, the precipitates were stored at −40 °C until used for DNA extraction, whereas, the supernatants were used to measure the metal concentrations. The rice roots after Fe plaque extraction were dried at 60 °C to a constant weight. The fresh and dry weights of rice roots were recorded, and they were then ground in a carnelian mortar. The paddy soils were air dried and crushed with a wooden hammer to pass through a 100 mesh sieve for subsequent analysis. The concentration of As in soil and rice root was determined using atomic fluorescence spectrometry (AFS, SA-10, Beijing Titan Instruments Co., Ltd., China) after digestion with HNO_3/H_2O_2 in a microwave oven. The digested samples were further dissolved in 2% HCl. As in Fe plaque samples was determined using DCB-extraction with the same instrument after being filtered through a Millipore membrane (0.45 mm) and acidified with HCl. For the detection of Cd, Ni, Pb, and Cr in soil, rice root, and plaque, the ground soil, root, and DCB extracts were digested with a HF-$HClO_4$ mixture in a microwave oven. Cd and Pb were analyzed using graphite furnace atomic absorption spectroscopy (Perkin Elmer PinAAcle 900Z), and Ni and Cr were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Perkin Elmer Optima8000). To verify the accuracy of the metal analyses, standard reference materials (from the National Research Center for Standards in China) were used. The standard reference materials used in the present study were soil GBW (E) 070008, GBW (E) 070009, and GBW 07402. Reagent blanks and three analytical duplicates were also used in each sample batch to ensure the accuracy and precision of the analysis. The details of the procedure were described previously by Liu et al. (2010).

2.2. Total DNA extraction

The DCB-extract solution was divided into three parts with equal volumes and centrifuged individually at 16,000 ×*g* for 10 min to pellet any microorganisms present in the plaque. Genomic DNA was extracted from the precipitated product of the DCB-extracted Fe plaque using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. The DNA was eluted with