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Arsenate reduction and mobilization in the presence of indigenous aerobic bacteria obtained from high arsenic aquifers of the Hetao basin, Inner Mongolia

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ABSTRACT

Intact aquifer sediments were collected to obtain As-resistant bacteria from the Hetao basin. Two strains of aerobic As-resistant bacteria (*Pseudomonas* sp. M17-1 and *Bacillus* sp. M17-15) were isolated from the aquifer sediments. Those strains exhibited high resistances to both As(III) and As(V). Results showed that both strains had *arr* and *ars* genes, and led to reduction of dissolved As(V), goethite-adsorbed As(V), scorodite As(V) and sediment As(V), in the presence of organic carbon as the carbon source. After reduction of solid As(V), As release was observed from the solids to solutions. Strain M17-15 had a higher ability than strain M17-1 in reducing As(V) and promoting the release of As. These results suggested that the strains would mediate As(V) reduction to As(III), and thereafter release As(III), due to the higher mobility of As(III) in most aquifer systems. The processes would play an important role in genesis of high As groundwater.

1. Introduction

Arsenic is one of the most common and harmful carcinogens in the environment. The problem of As contamination in groundwater has become a worldwide environmental issue (Ravenscroft et al., 2009). It directly endangers human health, and influences the sustainable development of society. Therefore, the source and fate of groundwater As is one of the hottest topics in the field of environmental science. Several biogeochemical processes control the release and transport of As in natural waters, including adsorption, oxidation–reduction, and microbe-mediated electron transfer (Diesel et al., 2009). More and more studies have shown that microbes play an important role in the release, migration and transformation of As in aqueous systems (Oremland and Stolz, 2005; Anderson and Cook, 2004; Chang et al., 2008; Pepi et al., 2007; Lievremont et al., 2009; Chang et al., 2012; Mirza et al., 2014).

Although As is generally toxic to life, a lot of microorganisms can get the energy for growth through metabolizing As (Oremland and Stolz, 2003). These microorganisms have evolved the necessary genetic components which confer resistance mechanisms, including arsenite-oxidation, arsenate-reduction and As(V) resistance minimizing the amount of As that enters the cells (Cervantes et al., 1994; Ji and Silver, 1992a,b). Additionally, the microorganisms can use As compounds as electron donors or electron acceptors, and possess As detoxification mechanisms (Ahmann et al., 1994; Johnson et al., 2003). These microorganisms are taxonomically diverse and metabolically versatile, mainly including the dissimilatory arsenate-respiring prokaryotes (DARPs), chemoautotrophic arsenite-oxidizing bacteria (CAOs), heterotrophic arsenite-oxidizing bacteria (HAOs), and arsenate-resistant microbes (Oremland and Stolz, 2005; Anderson and Cook, 2004; Chang et al., 2008; Pepi et al., 2007). Since As(III)-oxidizing bacteria have firstly been found by Green (1918), a series of As(III)-oxidizing bacteria have been isolated (Anderson et al., 1992; Santini et al., 2000; Kashyap et al., 2006; Muller et al., 2007; Duquesne et al., 2008).

Arsenic(V)-reducing bacteria are one of the dominant bacterial groups involved in the cycle of As (Lievremont et al., 2009). A series of As(V)-reducing bacteria have been isolated in previous studies (Santini et al., 2004; Handley et al., 2009; Chang et al., 2012;

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Anderson and Cook, 2004; Liao et al., 2011). Microbial As(V) reduction occurs via two common mechanisms, respiration and detoxification. Respiration occurs only under anaerobic conditions. During the respiration, the *arrA* gene, which encodes for a reductase, catalyzes respiratory As(V) reduction (Saltikov and Newman, 2003; Afkar et al., 2003; Muphy and Saltikov, 2009). Detoxification is an efficient As(V)-reducing mechanism that occurs under both aerobic and anaerobic conditions (Silver and Phung, 2005; Murphy and Saltikov, 2009). During the detoxification process, the *arsC* gene, which is responsible for the biotransformation of As(V) to As(III) (Krumova et al., 2008; Musingarimi et al., 2010), and the *arsB*, which extrudes As(III) from the cytoplasm (Rosen, 2002; Silver and Phung, 1996), would be involved. Furthermore, organoarsenicals are presumed to be formed via microbial activities (Cullen and Reimer, 1989). Bacteria have a function of As methylation, i.e., having arsenite methylation transferases that transfer As (III) to As methyl products, such as MMA, DMA (Oremland and Stolz, 2003; Hong, 2006; Cai and Wang, 2009).

The Hetao basin, as one of the important commodity grain bases in China with more than 10,000 km^2 of farmland and 1 million residents, has been severely affected by high-As groundwater (Guo et al., 2008a, 2014a). Groundwater has been used as a source for drinking water and irrigation water (Guo et al., 2012, 2013a). This has led to many cases of chronic As poisoning in this area (Jin et al., 2003). Previous studies have investigated characteristics of sediment, high As groundwater distribution, chemical and isotopic characteristics, and migration and transformation of groundwater As (Guo et al., 2008b, 2010, 2011a, 2011b, 2012, 2013a, 2013b). However, few studies have been conducted to investigate influences of microbiological processes on As speciation and mobility in the aquifers of the Hetao basin (Li et al., 2014). Li et al. (2014) observed that *Pseudomonas*, *Dietzia* and *Rhodococcus* widely occurred in aquifer systems and anaerobic NO_3^--reducing bacteria *Pseudomonas* sp. was the largest group, followed by Fe(III)-reducing, SO_4^{2-}-reducing and As(V)-reducing bacteria, although bacterial diversity was dependent on groundwater As concentration. However, the roles of indigenous aerobic bacteria in As cycling are unclear and need more investigation. Therefore, it is important to understand As resistance of indigenous aerobic bacteria and their roles in As transformation and mobility. The objectives of this study are to 1) characterize effects of As on indigenous aerobic bacteria from aquifer sediments hosting high As groundwater, 2) evaluate As(V) reduction in As(V)-containing media in the presence of the indigenous aerobic bacteria, and 3) assess As mobilization during bacteria-solid-water interactions.

2. Materials and methods

2.1. Sample collection

Sediment samples M14 and M17 were collected at depths of 12.2 and 25.0 m, respectively, from the borehole (40°58.020′N, 107°00.521′E) in the Hetao basin, Inner Mongolia, in 2009. Immediately after removal from the borehole, the sediment samples were put into sterilized plastic bags and sealed under pure N_2 gas (N_2 > 99.999%). They were transported at 4 °C to the laboratory, and stored in a refrigerator at −20 °C for experiments. M17 is gray sand, and used for isolation of aerobic As-resistant bacteria, since gray sand universally occurs in aquifers hosting high As groundwater (Guo et al., 2008a). M14 is brown clay with 100% As as As(V) species, which allowed us to investigate the reduction of sediment As(V). Therefore, it was used as the solid phase in microcosm experiments.

In comparison with M14, more SiO_2, but lower Al_2O_3, Fe_2O_3, As, F and Mn were observed in sediment M17 (Table 1). Arsenic content was 88.2 and 7.9 mg/kg in M14 and M17, respectively. At the depth around 25 m, groundwater had high As concentrations, with As(III) of 528 μg/L and As(V) of 54.0 μg/L.

Table 1
Chemical components of sediment samples (M14 and M17).

Chemical components (%)								
	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	OC
M14	54.5	14.9	6.0	3.1	6.2	1.6	3.1	2.7
M17	82.2	7.8	2.0	0.7	2.5	1.6	2.3	0.5

Chemical components (mg/kg)					
	As	F	Mn	P	S
M14	88.2	892	590	575	218
M17	7.9	238	259	202	83.0

2.2. Isolation of aerobic As-resistant bacteria

Aerobic As-resistant bacteria were cultivated in CDM medium (Text 1 in Supplementary Materials). Sediment sample M17 (10 g) was mixed with 3 mL solution with 1.5 mg/L As(III) or As(V), which was filtered by 0.22 μm filter membrane to remove microorganisms beforehand, in 100 mL glass flasks sealed with sterilized air-permeable polypropylene membranes (Thermo Scientific ABgene). The mixtures were incubated for 10 d in a shaking water bath at 150 rpm at 28 °C. In order to keep the humidity of samples, sterilized water was added each 1–2 days (Zhao, 2009). After that, 50 mL sterilized water was added to the mixtures. The suspension was incubated for 1 h at 150 rpm at 28 °C. The supernatant was diluted to 10^{-2}–10^{-5}. For each dilution, 100 μL supernatant was added to CDM plates. The plates were incubated for 2 d at 28 °C. Then, single strains were selected and plate streaking method was used to obtain pure bacteria (Liao et al., 2011). The pure strains were stored in 4 °C.

2.3. Identification of aerobic As-resistant bacteria

The aerobic As-resistant strains were identified, using the 16S rRNA and As marker gene sequence analysis method. Sequences of 16S rRNA gene and As marker genes are shown in Table 2. After the DNA extraction, bacterial 16S rRNA gene was amplified using bacterial universal primers. Degenerate primers used to amply the As marker genes were designed especially for *arrA*, *arsB*, and *arsC*. The amplification procedure was provided in details in Text 2 of Supplementary Materials.

The clone sequencing was detected by the Beijing ZhongKe Xilin Biotechnology Company Limited. The DNA sequencing results were analyzed for similarities and aligned in the BLAST program packages (http://blast.ncbi.nlm.nih.gov). The NCBI (USA) was used as the reference database to identify 16S rRNA gene and amplified As marker gene sequences.

2.4. Experimental procedure

The pure strains were cultured in the CDM medium. The strain suspensions were used in microcosm experiments. To examine effects of bacteria on As(V) reduction and mobilization, four As(V) sources were prepared. One is dissolved As(V) with As concentrations of 0.5 and 7.5 mg/L (Treatment I); one is As(V)-adsorbing goethite (α-FeOOH) with As content of 5.6 mg/g (Text 3 in Supplementary Materials) (Treatment II); one is crystalline scorodite ($FeAsO_4 \cdot 2H_2O$) (Treatment III), and the other sediment M14 with As content of 88.2 mg/kg (Treatment IV). In each treatment, blank