Roles of iron and/or arsenic reducing bacteria in controlling the mobilization of arsenic in high arsenic groundwater aquifer

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ABSTRACT: To investigate microbial mediated As release and mobilization in the high arsenic groundwater, indigenous iron/As reducing bacteria were isolated from high arsenic groundwater in the Hetao Basin of Inner Mongolia in China. These isolates were identified to be Fe(III) and As(V) reducing (IAR) *Shewanella putrefaciens* IAR-S1, Fe(III) reducing (IR) *S. xiamenensis* IR-S2 and *Klebsiella oxytoca* IR-ZA, and As(V) reducing (AR) *Citrobacter freundii* AR-C1, *Paralostridium bifermentans* AR-P1, *Klebsiella pneumoniae* AR-K1 and *Aeromonas hydrophila* AR-A1. The incubation results showed that strains IAR-S1, IR-S2 and the control *S. oneidensis* MR-1 reduced ferrihydrite quickly and re-immobilized As. Strains AR-C1, AR-P1, AR-K1 and AR-A1 reduces As(V) to As(III), and arsenic (As_{tot}) was released into the aqueous phase with strain IR-ZA and strain AR-C1.

1 INTRODUCTION

Arsenic (As) as a toxic metalloid element threatens the health of more than 140 million people worldwide. It is well known that microbes play an important role on mediating As-bearing Fe(III) oxyhydroxides reduction and As mobilization. Whether DIR results in As retention or release is still an international debate topic. Therefore, to investigate microbially-mediated DIR affecting on As release and mobilization in high arsenic groundwater environment in Hetao Basin of Inner Mongolia, the primary objectives of this study were to: 1) isolate and characterize the dissimilatory iron and/or arsenic reducing strains from the high arsenic groundwater area of Hetao Basin; 2) investigate the arsenic mobilization from As-bearing ferrihydrite with different strains.

2 METHODS/EXPERIMENTAL

2.1 Site description and sample collection

Water samples for bacterial isolation were collected from two tube wells in Hangjinghouqi County of Hetao Basin, where endemic arseniasis is most serious. Groundwater from this region contains high As concentrations up to 1.74 mg L⁻¹.

2.2 Bacterial isolation and identification Bacterial isolation and identification

Iron-reducing medium (IRM) or chemically defined medium (CDM) were used to screen iron-reducing and arsenic-reducing bacteria. For isolation, the Hungate roll tube technique was used (Hungate, 1969). All transfers and cultures sampling were performed in an

anaerobic chamber with tubes and bottles flushed with O₂-free N₂ gas.

DNA was extracted from over-night cultures using FastDNA® SPIN Kit for Soil according to manufacturer's protocols (Qbiogene, Inc. CA, USA) and 16S rRNA gene was amplified with the primers 27F and 1492R. The 16S rRNA gene sequences were aligned with the closely related sequences in the GenBank database using the BLAST program.

2.3 Arsenate and ferric reduction assays

Iron and As(V) reduction activities of isolated strains were assayed with IRM or CDM, respectively, under anaerobic conditions as previously described by Dai *et al.* (2016).

0.8 mL pre-culture of strain and As-bearing ferrihydrite (As/Fe molar ratios was 1%) was transferred into 100 mL bottles with 80 mL basal medium (BM) under strictly anaerobic conditions and incubated at 30°C in dark with 120 rpm. The control experiment was conducted using an autoclaved abiotic sample under the same conditions to confirm only the chemical effect. In order to detect As mobilization with microbial DIR under groundwater conditions of our study area, strains were incubated with artificial groundwater medium (AGM) and natural groundwater instead of BM.

3 RESULTS

3.1 Isolation and identification

Three iron reducing strains were obtained and referred to as IAR-S1, IR-S2 and IR-ZA, respectively. BLAST analysis in GenBank identified that

strain IAR-S1 belonged to *Shewanella putrefaciens* (99.57%), strain IR-S2 belonged to *Shewanella xiamenensis* (99.36%), and strain IR-ZA belonged to *Klebsiella oxytoca* (99.54%).

Four arsenate reducing strain was obtained and was named as AR-C1, AR-P1, AR-K1 and AR-A1. Phylogenetic analysis places these four strains closest to organisms referring to as *Citrobacter freundii*, *Paralostrium bifermentans*, *Klebsiella pneumoniae* and *Aeromonas hydrophila* with the similarity of 99.93%, 100%, 99.94% and 99.97%, respectively.

3.2 Iron and arsenic reduction assays

Three Fe(III)-reducing strains IAR-S1, IR-S2 and IR-ZA performed relatively quick reducing rates in the initial 1.0 day, and then slowed down in the remaining incubation. Strain IAR-S1 has a comparatively strong iron reducing capability and could reduce 20.00 mM Fe(III) into Fe(II) in 1.5 days, while both strain IR-S2 and IR-ZA only could reduce 16 mM and 10 mM in 1.5 days.

In CDM medium, strain AR-C1 could reduce 100% of 1 mM As(V) in 72 h and 75% of 2.5 mM As(V) in 120 h and 23.1% of 5 mM in 48 h. Strain AR-P1 could reduce 100% of 1 mM As(V) in 96 h, 77.6% of 2.5 mM As(V) in 96 h, and 36.9% of 5 mM As(V) in 72 h. Strain AR-K1 could reduce 100% of 1 mM As(V) in 48 h, 55.8% of 2.5 mM As(V) in 84 h, and 33.7% of 5 mM As(V) in 48 h. Strain AR-A1 could reduce 100% of 1 mM As(V) in 48 h, 70.8% of 2.5 mM As(V) in 60 h, and 38.3% of 5 mM As(V) in 48 h. However, strain IR-S2 and IR-ZA did not show any As(V) reducing ability. IAR-S1 produced 1.00 mM As(III) within 1.0 days, which can reduce both Fe(III) and As(V) with glucose as a carbon source.

3.3 Reduction of As(V)-bearing ferrihydrite

S. oneidensis MR-1 was chosen as the positive control to detect the As mobilization from As-bearing ferrihydrite by DIR. Under H_2PO_4^- conditions, at the beginning of incubation, the concentration of aqueous total arsenic (As_{tot}) increased quickly by all strains. However, the decrease of As_{tot} was observed along with the culture of strain IAR-S1, IR-S2 and MR-1 after the peaks appeared. The fluctuation of As concentration might be due to the aqueous As captured by the secondary minerals gradually. In contrast, As_{tot} with strain IR-ZA and AR-C1 was gradually increased after 12.0 days incubation. The As release with strain IR-ZA could be attributed to the decrease of the surface area of ferrihydrite with the reductive dissolution. On the other hand, more mobile As(III) produced by the As reducing strain AR-C1 increased the aqueous As.

Strains IAR-S1, IR-S2, IR-ZA AR-C1 and MR-1 incubated in the HCO_3^- conditions, the AGM and the natural groundwater, also performed similar As release

and As(III) reduction patterns with those of H_2PO_4^- . However, the concentrations of aqueous As_{tot} released by strains were much lower than those of H_2PO_4^- .

3.4 Effect of As(V)-reduction or As(V) concentration on DIR

To detect the effect of microbial Fe(III) and As(V) reductions on each other, As(V) reducing strain AR-C1 and Fe(III) reducing strains IR-ZA and IR-S2 (without As(V) reducing capability) were chosen to co-incubate with As-bearing ferrihydrite. Results showed that both Fe(III) and As(V) reductions were improved in the co-incubation. Fe(II) concentrations produced by strains AR-C1 and IR-ZA after 8.0 days was 0.52 mM higher than sole incubation of strain IR-ZA. More 0.33 mM of Fe(II) were detected after 8.0 days co-culture of strains AR-C1 and IR-S2, which is higher than sole incubation of strain IR-S2. Aqueous As_{tot} concentrations increased with co-incubation of strain IR-ZA and AR-C1 after 18.0 days incubation, which is higher than the separated incubation of these two strains.

The effect of As(V) on DIR was also investigated, through incubation strain IR-S2 with different As/Fe molar ratio of As(V)-bearing ferrihydrite. The quantity of Fe(II) release was found to increase with the As/Fe molar ratio of ferrihydrite. No Fe release or reduction was observed in the blank control.

4 CONCLUSIONS

Our results suggested that the microbial DIR in the groundwater aquifer enhance not only the release but also retention of arsenic. Fe(III) and As(V) reductions mediated by the indigenous bacteria were improved each other. Both microbial As reduction and reductive dissolution of Fe(III) oxyhydroxides were important mechanisms of As enrichment in groundwater aquifers in the Hetao Basin of Inner Mongolia.

ACKNOWLEDGEMENTS

The research work was financially supported by the National Natural Science Foundation of China (No. 41521001 and No.41772260).

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