Functional microbial communities in high arsenic groundwater

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ABSTRACT: Microbial functional potential in high arsenic (As) groundwater remains largely unknown. In this study, the microbial community functional composition of high arsenic groundwater samples from Hetao Basin, China, was investigated using integrated methods including DGGE, gene clone library, qPCR, and GeoChip. The results showed that As-related genes (*arsC* and *arrA*), sulfate-related genes (*dsrA* and *dsrB*), and methanogen genes (*mcrA*) were correlated with As, SO_4^{2-} , or CH_4 concentrations in groundwater, respectively. Arsenic, total organic content, SO_4^{2-} , NH_4^+ , oxidation-reduction potential (ORP), and pH were important factors shaping the functional microbial community structure. Alkaline and reducing conditions associated with microbially-mediated geochemical processes could be linked to As enrichment in groundwater.

1 INTRODUCTION

Arsenic (As) in groundwater is a serious environmental issue due to its widespread distribution and high toxicity, which threatens the health of millions of people worldwide. Previous studies showed that As mobilization and transformation could be ascribed to the complex interactions between microbes and geochemical processes and that microbes play key roles in driving the biogeochemical cycle in high As groundwater aquifers (Li *et al.*, 2014, 2017; Wang *et al.*, 2015). The integrated role of various functional microbial populations on As mobilization in groundwater remained unknown. To investigate the functional potential microbial community structures in high As groundwater aquifers, we used a series of technologies including DGGE, gene clone library, qPCR, and GeoChip, which targets functional gene families involved in As, C, and S geochemical cycling.

2 METHODS

2.1 Sample collection and geochemistry measurements

Groundwater samples were collected from tubewells with a depth range of 20–30 m from Hetao Basin, Inner Mongolia. Microbial samples were collected by on-line filtering of 5–10 L water through 0.22- μm filters. Water pH, electrolytic conductivity (EC), oxidation-reduction potential (ORP), total dissolved solids (TDS), dissolved oxygen (DO), ammonium, sulfide, ferrous iron (Fe(II)), and total iron were measured in the field using portable hand-held meters. The measurements of cation, anion, dissolved organic carbon

(DOC), total carbon (TC), total nitrogen (TN), CH_4 , and As species were performed in the laboratory.

2.2 DNA extraction and functional gene detection

DNA for all groundwater samples was extracted using FastDNA spin kits for soil according to the manufacturer's manual. DNA concentrations were quantified by PicoGreen, using a FLUO star Optima instrument. The *dsrB* gene structure were investigated using denaturing gradient gel electrophoresis (DGGE). The functional communities of *mcrA* genes and arsenic related genes including *aioA*, *arsC*, and *arrA* were detected with gene clone library method. N, C, S and As related genes were qualified by qPCR and geochip. For geochip detection, ~500 ng of DNA was labeled with Cy. The labeled DNA was re-suspended in hybridization solution, and then hybridized in an Agilent hybridization oven at 67°C for 24 h. After hybridization, the arrays were scanned with a NimbleGen MS200 Microarray Scanner. The images were extracted by the Agilent Feature Extraction program. Poor quality spots with a signal-to-noise ratio of less than 2.0 were removed before statistical analysis. The positive signals were normalized within each sample and across all samples.

2.3 Statistical analysis

Data were further analyzed by the Vegan package in R 3.1.1 (<http://www.r-project.org/>). Hierarchical clustering was performed with CLUSTER 3.0 were visualized in TREEVIEW. Canonical correspondence

analysis (CCA) were performed to link microbial communities to environmental variables.

3 RESULTS AND DISCUSSION

3.1 Geochemistry

Groundwater samples could be divided into well-defined high and low As groups based on geochemistry. Samples with high As concentrations generally had low concentrations of sulfate, negative ORP, and relatively high concentrations of As(III), Fe(II), CH₄, NH₄⁺, and TOC contents. These geochemical characteristics indicated that alkaline and reducing conditions could be linked to natural As enrichment in groundwater.

3.2 Functional microbial communities

Analysis of *aioA* gene clone library found that As-oxidizing bacteria in high As groundwater of Hetao Plain belonged to *Rhodospirillum rubrum*, *Leptothrix* sp., *Acidovorax* sp., *Pseudomonas*, *Acinetobacter*, *Xanthobacter autotrophicus*, *Bradyrhizobium* sp., *Bosea* sp., *Aminobacter* sp. and *Nitrobacter hamburgensis*. As-reducing populations in high As groundwater mainly belonged to *Geobacter* sp., *Desulfosporosinus* sp., *Chrysiogenes* and *Desulfurispirillum*. Most of these populations were found capable of dissimilatory arsenate reducing or sulfur oxidizing in previously studies.

Clone library result of *mcrA* gene indicated methanogens in high As groundwater were mainly composed of *Methanomicrobia*, *Methanobacteria* and the uncultured group, among which 83% clones belonged to *Methanomicrobia* including *Methanomicrobiales* and *Methanosarcinales*.

Sulfate reducing bacteria in high As groundwater were mainly dominated by *Desulfotomaculum*, *Desulfobulbus*, *Desulfosarcina* and *Desulfobacca*. Most of the functional populations were found capable of both arsenate reducing and sulfur reducing.

3.3 The abundance of functional genes

The number of detected As related genes *arsC* ($r = 0.462$, $p < 0.01$) and *arrA* ($r = 0.486$, $p < 0.01$) have positive correlations with As concentration. More *arsC/arsB* genes were detected than the other genes, such as *arrA* (around 5–8 folds), indicating that the most common detoxification mechanism was reduction of As(V) to As(III) rather than respiratory arsenate reduction in the high arsenic groundwater aquifers. Besides, As detoxification genes *arsC* and *arsB*, and the respiratory arsenate (As(V)) reductase gene *arrA* had higher relative abundances in the high As group than low As group.

There were positive correlations between As and sulfur reduction genes (*dsrB* and As: $r = 0.327$, $p < 0.05$). Positive correlations were also observed between methanogenesis genes and As and methane concentrations (*mcrA*: $r = 0.549$, $p < 0.05$; $r = 0.314$, $p < 0.05$, respectively). These results indicated that sulfur reduction and methanogenesis might be an important metabolic process and accelerate As release and accumulation in high arsenic aquifers.

3.4 Functional microbial community structure in relation to geochemistry

Significant differences were observed in functional microbial structure between low and high As groups ($r = 0.4776$, $p = 0.007$). Six environmental variables, As_{Tot}, SO₄²⁻, NH₄⁺, pH, ORP, and TOC were the most significant environmental variables shaping the microbial community structure ($p = 0.021$). Group 1 (SO₄²⁻, ORP) explained 22.63% ($p = 0.010$), and group 2 (As_{Tot}, TOC and NH₄⁺) explained 24.98% ($p = 0.010$). The pH (group 3) independently explained 17.64% ($p = 0.005$) of the observed variation. About 58.80% of the community functional variation remained unexplained by the above selected variables.

4 CONCLUSIONS

Our results reveal that environmental variables account for the majority of variation in microbial functional potential and provides evidence for possible links between As, S, and C related microbial functional gene abundances and As geochemistry. Alkaline and reducing conditions associated with microbially-mediated geochemical processes could be linked to As enrichment in groundwater in this study area.

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