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Evidence of arsenic mobilization mediated by an indigenous iron reducing bacterium from high arsenic groundwater aquifer in Hetao Basin of Inner Mongolia, China

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ABSTRACT

A facultative anaerobic iron reducing bacterium IMCUGIR-ZA was isolated from high arsenic (As) groundwater in Hetao Basin of Inner Mongolia and was identified as *Klebsiella oxytoca*. Strain IMCUGIR-ZA could reduce more than 96.8% of 2.78 mmol L^{-1} soluble Fe(III)-citrate in 7 days and 0.42 mmol solid iron from ferrihydrite into aqueous phase in 10 days using glucose as an electron donor under strictly anaerobic conditions. Appropriate concentrations of As could improve microbially-mediated iron reduction and release from ferrihydrite. Nearly threefold iron was reduced and released by strain IMCUGIR-ZA from As(V)-doped ferrihydrite (As 100 μmol L^{-1}). When the strain was incubated with high arsenic sediments from Hetao Basin of Inner Mongolia under anaerobic conditions, 0.894 mmol L^{-1} Fe(III) and 12.44 μmol L^{-1} As were released to aqueous phase in 7 days. The results provided evidence of microbially-mediated mobilization of As in high arsenic groundwater aquifer in Hetao Basin of Inner Mongolia, China.

1. Introduction

Arsenic (As) widely exists in the nature as a toxic metalloid element. Long term ingestion of high arsenic groundwater can result in serious chronic diseases (Jomova et al., 2011). High arsenic groundwater is distributed in many countries including the USA, Argentina, Chile, Mexico, Thailand, Bangladesh, India, Japan, and China (Anawar et al., 2011; Chauhan et al., 2009; Ferreccio and Sancha, 2006; Guo et al., 2008b). In China, Hetao Basin of Inner Mongolia is one of the most serious and representative areas affected by arsenic, resulting in over 250, 000 cases of arsenicosis. The concentrations of As in groundwater are more than 50 μg L^{-1} in most parts of this region, with the maximum up to 1740 μg L^{-1} (Deng et al., 2009).

Previous studies have demonstrated that microorganisms played an important role in the process of mobilization and transformation of arsenic (Bahar et al., 2016; Liu et al., 2013). Over the last decades, many hydrological, mineralogical and geochemical studies have been conducted to investigate the source, distribution, mobilization and transformation mechanisms of As in groundwater of Hetao Basin (Deng et al., 2011; Guo et al., 2001, 2013; Neidhardt et al., 2012; Yu et al., 2007). These investigations suggested that iron (Fe) oxyhydroxides, clay minerals and organic matter was related to As release from sediments to groundwater under alkaline pH and reducing conditions. Indigenous microbes have been demonstrated to play important roles in the As mobilization and transformation in high arsenic aquifers (Maizel et al., 2016; Park et al., 2006; Xie et al., 2011). However, As mobilization mediated by iron reducing bacteria was not fully understood in high arsenic groundwater aquifers in Hetao Basin of Inner Mongolia. In this study, an facultative anaerobic iron reducing bacterium IMCUGIR-ZA was isolated from high arsenic groundwater in Hetao Basin of Inner Mongolia, and was found to be able to reduce Fe(III) and thus cause As release from minerals and high arsenic sediments from Hetao Basin of Inner Mongolia.

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2. Materials and methods

2.1. Medium

Iron-reducing medium (IRM) was used to screen and isolate bacteria. Its ingredients were (g L^{-1}) $NaHCO_3$ 2.5, KCl 0.1, NH_4Cl 1.5, NaH_2PO_4 0.6, Yeast extract 0.5, sodium acetate 20 mmol, ferric citrate 20 mmol, and the pH was around 6.7. LB medium was used to pre-culture bacterial strains. It contained (g L^{-1}): NaCl 10, Tryptone 10, Yeast extract 5, and the pH was 7.0. Fe(III)-citrate reaction solution (FRS) was used for Fe(III)-citrate reduction to determine the aqueous Fe(III) reducing capability of strains. It was a mixture prepared as follows: Fe(III)-citrate stock solution 20 mmol L^{-1}, NH_4Cl stock solution 5 g L^{-1}, phosphate buffer (Na_2HPO_4 and NaH_2PO_4) stock solution 25 mmol L^{-1} and then 5 ml of each stock solution was dispensed to a 50-ml anaerobic bottle. MS medium was used as basic medium in the experiments of arsenic release due to the microbially-mediated reduction of ferrihydrite. The medium contained (g L^{-1}): $NaHCO_3$ 2.5, NH_4Cl 0.25, $NaH_2PO_4 \cdot H_2O$ 0.6, KCl 0.1, and 10 mL of each of Wolfe's vitamin solution and Wolfe's mineral solution (Roller, 2004). The pH was adjusted to 7.0. 20 mmol L^{-1} PIPES (Piperazine-1,4-bisethanesulfonic acid) buffer (pH 7.0) was used to prepare high arsenic sediments suspension, and it was amended with the following ingredients (g L^{-1}): NH_4Cl 1.5, NaCl 5, $CaCl_2$ 0.1, KCl 0.1, and 10 mL modified Wolfe's vitamins and trace minerals (Balch et al., 1979; Lovley and Phillips, 1988).

All the mediums were prepared with a gassing station using standard anaerobic techniques (Balch et al., 1979; Miller and Wolin, 1974). Briefly, 99.9999% N_2 were passed through a column of hot reduced copper filings to remove traces of oxygen. All transfers and sampling of cultures were performed in an anaerobic chamber with syringes and needles that had been flushed with O_2-free gas (Roden and Lovley, 1993).

2.2. Isolation and identification

Water samples were collected from a tube well (106°49′43″E, 40°54′29″N) located in Sandaoqiao Guanglinyishe in high arsenic contaminated area in Hangjinghouqi County of Hetao Basin. Arsenic concentration in the tube well was 304 μg L^{-1}. 1 ml groundwater was inoculated into a 25-ml sterilized anaerobic Hungate tube containing 9 ml IRM in site. 0.5 ml suspension was transferred into another sterilized anaerobic Hungate tube containing 9.5 ml IRM every three days under anaerobic conditions. The tubes were kept in the dark at 30 °C with the shaking rate of 120 rpm. After three times of transfer, the enrichment was serially diluted and inoculated into anaerobic tubes containing 4.5 ml IRM and 1.8% agar following the Hungate roll tube technique. After incubation for 3 days at 30 °C, colonies were picked up and inoculated into new anaerobic tubes. After being isolated for 5 times, pure culture was obtained. The pure culture was grown anaerobically on LB medium and then stored at 4 °C for further investigations.

Colonial morphology in the roll tube of IRM was observed. The main physiological and chemical features of the isolate were investigated by comparing with the characteristics from Bergey's Manual of Systematic Bacteriology (Brenner et al., 2005). The genomic DNA was extracted using FastDNA® SPIN Kit for Soil (Qbiogene, Inc. CA, USA) according to the manufacturer's protocols. DNA yields were quantified with a NanoDrop 2000 spectrophoto meter (Thermo Scientific, USA). All PCRs were performed in Master cycler ep Gradient S Thermal Cycler block (Eppendorf, Hamburg, Germany). The 16S rRNA gene was amplified with universal bacterial primers 27f (5′ AGAGTTTGATCCTGGCTCAG3′) and 907r (5′ CCGTCAATTCMTTTRAGTTT3′) (Marchesi et al., 1998). A 25 μl PCR mixtures contained 1 μl DNA template, 0.5 μl of 20 μM each primers, 2.5 μl 10× reaction buffer, 1 μl 10 × dNTP, 0.5 U 10 × DNA Taq polymerase (TaKaRa, Japan). PCR was performed with the following thermo cycler program: denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min; and final extension at 72 °C for 10 min. The amplified product was screened by electrophoresis in a 1% agarose gel, then excised and purified using AxyPrep DNA Gel Extraction Kit (Axygen, USA). Purified PCR product was commercially sequenced. The 16S rRNA gene sequence was aligned with the closely related sequences in the GenBank. The phylogenetic tree was constructed with the neighbor-joining method in MEGA 5.0 (Tamura et al., 2011). The 16S rRNA gene fragment nucleotide sequence of IMCUGIR-ZA has been deposited in the GenBank database under accession number KP769536.

2.3. Fe(III) reduction experiments

Fe(III) reduction experiments were performed under anaerobic conditions. During Fe(III)-citrate reduction, anaerobic bottles containing FRS were flushed with N_2 to remove O_2, capped with thick butyl rubber stoppers, sealed with aluminum crimps, and autoclaved for 20 min at 121 °C. 5 ml glucose sterilized by filtration at an initial concentration of 300 mmol L^{-1} was added into cooled anaerobic bottles. 5 ml of the LB medium pre-cultured iron reducing strain was centrifuged, washed and resuspended in 5 ml anaerobic sterilized phosphate buffer, and then inoculated into a 50 ml anaerobic bottle. 5 ml anaerobic sterilized phosphate buffer without the pre-culture was inoculated as a control. The bottles were kept in the dark at 30 °C and 0.3 ml of the solution was sampled every 12 h for Fe(II), total Fe and Fe(III) detection. The concentrations of Fe(II) and total Fe were measured with ferrozine assay according to Stookey (Stookey, 1970) with an UV-VIS spectrophoto meter (UV-1750; SHIMADZU Corp., Kyoto, Japan), and Fe(III) concentration was calculated by subtracting Fe(II) from total Fe. All experiments were run in duplicate.

2.4. Arsenic release from ferrihydrite

Synthetic As(V)-doped ferrihydrite was used to investigate the release of As with the reduction of Fe(III) by the iron reducing bacterium. Ferrihydrite synthesization was referenced with the method of Schwertmann and Cornell (2007). Briefly, synthetic As(V)-doped ferrihydrite was obtained by adsorption (Burnol et al., 2007). The concentrations of Fe(III) was approximate 400 mmol L^{-1}. The ferrihydrites were prepared with As(V) at final concentrations of 10 mmol L^{-1} and 20 mmol L^{-1}, and the As/Fe molar ratios were 1.8% and 3.4% respectively. Four incubation experiments were conducted to determine Fe(III) reduction rates and As mobilization from ferrihydrite in the presence and absence of iron reducing bacteria. Experiments were performed with incubation of 0.3 ml ferrihydrite with As/Fe molar ratio of 1.8% or 3.4%, respectively, 0.3 ml of 1 mol L^{-1} glucose, and 0.3 ml pre-culture of strain IMCUGIR-ZA under strictly anaerobic conditions. 0.3 ml ferrihydrite without As(V), 0.3 ml of 1 mol L^{-1} glucose, and 0.3 ml pre-culture of strain IMCUGIR-ZA were incubated as control. Incubation of 0.3 ml ferrihydrite with As/Fe molar ratio of 1.8%, 0.3 ml of 1.0 mol L^{-1} glucose, without inoculation of strain IMCUGIR-ZA was used as blank. The constituents of each trial described previously were added into 50-ml anaerobic bottles with MS medium, and sterilized anaerobic MS medium were added to keep the final volume 30 ml. The bottles were kept in dark at 30 °C and sampled periodically for Fe(II) and As in the solution. All experiments were performed in duplicate using standard anaerobic techniques. The concentrations of Fe(II) were measured with Ferrozine method as described

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