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Inhibitory effect of nitrate/nitrite on the microbial reductive dissolution of arsenic and iron from soils into pore water

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Abstract

It was well established that microbial communities are the major drive for the formation of arsenic-contaminated groundwater. However, it remains to be elucidated for how nitrate/nitrite affects the microorganisms-catalyzed dissolution and reduction of arsenic. To address this issue, we collected soil samples containing high-contents of arsenic from the Shimen Realgar Mine area. Microcosm assay indicated that addition of nitrate/nitrite significantly inhibited the dissolution, reduction and release of As and Fe caused by the biological catalysis of microbial communities in the soils, meanwhile nitrate/nitrite was reduced into N_2. To further investigate the molecular mechanism of this finding, we used a representative dissimilatory arsenate-respiring strain *Shewanella* sp. GL90 from the soils to perform the arsenic release assay. GL90 can efficiently catalyze the reductive dissolution, and promote the release of As and Fe in soils. It is interesting to see that the addition of nitrate/nitrite to the soils led to marked decreases in the GL90-mediated dissolution of As and Fe in the soils. Moreover, we found that this finding was attributed to that nitrate/nitrite significantly inhibited the transcription of the gene of the respiratory arsenate reductase protein in GL90 cells. This work provided new insights into the mechanisms for the coupling of As, N and Fe geochemical cycles in arsenic-rich soils, and for how environmental factors affect As concentration in groundwater.

Keywords Arsenate- · Ferric iron-reducing bacterium · Nitrate/nitrite · Bioremediation · Arsenic contamination · Arsenic in groundwater

Introduction

Increasing geochemical surveys have suggested that As-contaminated groundwater exists in many countries worldwide, such as China, United States, India, Bangladesh, Vietnam, Pakistan, Germany, Greece, and Spain (Fendorf et al. 2010; Nordstrom. 2002; Harvey et al. 2002). At least 300 millions of people in the world are utilizing groundwater that contains unsafe level of As (>10 μg/L) (Rodríguez-Lado et al. 2013; Nordstrom et al. 2002). Arsenic compounds are extremely toxic and pose a serious damage to human health. It was well established that arsenic can induce the cancers of multiple organs and tissues, such as skins, liver, lung and bladder. Arsenic also causes non-cancerous diseases, like diabetes, cardiovascular diseases, peripheral vascular diseases and neurological effects (Chen 2014; Chung et al. 2013; Roh et al. 2017). Therefore, it is necessary to fully understand the biogeochemical reactions that affect the arsenic concentrations in groundwater.

The arsenic compounds in groundwater largely come from weathering and mobilization of arsenic that originally exists in insoluble phase, including soils, minerals, sediments and rocks (Lièvremont et al. 2009; Clancy et al. 2013). Increasing evidences suggest that microorganisms are the catalyst for the solubilization of arsenic from mineral phase to groundwater (Burton et al. 2014; Haque et al. 2008). These microorganisms mainly include arsenite-oxidizing bacteria and dissimilatory arsenate-respiring prokaryotes (DARPs) (Zhang et al. 2015; Barringer et al. 2010). Arsenite-oxidizing bacteria can oxidize As(III) into As(V) under aerobic or anaerobic conditions. Under aerobic

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conditions, arsenite-oxidizing bacteria convert As(III) into As(V) using oxygen as the terminal electron acceptor, whereas under anaerobic conditions, some arsenite-oxidizing bacteria can oxidize As(III) to As(V) using nitrate, chlorate or selenate as the terminal electron acceptor (Zhang et al. 2017; Zeng et al. 2016). DARPs are the different group of arsenic-resistant microbes that can reduce As(V) into As(III) under anaerobic conditions, using lactate, formate, aromatic compounds, acetate, hydrogen, or other organic/inorganic compounds as the sole electron donor (Ghosh and Sar 2013; Wang et al. 2014). Although arsenite/sulfide-oxidizing bacteria may also play roles in arsenic solubilization from geologic materials, it was well established that dissimilatory arsenate-respiratory prokaryotes (DARPs) are the key catalyst for the observed mobilization of arsenic from mineral materials (Mumford et al. 2012; Fisher et al. 2008; Kudo et al. 2013), DARPs can dissolve and convert As(V) absorbed on the soil or mineral surfaces into As(III) under anaerobic conditions, using acetate, lactate, pyruvate or other substances as the electron donor (Ohtsuka et al. 2013; Jiang et al. 2013; Wang et al. 2017a, 2017b). DARPs were found to widely exist in arsenic-contaminated soils, aquifer sediments, groundwater and tailings (Chen et al. 2017; Wang et al. 2017a, 2017b; Song et al. 2009).

Some environmental factors were found to have apparent effects on the microbial community-mediated solubilization of arsenic from soil phase into solution. It was found recently that biochar, sulfate and organic matters significantly enhance the copies of the As(V)-respiratory reductase genes of DARPs in the arsenic-rich soils or sediments, and thus increase the microbial mobilization and reduction of arsenic (Sharma et al. 2010).

Nitrate is one of the most ubiquitous contaminants in the environment, and its levels of contamination are increasing (Xu et al. 2017; Hudak 2000). Nitrate comes from natural biological/geochemical processes or resulted from anthropogenic impacts. In the areas of human settlements, nitrate contamination in soils are largely attributed to anthropogenic activities, including nitrate fertilizers, manure, and septic wastes, and nitrate are easily converted into nitrite by microorganisms under anaerobic condition (Kaushal et al. 2011). Many biogeochemical reactions, such as nitrogen fixation, nitrification, denitrification, anammox and feammox, significantly affect the concentrations of nitrate/nitrite in the environment, especially in soils (Yu et al. 2016; Cui et al. 2013; Weng et al. 2017). Geochemical surveys indicated that nitrate concentrations are highly correlated with arsenic concentrations in the arsenic-contaminated environment (Yao et al. 2018; Park et al. 2018); however, the mechanism remains to be elucidated. Considering that DARPs are the major drive for the dissolution, transformation and release of arsenic from solid phase into groundwater, it is of great importance to investigate how nitrate/nitrite affects the DARPs-catalyzed dissolution of arsenic from soils into soluble phase (Wang et al. 2017a, 2017b; Osborne et al. 2015; Islam et al. 2004). Hence, it is of great importance to detect whether and how nitrate/nitrite impacts DARPs-catalyzed dissolution of As from soils into soluble phase.

In this study, we observed that nitrate/nitrite significantly inhibited the DARPs-catalyzed solubilization of As and Fe from As contaminated soils. Our group further observed that this finding is attributed to that nitrate/nitrite involves in the expression regulation of the gene encoding As-respiratory reductase protein in DARPs. This work provided new insight into the mechanism by which the concentrations of arsenic in groundwater changed dynamically and provided direct evidence that the biogeochemical cycles of N, Fe and As in arsenic-contaminated soils were coupled.

Materials and methods

Sampling and geochemical measurement

Soil samples were collected from an As-contaminated area, located in the Shimen Realgar Mine in the Changde City, China. A direct-mud rotary drilling method was used to drill a hole and collect soil samples from 2.0 and 4.5 m (Chen et al. 2017). The two samples were referred to as SM1 and SM2, respectively. Total As in the soils was solubilized and determined as described previously (Wang et al. 2017a, 2017b). The concentrations of soluble As(V) and As(III) were measured by high-performance liquid chromatography (HPLC, Agilent1220, USA) with a Hamilton PRP-X100 ion exclusion column linked to an atomic fluorescence spectrometry (AFS-9600, Haiguang, China) as described previously (Wang et al. 2017a, 2017b). The mobile phase was 15.0 mM $(NH_4)_2HPO_4$ at a flow rate of 1.0 mL/min; the retention times for As(III) and As(V) were 2.6 and 10.7 min, respectively (Georgiadis et al. 2006). The contents of total organic carbon (TOC) in the soils were determined using a TOC analyzer. Ammonium in the soils was detected with Nessler assay (Kim et al. 2006). The concentrations of nitrite and nitrate were determined using Griess and thymol reagents, respectively (Broderick et al. 2005). The small organic molecules were detected using HPLC-PDA technique (Fiedler et al. 2011). The mobile phase was 10 mM KH_2PO_4 at a flow rate of 0.3 mL/min for 21 min, 0.8 mL/min for 12 min, 0.3 mL/min for 4 min; the retention times for lactate and acetate were 19.02 and 20.44 min, respectively.

Table 1 PCR primers used in this study

Target genes	Sequences of primers	Reference
arrA	CGAAGTTCGTCCCGATHACNTGG (Forward)	Wang et al. 2017a, 2017b
	GGGGTGCGGTCYTTNARYTC (Reverse)	
	GTCCCNATBASNTGGGANRARGCNMT (Forward)	Wang et al. 2017a, 2017b
	ATANGCCCARTGNCCYTGNG (Reverse)	
T vector	CGCCAGGGTTTTCCCAGTCACGAC (Forward)	Zeng et al. 2016
	GAGCGGATAACAATTTCACACAGG (Reverse)	
16S rRNA	AGAGTTTGATCCTGGCTCAG (Forward)	Zeng et al. 2016
	TACGGCTACCTTGTTACGACTT (Reverse)	

Anaerobic microcosm assay

Three grams of arsenic-contaminated samples were inoculated to 10.0 mL of oxygen-free MMS (modified mineral salt, MMS) medium (Li et al. 2016). The mixtures were prepared in triplicate. The mixtures were each amended with 10.0 mM sodium lactate (as the terminal electron donor), and 10.0 mM NO_3^-/NO_2^- or with no nitrate/nitrite. To prepare triplicate control, 3.0 g of sterilized soils were mixed with 10.0 mL of oxygen-free MMS medium containing 10.0 mM lactate and NO_3^-/NO_2^-, or with no nitrate/nitrite. Anaerobic incubations were performed at 30 °C with moderate shaking. Approximately 0.5 mL of mixtures was taken out at an flexible interval of 1.0 to 8.0 days for measuring the concentrations of dissolved As(V) and As (III), as well as lactate and acetate.

Isolation of a novel DARP strain from arsenic-contaminated soils

About 1.0 g of soils from the depth of 2.0 m was mixed with 4.5 mL of MMS medium containing 2.0 mM As(V) and 10.0 mM lactate. Anaerobic incubations were performed at 30 °C for enrichment. Once 90–100% of arsenate was reduced into arsenite, approximately 0.5 mL of cultures was transferred into 4.5 mL of fresh MMS for second-round enrichment. Through 3–4 rounds of transferring and culturing, the cultures were subjected for isolation of single bacterial strains under strict anaerobic conditions (Chen et al. 2017).

Gene cloning and sequencing

The nucleotide sequences coding for the 16S rRNA and the catalytic subunit ArrA of the As-respiratory reductase protein were cloned, sequenced and analyzed (Song et al. 2009). Bacterial genomic DNA was extracted and purified with QuickExtract-Bacterial DNA Extraction Kit (Epicentre, Madison, Wisconsin). Two pairs of primers, 27F and 1492R, and AS1F1R and AS2F2R, were used for the amplifications of the 16S rRNA gene sequence, and the *arrA* gene sequence, respectively (Table 1). DNA bands were cut off, collected and subjected for DNA extraction and purification using standard method (Wang et al. 2017a, 2017b). DNA fragments with high purity were ligated into the pBlueScript SK T vectors that were subsequently transformed into *E. coli* competent cells. The clones with proper-size inserts were subjected for sequence analyzing. Phylogenetic trees were reconstructed with the 16S rRNA gene sequences from this study and their known homologues, or with the ArrA protein sequences from this study and their associated known ArrA proteins, by using the neighbor-joining method.

Examinations of the anaerobic reduction functions of the isolate

The bacterial anaerobic reduction activities for As(V), Fe (III) and NO_3^- were detected as described elsewhere (Wang et al. 2017a, 2017b). Briefly, the isolate was grown in 10.0 mL of MMS containing 10.0 mM sodium lactate (acting as the sole electron donor), and 2.0 mM As(V), Fe (III), or NO_3^- (as the terminal electron acceptor). Anaerobic incubations were performed at 30 °C. Approximately 0.5 mL of cultures was taken out from the anaerobic cultures at an interval of one day, for measuring the numbers of bacterial cells, and determining the contents of dissolved As, Fe or N compounds.

Arsenic release by cultivable DARPs in the presence of nitrate/nitrite

Microcosms of the isolate were prepared by mixing three grams of sterilized arsenic contaminated soil slurries with 10.0 mL of sterile MMS medium containing 10.0 mM sodium lactate (acting as the sole electron donor), and 10.0 mM NO_3^-/NO_2^- or containing no nitrate/nitrite. Anaerobic incubations were performed at 30 °C. About 1.0 mL of mixtures was taken out from the cultures at a flexible interval of 1.0 to 8.0 days, for measuring the concentrations of dissolved As, Fe and N compounds, as well as lactate and acetate.

Effects of nitrate/nitrite on the expression of bacterial arsenate-respiring reductase gene

The isolate was grown in 10.0 mL of MMS (without NH_4Cl) containing 10.0 mM sodium lactate (acting as the sole electron donor) and 2.0 mM As(V) (acting as the terminal electron acceptor,) as well as 10.0 mM NO_3^-/NO_2^- or without nitrate/nitrite. Anaerobic incubations were performed at 30 °C. About 1.0 mL of cultures was removed from the tubes at an interval of one day, for determining contents of As compounds. After 4.0 days of anaerobic incubation, the bacterial cells were washed two times using the oxygen-free MMS medium. The cells were re-suspended in the fresh MMS containing 2.0 μM As(V) and 10.0 mM sodium lactate for examining the arsenate-respiring activities. After 3.0 h, about 0.6 mL of mixtures was taken out for measuring the concentration of As species.

RT-qPCR

Reverse transcription-quantitative PCR (RT-qPCR) technique was used to detect the expressed mRNA levels of bacterial As-respiratory reductase genes (Giloteaux et al. 2013). Briefly, bacterial cells were cultivated into log phase in the MMS medium containing 2.0 mM As(V), 10.0 mM lactate, and 10.0 mM NO_3^-/NO_2^-, or without nitrate/nitrite. Cells of the isolate were precipitated by refrigerated centrifuge at 4 °C for 2.0 to 5.0 min. TRIzol reagent was used to isolate RNAs from the cell pellets. Contaminated DNA was degraded with Turbo DNAse in the light of the manual provided by manufacturer. cDNAs were synthesized with random primers using the TaKaRa reverse transcription kit in the light of the manual from manufacturer. Quantitative real-time PCR for the bacterial arsenate-respiring reductase gene was performed using the TaKaRa Real-Time PCR Core Kit according to the instructions of manufacturer. The 16S rRNA gene was amplified as internal control.

Results

Geochemical features

We collected two different arsenic-rich soil samples (SM1 and SM2) from the depths of 2.0 and 4.5 m, respectively, in an As-contaminated area located in the Changde City of Hunan Province, China. Geochemical investigations demonstrated that the samples SM1 and SM2 contained 1202.2 and 34840.1 mg/kg of total arsenic, 186.6 and 836.5 mg/kg dissolved arsenic, 0.4 and 0.2 mg/kg of nitrate, respectively. They also contained high contents of TOC (20.9 and 37.4 g/kg). Other geochemical parameters of the samples are listed in Table 2.

Table 2 Geochemical features of the arsenic-contaminated soil samples

Parameters	Soil samples	
	SM1	SM2
pH	6.7	6.8
TOC (g/kg)	20.9	37.4
TN (g/kg)	0.5	0.5
Total As (mg/kg)	1202.2	34840.1
Soluble As (mg/kg)	186.6	836.5
NH_4^+ (g/kg)	0.08	0.05
NO_3^- (g/kg)	0.4	0.2
SO_4^{2-} (g/kg)	131.2	50.0
PO_4^{3-} (g/kg)	0.9	1.1

TOC total organic carbon, *TN* total nitrogen

Nitrate/nitrite significantly inhibited the microbial dissolution of As from soils

We prepared active microcosms with the arsenic-rich soils of SM1 containing 10.0 mM sodium lactate. After 7.0 days of anaerobic incubation, approximately 2.25 mM As(III) and 0.08 mM Fe(II) were released from SM1 microcosms (Fig. 1a, b), meanwhile 1.14 mM lactate was converted into acetate (Fig. 1c); in comparison, very little arsenic and iron were dissolved and transformed in the sterilized soils. This suggests that the microbial communities significantly stimulated the solubilization and transformation of As and Fe in SM1 soils (Fig. 1a, b). To address how nitrate/nitrite affects the microbial community-catalyzed release of As and Fe from SM1 soils, we prepared active microcosms supplemented with 10.0 mM sodium lactate, and 10.0 mM $NaNO_3$/$NaNO_2$, or with no nitrate/nitrite. After anaerobic incubation for 7.0 days, in contrast to the As release assays in the absence of nitrate/nitrite, addition of 10.0 mM $NaNO_3$/$NaNO_2$ led to 21/17, and 45/73% decreases in the microbial community-catalyzed releases of arsenite and ferrous ion, respectively (Fig. 1a, b), meanwhile, approximately 9.23/7.61 mM lactate were oxidized into acetate (Fig. 1c).

We also determined the fate of NO_3^- in the liquid phase of SM1 microcosm cultures during the anaerobic reactions (Fig. 1d). In the active microcosms containing 10.0 mM sodium lactate and 10.0 mM $NaNO_3$, after 3.0 days of anaerobic incubation, approximately 6.93 mM NO_3^- was reduced into NO_2^-, which was rapidly converted into volatile N_2; after 14.0 days, all NO_3^- was converted into N_2 (Fig. 1d). This suggests that NO_3^- in the microcosms was rapidly reduced into NO_2^-, and finally converted into N_2

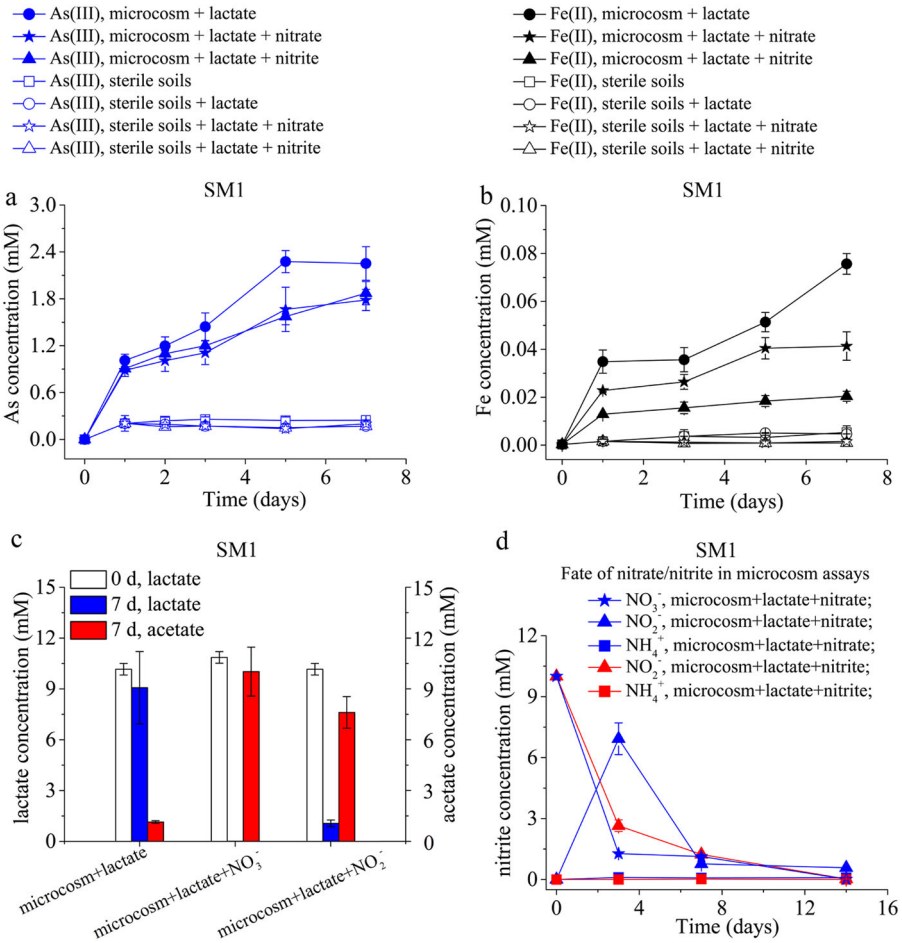

Fig. 1 Effects of nitrate and nitrite on the microbial communities-catalyzed mobilization, reduction and release of arsenic and iron from the arsenic-rich soils of SM1 as examined using microcosm assays. Control soils were sterilized using Co60 irradiation. **a** Microbial release of As(III) from SM1. **b** Microbial release of Fe(II) from SM1. **c** Fate of lactate during the microbial reactions in SM1. **d** Fate of nitrate/nitrite during the microbial reactions in SM1

during the reactions. In comparison, in the active microcosms containing 10.0 mM $NaNO_2$, after 3.0, 7.0, and 14.0 days of incubation, approximately 2.65, 1.23, and 0.02 mM NO_2^- were detected, respectively (Fig. 1d), suggesting that nitrite was also rapidly reduced into N_2.

Similar phenomenon was observed in the microcosm assays with SM2 soils. As shown in Fig. S1a–d, the presence of nitrate/nitrite caused 32/48, and 57/67% decreases in the microbial community-catalyzed dissolution and reduction of As and Fe in the microcosms of SM2 after 7.0 days of anaerobic incubation; meanwhile, both nitrate and nitrite were rapidly reduced into N_2, and lactate was oxidized into acetate.

Taken together, it is most likely that the anaerobic reduction of As/Fe/nitrate or nitrite, each of which was coupled with the lactate oxidation, occurred in the microcosms of SM1 and SM2, and nitrate/nitrite significantly inhibited the reduction and release of As and Fe from the soils. The stoichiometry of the anaerobic redox reactions are:

$$\text{lactate}^- + 2\text{HAsO}_4^- + \text{H}^+ \rightarrow \text{acetate}^- + 2\text{H}_2\text{AsO}_3^- + \text{HCO}_3^-$$

$$\text{lactate}^- + 4\text{Fe}^{3+} + \text{H}^+ \rightarrow \text{acetate}^- + 4\text{Fe}^{2+} + \text{HCO}_3^-$$

$$\text{lactate}^- + 2\text{NO}_3^- + \text{H}^+ \rightarrow \text{acetate}^- + 2\text{NO}_2^- + \text{HCO}_3^-$$

$$3\text{lactate}^- + 4\text{NO}_2^- + \text{H}^+ \rightarrow 3\text{acetate}^- + 2\text{N}_2 + 3\text{HCO}_3^-$$

$$5\text{lactate}^- + 4\text{NO}_3^- + \text{H}^+ \rightarrow 5\text{acetate}^- + 2\text{N}_2 + 5\text{HCO}_3^-$$

Molecular features of the isolate

To elucidate the mechanism of action of the inhibitory effects caused by nitrate/nitrite in the microbial community-mediated solubilization of As in the arsenic-rich soils, we isolated a representative As(V)-respiring bacterium (referred to as GL90) from arsenic-rich soils. A phylogenetic analysis was performed with the 16S rRNA genes of GL90 and its associated microorganisms (Fig. 2a). It illustrates that GL90 was clustered together with the members of *Shewanella*; this clearly indicated that GL90 is affiliated to *Shewanella* (genus). The isolate was accordingly named as *Shewanella* sp. GL90.

The DNA sequence encoding an ArrA subunit of the As (V)-respiratory reductase in GL90 cells was obtained and

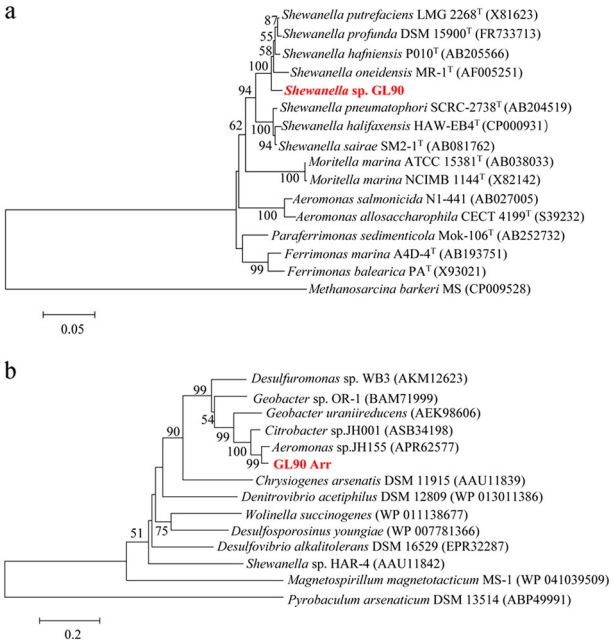

Fig. 2 Cloning and phylogenetic characterization of the 16S rRNA (**a**) and arsenate-respiring reductase (Arr) genes (**b**) from *Shewanella* sp. GL90. The tree was constructed using the neighbor-joining method. Numbers on the branches are bootstrap values based on 1000 replicates. Only the bootstrap values greater than 50% are shown

analyzed. The encoded enzyme was referred to as GL90_ArrA. The amino acid sequence of GL90_ArrA shares 96, 91, 82, and 74% identities with those of the ArrA proteins from *Aeromonas* sp. JH155, *Citrobacter* sp. JH001, *Geobacter uraniireducens*, and *Geobacter* sp. OR-1, respectively. The phylogenetic tree also illustrates that GL90_ArrA is clustered together with these bacterial ArrA proteins (Fig. 2b). These results showed that GL90 possesses a new As(V)-respiratory reductase, and it can be inferred that this strain is a new DARP strain.

Anaerobic reduction activities of *Shewanella* sp. GL90

We examined whether *Shewanella* sp. GL90 has the activities to anaerobically respire arsenate, ferric iron, or nitrate.

As shown in Fig. 3a, GL90 possesses high As(V) reduction activity under anaerobic conditions. The bacterial cells were grown into stationary phase in 5.0 days in MMS medium containing 2.0 mM arsenate and 10.0 mM sodium lactate. After 2.0 days of anaerobic incubation, GL90 quickly catalyzed the reduction of arsenate; after 7.0 days of anaerobic cultivation, arsenate was totally converted to arsenite. No growth was found without arsenate; this suggests that anaerobic respiration is required for the bacterial growth.

GL90 is also able to respire NO_3^-. As shown in Fig. 3b, in the presence of 10.0 mM NO_3^-, the bacterial cells were grown into stationary phase in 21.0 h. As the bacterial cells started to proliferate, NO_3^- was quickly reduced into NO_2^-; this was coupled with the oxidation of lactate; after 21.0 h of anaerobic incubation, 10.0 mM NO_3^- was completely converted into NO_2^- (Fig. 3b); this suggests that GL90 possesses efficient nitrate reduction activity.

Moreover, GL90 has apparent activity to reduce Fe(III) under anaerobic conditions. As shown in Fig. 3c, the bacterial cells of GL90 completely converted 1.5 mM Fe(III) into Fe(II) in 6.0 days.

Therefore, *Shewanella* sp. GL90 is able to efficiently respire arsenate, ferric iron and nitrate coupled with the oxidation of lactate, and it is a representative bacterial strain from the soils.

Nitrate/nitrite inhibited GL90-catalyzed reduction and release of As and Fe from soils

We measured if *Shewanella* sp. GL90 cells drive the dissolution and reduction of As and Fe in the As-contaminated soils using microcosm technique. The data showed that after anaerobic incubations, very little arsenic and iron were dissolved in the sterilized soil slurries of SM1 without GL90 cells (Fig. 4a, b); in contrast, when GL90 cells in exponential period were added to the sterilized slurries, approximately 1.13 mM As(III) and 0.12 mM Fe(II) were dissolved in SM1 microcosms. This suggests that GL90 cells markedly stimulate the reductive mobilization of As and Fe in the soils.

Interestingly, in comparison with the microcosm assays in the absence of NO_3^-, the addition of NO3$^-$ to the microcosms led to 67, 61, 67, and 67% decreases in the GL90-catalyzed releases of As(III), and 76, 47, 56, and 53% decreases in the GL90-catalyzed releases of Fe(II) from the slurries of SM1, after 3.0, 5.0, 7.0 and 14.0 days of anaerobic incubations, respectively (Fig. 4a); similarly, the addition of NO_2^- also caused 79, 59, 69, and 71% decreases in the GL90-catalyzed releases of As(III), and 73, 65, 71, and 72% decreases in the GL90-catalyzed releases of Fe(II) from the slurries of SM1, after 3.0, 5.0, 7.0 and 14.0 days of anaerobic incubations, respectively (Fig. 4b). This suggests that NO_3^-/NO_2^- significantly inhibited the GL90 cells-catalyzed reductive dissolution of As and Fe in the As-contaminated soils.

We examined the fate of NO_3^-/NO_2^- during the microcosm assays of SM1 (Fig. 4d). In the microcosms amended with NO_3^-, the majority of NO_3^- was rapidly reduced into NO_2^- in 7.0 days by GL90 cells; afterwards, NO_2^- was slowly reduced into NH_4^+, and after 21.0 days, approximately 2.73 mM NH_4^+ was generated (Fig. 5d). In the microcosms amended with NO_2^-, NO_2^- was gradually reduced into NH_4^+, and after 21.0 days of anaerobic incubation, approximately 4.78 mM NO_2^- was reduced into

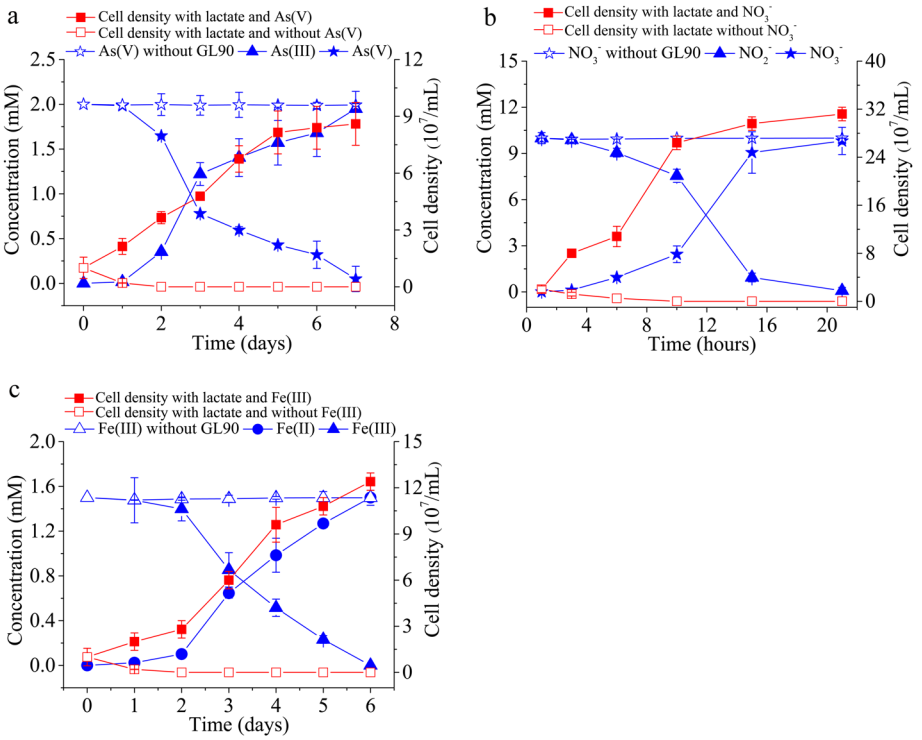

Fig. 3 Arsenate-, nitrate-, ferric ion-respiring activities of *Shewanella* sp. GL90. **a** Bacterial arsenate-respiring curve. **b** Nitrate-respiring curve. **c** Ferric ion-respiring curve

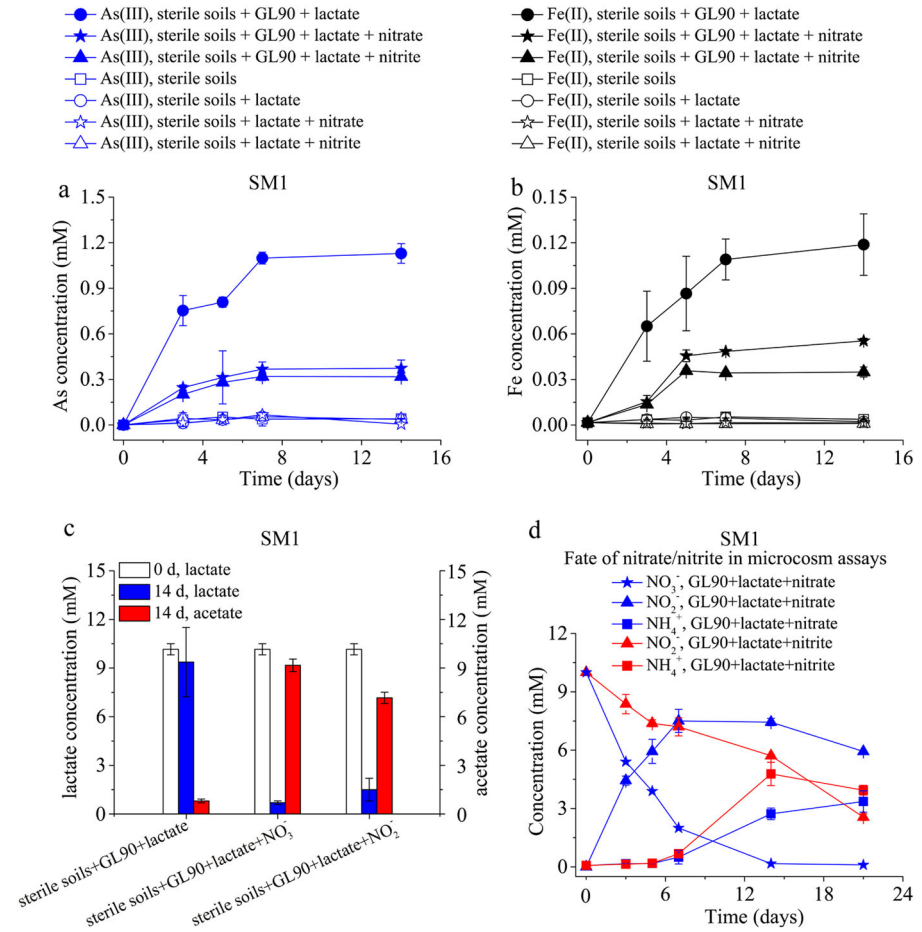

Fig. 4 Effects of nitrate and nitrite on the *Shewanella* sp. GL90-catalyzed mobilization, reduction and release of insoluble arsenic and iron from the sterilized soil slurries of SM1, under strict anaerobic condition. Control soils were sterilized using Co60 irradiation. **a** Microbial release of As(III) from SM1. **b** Microbial release of Fe(II) from SM1. **c** Fate of lactate during the microbial reactions in SM1. **d** Fate of nitrate/nitrite during the microbial reactions in SM1

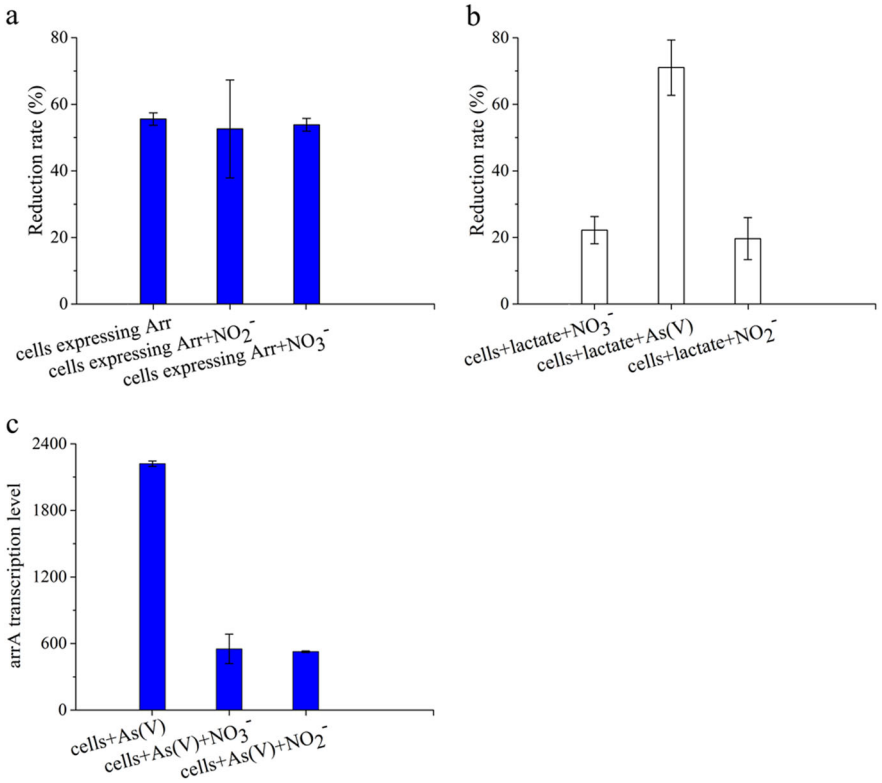

Fig. 5 Inhibitory effects of nitrate/nitrite on the expression level of arsenate-respiring reductase proteins in the *Shewanella* sp. GL90 cells. **a** Nitrate/nitrite has no significant effects on the bacterial activity of arsenate-respiring reductase. **b** The presence of nitrate/nitrite inhibited the expression levels of the arsenate-respiring reductase proteins in the bacterial cells as examined using functional assays. **c** The presence of nitrate/nitrite inhibited the transcription levels of arsenate-respiring reductase gene in GL90 cells as detected using RT-qPCR technique

NH_4^+. We also measured the fate of lactate during anaerobic reactions (Fig. 4c). We found that after 14.0 days of anaerobic incubation, approximately 0.81, 9.17 or 7.17 mM lactate were oxidized into acetate by the GL90 cells in SM1 slurries amended with lactate only, lactate and NO_3^-, and lactate and NO_2^-, respectively. Therefore, it can be concluded that the anaerobic reduction of As(V)/Fe(III)/NO_3^-/NO_2^- was each coupled with the oxidation of lactate.

Similarly, the supplement of NO_3^-/NO_2^- in the slurries of SM2 also caused significant inhibitions of the GL90-mediated reductive dissolution of As and Fe in the soil phase of SM2 (Fig. S2a, 2b). During the anaerobic reactions, nitrate and nitrite were also reduced into ammonium (Fig. S2d), and lactate was oxidized into acetate (Fig. S2c).

Taken together, it can be concluded that nitrate and nitrite are two powerful inhibitors of the DARP cells-catalyzed reductive dissolution of As and Fe in the As-contaminated soils.

Inhibition of the expression of arsenate-respiring reductase gene by nitrate/nitrite

Two mechanisms could be responsible for the inhibitory effects of nitrate/nitrite on the microorganisms-catalyzed reductive dissolution of As and Fe in the As-contaminated soils: (a) Nitrate/nitrite inhibited the bacterial arsenate-respiring activity; (b) Nitrate/nitrite inhibited the transcription level of the gene of the As(V)-respiratory reductase in the cells of DARPs. To address this issue, we conducted bacterial arsenate respiring assays with or without external nitrate/nitrite to the microcosms. Our results showed that either nitrate or nitrite has no significant effects on the arsenate-respiring reductase activity of GL90 (Fig. 5a).

We also examined how nitrate/nitrite affects the expression level of arsenate-respiring reductase proteins in GL90 cells. We inoculated a colony of GL90 cells into the MMS medium containing Na_3AsO_4 and sodium lactate, with or without nitrate/nitrite. After 24.0 h of anaerobic incubation, the cells were collected and washed for measuring the arsenate-respiring activities of GL90 cells. We found that the arsenate-respiring activity of the nitrate or nitrite-treated GL90 cells is 68.8 or 72.3% lower than that of the non-treated cells (Fig. 5b). This suggests that the presence of nitrate/nitrite in the bacterial cultures inhibited the expression of the As(V)-respiratory reductase proteins in GL90 cells during anaerobic incubation.

We further detected how nitrate/nitrite affects the transcription levels of the As(V)-respiratory reductase genes in the cells of GL90 strain with Reverse transcription quantitative real-time PCR. We found that the transcription levels of the As(V)-respiratory reductase genes in the microcosms amended with nitrate or nitrite are 4.02- or 4.22-fold lower than those in the cultures without addition of nitrate/nitrite (Fig. 5c).

Discussion

Environmental implications

Nitrate is one of the most ubiquitous contaminants in soils, sediments and groundwater (Hudak 2000). Many investigations suggested that geochemical processes, biological reactions, and anthropogenic activities create a continuous input of nitrate sources into the environment, particularly into groundwater (Gu et al. 2012). Nitrate accumulation typically occurs when the supply of nitrate exceeds the denitrification capacity of the soils and aquifer. Geochemical survey indicated that approximately 28% of detected groundwater samples were contaminated by NO_3^- in China (Liu et al. 2013). Increasing evidences suggested that there were correlations between the concentrations of nitrate and the levels of arsenic contamination in groundwater (Yao et al. 2018; Park et al. 2018; Kim et al. 2006). Because DARPs play key roles in the formation of arsenic-contaminated groundwater, it is very interesting to investigate the effects of nitrate/nitrite on the DARPs-catalyzed reductive dissolution of As in the As-contaminated soils (Fisher et al. 2008; Islam et al. 2004; Kudo et al. 2013; Osborne et al. 2015).

We found that nitrate/nitrite significantly inhibited the microbial community-catalyzed, reductive dissolution of As and Fe in the As-contaminated soils. Moreover, we used a representative arsenate-respiring bacterium *Shewanella* sp. GL90, which was isolated from the arsenic-rich soils, to perform the microcosm assays. Microcosm assays showed that nitrate/nitrite was reduced into NH_4^+ by GL90 cells, and both nitrate and nitrite dramatically inhibited the GL90-catalyzed reductive dissolution of As and Fe in the As-contaminated soils. We further observed that these observations were attributed to that nitrate/nitrite markedly inhibited the expression levels of As(V)-respiratory reductase ArrA subunit gene of GL90 cells. The discoveries of this study firstly provided new insights into the microbial mechanisms by which the biogeochemical reactions of As is coupled with those of Fe and N in the arsenic-contaminated soils and provided new insights into the mechanisms by which As concentrations in groundwater dynamically changed.

Conceptual model for inhibition of the microbial dissolution of As by nitrate/nitrite

Based on the biological activities of *Shewanella* sp. GL90 and the data achieved from the microcosm assays, a conceptual model was proposed to explain the inhibitory effects of nitrate/nitrite on the GL90-catalyzed reductive dissolution of As in arsenic-rich soils.

As shown in Fig. 3, the GL90 cells were not capable of growing in the absence of As(V) or nitrate; this suggests that a terminal electron acceptor, such as As(V), nitrate or Fe(III), is required to support the bacterial growth. Comparisons of the bacterial reduction activities for As(V), nitrate or Fe(III) showed that the respiring efficiency for nitrate is much higher than those for As(V) or Fe(III). We thus proposed that if nitrate co-exists with As(V) and Fe(III) in the environment, GL90 cells would take the first priority to utilize nitrate as the sole electron acceptor; this would inhibit the electron acquirement by As(V), causing no As (III) available inside the GL90 cells. However, it was established that the expression of the arsenate-respiring reductase genes is regulated by *arr* operon, of which activation is As(III)-dependent (Giloteaux et al. 2013). Therefore, the expression of the arsenate-respiring reductase gene would be inhibited due to lack of As(III) in GL90 cells (Fig. 6).

However, it remains to be elucidated how the bacterial cells sensed and utilized nitrate, and meanwhile abandoned utilization of As(V).

Can nitrate/nitrite be used for bioremediation of As-contaminated groundwater?

Our work clearly indicated that nitrate/nitrite dramatically inhibits the DARPs-catalyzed reductive dissolution of As and Fe in the As-contaminated soils. Considering that DARPs are the major drive of the reductive dissolution and release of As from mineral phase into solution, it can be inferred that nitrate/nitrite could significantly inhibit the DARPs-induced arsenic dissolution and release. Thus, nitrate/nitrite seems to have potential to be used for remediation of As contaminations in groundwater.

However, it is well known that nitrate may be involved in multiple microorganisms-mediated anaerobic redox reactions in the arsenic-rich soils and aquifers: nitrate can be reduced into nitrite/ammonium, which is coupled with the oxidations of multiple electron donors, including Fe(II), As

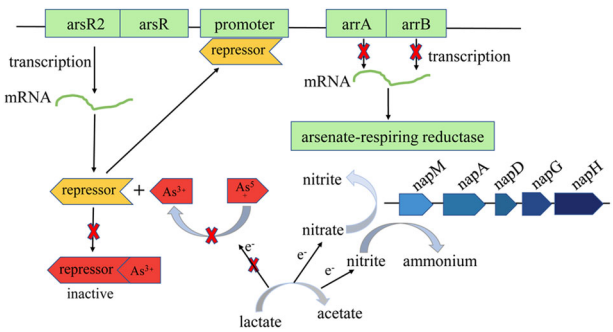

Fig. 6 Conceptual model for the inhibitory effects of nitrate/nitrite on the *Shewanella* sp. GL90-catalyzed reduction of As(V) under anaerobic conditions

(III), hydrogen, sulfide, lactate, acetate, pyruvate, and other inorganic/organic materials (Ohtsuka et al. 2013; Jiang et al. 2013; Zhang et al. 2017). The effects of these reactions on the reductive dissolution of As from mineral phase are complicated, and have been poorly understood so far. Therefore, we have to be cautious to utilize nitrate/nitrite for the bioremediation of As-contaminated groundwater. At least, before the remediation is performed, we have to conduct a thorough investigation on the structures and functions of the indigenous microbial communities, as well as the geochemical features of the remediation site. We also have to pay attention to the secondary contaminations caused by nitrate/nitrite.

Conclusion

Nitrate is one of the most ubiquitous contaminants in soils, sediments and groundwater. In this study, we observed that nitrate/nitrite significantly inhibited the microbial communities and GL90-mediated mobilization, reduction and release of As and Fe from arsenic-rich soils. During the microbial reactions, nitrate/nitrite was reduced to ammonium and nitrogen. We further found that these findings are attributed to that nitrate/nitrite significantly inhibited the transcription of the gene of the respiratory arsenate reductase protein in GL90 cells. This work suggests that natural and anthropogenic inputs of nitrate into soils may significantly reduce arsenic contaminations in groundwater. This discovery provided new insights into the mechanism by which As concentrations in groundwater changed dynamically.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the author.

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