Comparison of arsenate reduction and release by three As(V)-reducing bacteria isolated from arsenic-contaminated soil of Inner Mongolia, China

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HIGHLIGHTS

- We isolated three anaerobic As(V)-reducing bacterium from soil with high ratio of As(III).
- Strains JQ, DJ-3 and DJ-4 could transform very high concentration of As(V) to As(III).
- All the three strains could release goethite-adsorbed As(V) into solution.
- We compared the three strains with other previously isolated strains.
- The existence of the strains will cause the mobilization and transformation of As in soil.

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ABSTRACT

Arsenic (As) contamination has become a worldwide environmental problem: arsenite (As(III)) especially has posed a major threat to human health. Here, we report the first three isolates of anaerobic As(V)-reducing bacterial strains (strains JQ, DJ-3 and DJ-4) from a soil sample containing 48.7% of total As in the form of As(III) collected in Chifeng, Inner Mongolia, China. Strains JQ, DJ-3 and DJ-4 were phylogenetically closely related to *Bacillus*, *Desulfitobacterium* and *Exiguobacterium*, respectively. Among these strains, JQ and DJ-3 have the *arsC* gene, DJ-4 possesses the *arrA* gene. The three strains could all resist and reduce high concentrations of As(V) under anoxic conditions. The order of resistance to As(V) was DJ-3 > JQ > DJ-4. Strain DJ-3 not only possesses the strongest resistance to As(V) but could also reduce 53% of the As(V) to As(III) in the treatment of 60 mM As(V) in 5 d. All three strains could release As from goethite; strain DJ-4 has the highest ability to promote the release of As (90.5%) from goethite. These results suggested that strains JQ, DJ-3 and DJ-4 may play an important role in the mobilization and transformation of As in soil.

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1. Introduction

Arsenic (As) is a strong carcinogen. Excess ingestion of As would cause internal organ cancers and skin diseases such as skin lesions, hyperkeratosis and blackfoot disease (Berg et al., 2001; Nordstrom,

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2002). Over the past few decades, As contamination has become a worldwide environmental problem (Mandal and Suzuki, 2002). In Bangladesh, for example, the As concentration in groundwater in parts of West Bengal is approximately 3.7 mg/L (Samal et al., 2011), which severely exceeds the World Health Organization guideline for As in drinking water (0.01 mg/L) (WHO, 2011). Ingestion of As-polluted groundwater threatened the health of 42 million people in Bangladesh alone in 2009 (Osborne et al., 2015). In China, because of mining, pesticide usage, smelting activities and other natural factors, many provinces such as Hunan, Yunnan and Inner Mongolia are also exposed to severe soil As contamination (Guo et al., 2015; Zhao et al., 2015; Zhu et al., 2008). These As-contaminated soils

have caused serious pollution of agricultural products: As concentrations in some vegetables grown there were up to 7.9–16.6 mg/kg (Liu et al., 2005).

In the soil environment, As exists mainly as two inorganic forms, arsenate (As(V)) and arsenite (As(III)). Arsenate is easily associated with soil minerals (Dixit and Hering, 2003; Liu et al., 2015), especially iron (oxy) hydroxides such as goethite, hematite and ferrihydrite. However, arsenite is much less adsorptive, more mobile and approximately 60 times more toxic than arsenate (Liu et al., 2015; Mueller et al., 2010), which means that As(III) more feasibly enters groundwater or be taken up by agricultural crops and thus threatens human health. Arsenate and arsenite can be inter-converted. Apart from some abiotic factors, several studies have indicated that As(V)-reducing bacteria play an important role in the release, mobilization and transformation of As in the soil-water environment (Chang et al., 2012; Guo et al., 2015; Ohtsuka et al., 2013; Wu et al., 2013).

Several strains of As(V)-reducing bacteria have been isolated in recent years. Microbial reduction of As(V) occurs by two main mechanisms, dissimilatory reduction and detoxification (Chang et al., 2008; Silver and Phung, 2005). Dissimilatory reduction is catalyzed by the respiratory As(V) reductase (ArrA), which is encoded by the *arrA* gene. This process occurs only under anaerobic conditions, and bacteria obtain energy from coupling the reduction of As(V) to the oxidation of some compound (Osborne et al., 2015) such as lactate, hydrogen and glucose (Chang et al., 2012). The second mechanism, detoxification, depends on the existence of the *ars* gene. When As(V) enters into the cell cytoplasm, the ArsC, which is encoded by the *arsC* gene, will transform As(V) to As(III). Thereafter, the ArsB, which is encoded by the *arsB* gene, will pump As(III) out of the cells via an efflux pump (Chang et al., 2008; Jackson and Dugas, 2003). This mechanism occurs under both aerobic and anaerobic conditions.

According to the study of Yin et al., the ratio of As(III) to total As of one soil in Chifeng, Inner Mongolia, China, was 48.7%, which is much higher than other As-contaminated soils (Yin et al., 2015). Considering the characteristics of As(III), this situation will cause more severe As contamination of groundwater and lead many people to suffer chronic As poisoning. Therefore, it is really important to investigate the microorganisms that are capable of reducing As(V) to As(III) in the Inner Mongolian soil. To date, no indigenous As(V)-reducing bacteria have yet been isolated from this As-contaminated soil with a high ratio of As(III) in Chifeng, Inner Mongolia, and no anaerobic As-resistant bacteria have yet been found there.

In this study, we report three isolates, strains JQ, DJ-3 and DJ-4, isolated from the soil of Chifeng, Inner Mongolia, China. Our study first describes and compares the physiological characteristics of the three As(V)-reducing bacteria, then we make a comparison of the isolates based on their ability to resist and reduce As(V). Finally, we assess As release from goethite by the isolates.

2. Materials and methods

2.1. Enrichment, isolation and growth of As(V)-reducing bacteria

A soil sample was collected from a mine in Chifeng, Inner Mongolia, China. The total soil As was approximately 300 mg/kg, and 48.7% of the total As was As(III) (Yin et al., 2015). The enrichment culture was prepared by mixing 1.0 g soil sample with 10 ml dissolved oxygen-free water in a 20-ml sterile serum bottle. After shaking well, 1 ml of the resulting suspension was inoculated into 19 ml of minimal medium. The anoxic minimal salt medium contained the following (per liter): KH_2PO_4 (0.14 g), NH_4Cl (0.25 g), KCl (0.5 g), CaCl_2 (0.113 g), NaCl (1.0 g) and $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (0.62 g). Five

mM lactate was added as a carbon source, with 0.05% yeast extract as a nutrient supplement and 0.1% resazurin solution (1%) as a dissolved oxygen indicator. The medium was dispensed into 100 ml serum bottles under a high purity nitrogen atmosphere and autoclaved under 121 °C for 20 min. Arsenate (5 mM), cycloheximide (0.2 g/L) and L-cysteine (1 mM) were added separately into the culture system after filtering through 0.22 μm Millex (Millipore). The pH of the medium was accurately adjusted to 7.0 using HCl or NaOH with a portable pH meter (HQ11d, Hach, USA). The capped serum bottles were incubated in a rotary shaker at 30 °C in the dark.

During the cultivation, 1 ml of the culture medium was taken out, and the As species was analyzed by combined utilization of high-performance liquid chromatography and inductively coupled plasma mass spectrometry (HPLC-ICP/MS) (7500a, Agilent, USA). After confirming that As(V) was completely reduced to As(III), 1 ml of the enrichment was transferred to 19 ml of the fresh above-mentioned medium. After several such transfers and incubations, a stable enrichment culture of As(V)-reducing bacteria was obtained. The enrichment was serially diluted, and 100 μl of the supernatant of each dilution was inoculated into the minimal medium containing 1.5% (w/v) purified agar (K1001, Japan) in anaerobic tubes. The Hungate technique was used in this experiment. Single colonies were picked and inoculated into new anaerobic tubes, and this procedure was repeated several times to ensure purity. The pure cultures were then inoculated into the minimal medium to test their ability to reduce As(V).

The manipulations and preparations described above were performed in the anaerobic glove box to create a strict anoxic environment. All transfers were carried out using sterile syringes and needles.

2.2. Identification of As(V)-reducing bacteria

DNA was extracted from the As(V)-reducing strains using a FastDNA Spin Kit (MP Biomedicals, Irvine, CA) according to the manufacturer's instructions. The extracted DNA was used as the template for PCR amplification. The 16S rRNA gene was amplified using universal primers, 27F (5'-AGAGTTGATCTGGCTCAG-3') and 1492R (5'-TACGGTTACCTTGTTACGACTT-3'). The PCR protocol was as follows: initial denaturation at 95 °C for 5 min, melting at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min 30 s, with a final extension at 72 °C for 10 min. After 30 cycles, the amplified product was separated in a 1.0% agarose Tris-acetate-EDTA (TAE) gel by electrophoresis and visualized on a UV trans-illuminator (Agilent, USA).

The clone sequencing was detected by Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China. To analyze sequence similarity, the 16S rRNA gene sequences obtained were aligned in the BLAST program (<http://blast.ncbi.nlm.nih.gov>). The phylogenetic tree was constructed in MEGA 6.0. The gene sequence with those of selected representatives was performed using 1000 bases.

2.3. Amplification of putative *arrA* and *arsC* genes

The amplification of the putative *arrA* and *arsC* genes was attempted using four especially designed primers, including *amlt-42-f* and *amlt-376-r* (Sun et al., 2004), *arsC3F* and *arsC3R* (Macur et al., 2004), *ArrAfw* and *ArrArev* (Malasarn et al., 2004), *HAArrAD1F* and *HAArrA-G2R* (Kulp et al., 2007). Sequences of these primers are shown in Table 1. The PCR protocol was different between the amplification of the putative *arsC* gene and the *arrA* gene.

The PCR conditions for amplifying *arsC* gene followed the procedure of Chowdhury et al. (2014): initial denaturation at 94 °C for 2 min, melting at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min. After 30 cycles, a final cycle was

followed: melting at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 5 min. For the *arrA* gene, the protocol was as follows: initial denaturation at 95 °C for 5 min, melting at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min after 30 cycles. The amplified products and the DL2000 DNA marker were confirmed on 2% agarose gel by electrophoresis. The bands of the amplifications were compared with the bands of the DL2000 DNA marker to determine the length.

2.4. Effect of As(V) and As(III) on bacterial growth

Resistance to As(V) and As(III) of the isolated strains was determined by culturing them in 20 ml liquid medium amended with increasing concentrations of As(V) (0 mM, 5 mM, 15 mM and 60 mM) or As(III) (2 mM, 8 mM and 16 mM), respectively. The cultures were incubated in a rotary shaker in the dark and sacrificed every day. The optical density of the medium samples at 600 nm (OD_{600}) was determined using an ultraviolet and visible spectrophotometer (UV-4802H, UNIC, China). All manipulations were carried out under anoxic conditions. After incubation for 7 d, the growth curves of the strains were drawn according to the OD_{600} .

2.5. Reduction of dissolved As(V) to As(III)

The strains were inoculated into a liquid medium amended with four As(V) concentrations: 0 mM, 5 mM, 15 mM and 60 mM. Treatments without the strains (sterilized at 121 °C for 20 min) were also carried out to detect the effect of the strains. All treatments were carried out under anoxic conditions.

The serum bottles were transferred to an anaerobic glove box (filled with ultrapure N_2) and solutions (1 ml) were taken from each bottle at different time intervals. The collected solutions were then passed through a 0.22 μ m filter for As species determination. Concentrations of As(V), As(III), MMA (monomethylarsonic acid, $CH_3AsO(OH)_2$) and DMA (dimethylarsinic acid, $(CH_3)_2AsO(OH)$) were analyzed using HPLC-ICP/MS (7500a, Agilent, USA). To assess whether the isolated strains could reduce As(V) under aerobic conditions, the three strains were then cultivated in an aerobic culture, and the As species were also determined.

2.6. Arsenic Release from the solid phase

Goethite synthesis was carried out by adding 90 ml 5.0 M NaOH (18 g) to 50 ml 1.0 M $FeCl_3$ (8.125 g) solution (Huang et al., 2011). The suspension was diluted to 1 L with deionized water and aged in a polypropylene flask at 70 °C for 3 d. Thereafter, the goethite suspension was centrifuged for 30 min at 2100g and washed several times with deionized water. After freeze-drying, the goethite was stored at 4 °C.

Three grams of the above-mentioned goethite were added to 100 ml of NaH_2AsO_4 solution (500 ppm) and stirred for 24 h to

prepare As(V)-adsorbed goethite. The suspension was centrifuged for 30 min at 2100g and washed twice with distilled water and freeze-dried. The adsorption of arsenate on goethite was approximately 4.75 mg/g.

The As(V)-reducing strains were pre-grown in the minimal medium with As(V) (5 mM) for 7 d. Then, the cultures (1 ml) were centrifuged at 15,000g for 10 min. After washing with 0.8% sterile NaCl, the cells were resuspended in 1 ml of 0.8% NaCl. Then, 1 ml of the cell suspensions were inoculated into 9 ml of fresh liquid medium containing 50 mg of As(V)-adsorbed goethite. Control groups (without bacteria) were set at the same time. Solutions were taken from each bottle after cultivating for 4 d. Arsenic species were determined using HPLC-ICP/MS to assess whether those three strains could reduce and release As that was adsorbed on goethite.

3. Results and discussion

3.1. Isolation and identification of As(V)-reducing bacteria

After inoculating the soil sample into the minimal medium and four rounds of subculturing, the microflora of As(V)-reducing bacteria were obtained. The Hungate technique was used to isolate pure cultures, and single colonies were picked and repeatedly inoculated five times to ensure purity. After affirming the ability to reduce As(V) to As(III), three organisms, strains JQ, DJ-3 and DJ-4, were isolated.

The 16S rRNA gene sequences of strains JQ, DJ-3 and DJ-4 were analyzed in BLAST. The results showed that strain JQ is closely related to *Bacillus* with a 16S rRNA gene sequence similarity of 99.7%. Strain DJ-3 and strain DJ-4 showed 99% similarity to *Desulfitobacterium* and *Exiguobacterium*, respectively. The high similarity suggested that strains JQ, DJ-3 and DJ-4 were *Bacillus* sp., *Exiguobacterium* sp. and *Desulfitobacterium* sp., respectively. The results of phylogenetic comparison are shown in Fig. 1.

Phylogenetically diverse organisms capable of reducing As(V) have been isolated from As-contaminated environments, including strains of *Bacillus* sp., *Anaeromyxobacter* sp., *Geobacter* sp., *Desulfohalobium* sp., *Desulfotomaculum* sp. and *Clostridium* sp. (Kudo et al., 2013; Mirza et al., 2014; Ohtsuka et al., 2013; Vaxevanidou et al., 2008). Among the As(V)-reducing bacteria, *Bacillus* is a common bacterial genus. Bacteria such as *Bacillus selenitireducens* (Blum et al., 1998), *Bacillus* sp. HT-1 (Kocar et al., 2006) and *Bacillus* sp. JMM-4 (Santini et al., 2004) have been isolated and studied in previous studies. In this study, strain JQ is also a member of the *Bacillus* family. In addition, Niggemyer et al. had isolated two As(V)-reducing bacteria, strain *Desulfitobacterium frappieri* and strain *Desulfitobacterium* sp. GBFII (Niggemyer et al., 2001). Strain DJ-3 is in the same family with those two strains.

There have been no reports on As(V) reduction by *Exiguobacterium* isolated from soil. The present study showed that strain DJ-4 is able to reduce As(V) to As(III). Therefore, strain DJ-4 is the first isolated and identified strain of the genus *Exiguobacterium* that

Table 1

The primer sequences used for the amplification of the *arsC* gene and the *arrA* gene.

Targeted gene	Primer	Primer sequences (from 5' to 3')	Length
<i>arsC</i>	<i>amlt-42-f</i>	TCGCGTAATACGCTGGAGAT	353
	<i>amlt-376-r</i>	ACTTCTCGCGTCTTCCTT	
<i>arsC</i>	<i>arsC3F</i>	AGTCCTGTTTCATGTGYAC	359
	<i>arsC3R</i>	TGCGGTSGAAYGCCG	
<i>arrA</i>	<i>ArrAfwd</i>	AAGGTGTATGGAATAAGCGTTTgtbgghgaytt	175
	<i>ArrArev</i>	CCTGTGATTTACAGGTGCCcaytyvgngnt	
<i>arrA</i>	<i>HAArrA-D1F</i>	CCGCTACTACCCGAGGCGWWYTGGGRNTA	418
	<i>HAArrA-G2R</i>	CGTGCGGTCTTGTAGCTCNWDRITCCACC

Fig. 1. Phylogenetic tree of 16S rRNA gene sequences of strains JQ, DJ-3 and DJ-4.

can actuate the mobilization and transformation of As in soil. Bacteria of the genus *Exiguobacterium* are Gram-positive and facultatively anaerobic. Many bacteria of *Exiguobacterium* sp. are thermophilic or psychrophilic, halophilic or alkaliphilic (Zhang et al., 2013). Based on the above characteristics of *Exiguobacterium*, strain DJ-4 may be able to reduce As(V) in many extreme environments.

3.2. Amplification of putative *arrA* and *arsC* genes

Results of amplifying the putative *arrA* gene and *arsC* gene are shown in Table 2.

Strains JQ and DJ-3 showed a single band when amplified with PCR primers *arsC3F* and *arsC3R*, and the size of the band was consistent with the expected size (359 bp) by comparing with the bands of the DL2000 DNA marker. In addition, a single band of expected size (175 bp) was observed in genomic DNA of strain DJ-4 when amplified with PCR primers *ArrA_{fwd}* and *ArrA_{rev}*.

The *arsC* gene was successfully amplified in strains JQ and DJ-3, indicating that these two strains can resist and reduce As(V) through the mechanism of detoxification. However, the *arrA* gene was successfully amplified in strain DJ-4, which means that this strain could obtain energy from coupling the reduction of As(V) to the oxidation of lactate.

According to the aerobic experiments, we discovered that all three strains could live in either aerobic or anaerobic environments,

but only strains JQ and DJ-3 could reduce As(V) in the presence of oxygen because strains JQ and DJ-3 are *arsC* gene carriers, and strain DJ-4 is an *arrA* gene carrier. These results gave a further verification of the theory that the *arsC* gene carriers can facilitate As redox transformation under both oxic and anoxic conditions, while the *arrA* gene carriers could reduce As(V) to As(III) only in an anaerobic environment.

Although the amplification of *arrA* gene from strains JQ and DJ-3, *arsC* gene from strain DJ-4 were unsuccessful, we cannot determine that strains JQ and DJ-3 do not possess *arrA* gene or strain DJ-4 do not possess *arsC* gene, for the putative *arrA* gene and *arsC* gene may not be amplified by previously designed degenerate primers (Kudo et al., 2013).

3.3. Growth of the As(V)-reducing bacteria on As(III) and As(V)

The growth of strains JQ, DJ-3 and DJ-4 in the treatments with different concentrations of As(III) or As(V) is shown in Fig. 2.

In the treatments with As(III), strain JQ showed a growth tendency different from strains DJ-3 and DJ-4. The maximum biomass of strain JQ was reached at 4 d in the treatments of 2 mM/8 mM As(III) and at 5 d with 16 mM As(III). Strains DJ-3 and DJ-4 achieved the maximum biomass at a shorter incubation time. In the treatments of 2 mM/8 mM As(III), the maximum biomass was reached at 1 d with strain DJ-3 and at 2 d with strain DJ-4. Both strains DJ-3 and DJ-4 failed to grow with 16 mM As(III). As shown in Fig. 2, strain JQ, strain DJ-3 and strain DJ-4 could resist 16 mM, 8 mM and 8 mM As(III), respectively. By comparing their maximum biomass in the treatments containing the same As(III) concentration, the resistance to As(III) of three strains was shown to be JQ > DJ-3 > DJ-4.

In the treatments with As(V), the maximum biomass of strain JQ tends to be uniform for each batch, and the maximum biomass was reached at 4 d. Strains DJ-3 and DJ-4 both had similar growth curves in the treatments of 5 mM As(V) and 15 mM As(V), all reached the maximum biomass at 1 d, but the maximum biomass of strain DJ-4 was much lower than the maximum biomass of strain DJ-3. When the concentration of As(V) increased to 60 mM, the maximum biomass of strain DJ-3 almost doubled in the above-mentioned treatments, which means that strain DJ-3 has a great tolerance for As(V). However, strain DJ-4 barely grows with 60 mM As(V). These results show that strain JQ, strain DJ-3 and strain DJ-4 could resist 60 mM, 60 mM and 15 mM As(V), respectively. Because strain DJ-3 showed a much higher maximum biomass than strain JQ with 60 mM As(V), the resistance to As(V) of the three strains was DJ-3 > JQ > DJ-4.

The growth of strain DJ-3 was significantly enhanced in the presence of 60 mM As(V), possibly because although the amplification of the *arrA* gene was unsuccessful from strain DJ-3, we speculate that strain DJ-3 may possess the *arrA* gene, and it may obtain energy from coupling the reduction of As(V) to the oxidation of lactate. Strain DJ-3 could tolerate high concentrations of arsenic. When the concentration of As(V) was raised to 60 mM, strain DJ-3 could obtain more energy from the respiration, which enhances the growth of strain DJ-3.

In addition, since strain DJ-4 could grow in the absence of As(V) (Fig. 2c), and there is no other electron acceptor in the culture medium, so strain DJ-4 may could obtain energy from the fermentation. That is to say, strain DJ-4 could reduce As(V) by respiratory action but it is not a dissimilatory As(V) reducer.

The reason why strain DJ-4 had the least capacity to resist As may be that the other two bacteria contain the *arsC* gene in a family of *ars* operons, which could confer a high resistance to a heavy metal (Tian et al., 2015). For those As(V)-reducing bacteria only owning the *arrA* gene (such as strain DJ-4), their limited tolerance

Table 2
Amplification of putative *arrA* and *arsC* genes in strains JQ, DJ-3 and DJ-4.

Genes	Isolates		
	Strain JQ	Strain DJ-3	Strain DJ-4
<i>arsC</i>	+	+	–
<i>arrA</i>	–	–	+

Fig. 2. Growth of strains JQ (a), DJ-3 (b) and DJ-4 (c) in the treatments with different concentrations of As(V) and As(III).

to As would confine its growth under a high concentration of As.

In other studies, different As(V)-reducing bacteria showed discrepant resistance to As. Anderson and Cook (2004) isolated several As(V)-reducing bacteria from the Barewood gold field. The strains could tolerate As(V) at varying concentrations from 30 to 50 mM or As(III) from 10 to 15 mM. Joshi et al. (2008) found that the growth of the isolated strain As-1 (*P. stutzeri*) was inhibited in the medium with 50 mM As(V) or 0.2 mM As(III) (Joshi et al., 2008). In this study, strain JQ could tolerate 60 mM As(V) or 16 mM As(III), strain DJ-3 could tolerate more than 60 mM As(V) or 8 mM As(III), indicating that the strains in our study had a relatively stronger resistance to As than other strains.

3.4. Dissolved As(V) reduction by As(V)-reducing bacteria.

Concentrations of dissolved As(V) and As(III) were investigated during incubation of the strains (Fig. 3). The results showed that As species did not change with time in the controlled treatments, indicating that the culture media do not have As(V)-reducing capacity.

In the treatments of 5 mM As(V), strains JQ, DJ-3 and DJ-4 all reduced As(V) quickly and completely transformed As(V) to As(III) after cultivating for less than 30 h (Fig. 3a–c). When the concentrations of As(V) rose to 15 mM and 60 mM, the three strains showed distinctly different reduction abilities. In the treatment of 15 mM As(V) (Fig. 3d–f), the three strains all reduced As(V) after a very short period of incubation and terminated the reducing action after 3 d. However, their reducing capacity showed a difference: strain JQ, strain DJ-3 and strain DJ-4 finally reduced 8.4 mM, 15 mM and 13.5 mM As(V) to As(III), respectively. The reduction rate of the three strains was DJ-3 (100%) > DJ-4 (97.2%) > JQ (56.8%) within 3 d.

With the treatment of 60 mM As(V) (Fig. 3g–i), the disparity between the reducing capacity of the three strains was more obvious. The three strains all began to reduce As(V) at 1 d, and strain JQ terminated the reducing action after 3 d. Strain DJ-3 reduced As(V) quickly during the logarithmic growth phase (the incubation time between 1 d and 5 d), but the reduction slackened after cultivating for 5 d and stopped in 7 d. As for strain DJ-4, because the biomass of strain DJ-4 seriously decreased in the treatment with 60 mM As(V), strain DJ-4 reduced As(V) at a

relatively low percentage and terminated the reducing action after 5 d. Strain JQ, strain DJ-3 and strain DJ-4 finally reduced 14.8 mM, 28.7 mM and 15.1 mM As(V) to As(III), respectively. The reduction rate of the three strains was DJ-3 (53.0%) > DJ-4 (22.0%) > JQ (18.7%). In conclusion, the As(V) reducing capacity of the three strains was DJ-3 > DJ-4 > JQ. Combining the results of the resistance experiments, strain DJ-3 not only possessed the strongest resistance to As(V) but also showed the greatest ability to reduce dissolved As(V).

Several As(V)-reducing bacteria had been isolated in previous studies. Here, we compare the reducing capacity of strains JQ, DJ-3 and DJ-4 with the previous isolated strains (Table 3).

By comparison, we find that when the initial concentration of As(V) was 5 mM (used by most studies), strains JQ, DJ-3 and DJ-4 had shown very strong As(V)-reducing capacity. The reduction rate of the three strains was obviously faster than the other reported strains. With regard to the studies in which initial As(V) concentrations were 10–15 mM, the three strains, especially strain DJ-3, also reduced As(V) completely with the fastest speed. In the treatment of 60 mM As(V), strain NC-1 (isolated by Chang et al.) transformed 20 mM As(V) to As(III) in 2 d, which was faster than strain DJ-3. However, strain NC-1 reduced less As(V) to As(III) than strain DJ-3 at the end of the incubation.

3.5. Release of arsenic adsorbed on goethite

The cell suspensions of three strains were cultured with As(V)-adsorbed goethite. In control groups without strains, a small amount of As(V) desorbed from the goethite. The concentration of desorbed As reached 11.32 mg/L (corresponding to 2.26 mg/g As) at 2 d and exhibited stability. Both strains JQ, DJ-3 and DJ-4 promoted the release of As(V) from the goethite (Table 4).

The initial adsorption of As on goethite was 4.75 mg g⁻¹, and 2.26 mg g⁻¹ As was desorbed in control, so the final adsorption of As on goethite was 2.49 mg g⁻¹. Because 2.34 mg L⁻¹ (corresponding to 0.47 mg g⁻¹) more As was released in the treatment with strain JQ than in control, so the percentage of As that was released from goethite by strain JQ was 18.8%. Similarly, the concentrations of released As by strain DJ-3 and strain DJ-4 were 1.61 mg g⁻¹ (64.6%) and 2.25 mg g⁻¹ (90.5%), respectively. The

Fig. 3. Biological reduction of As(V) to As(III) with different initial As(V) concentrations: (a) strain JQ with 5 mM As(V); (b) strain DJ-3 with 5 mM As(V); (c) strain DJ-4 with 5 mM As(V); (d) strain JQ with 15 mM As(V); (e) strain DJ-3 with 15 mM As(V); (f) strain DJ-4 with 15 mM As(V); (g) strain JQ with 60 mM As(V); (h) strain DJ-3 with 60 mM As(V); (i) strain DJ-4 with 60 mM As(V).

ability to release As adsorbed on goethite was DJ-4 > DJ-3 > JQ.

Among the three strains, strain DJ-4 has a relatively weaker ability to reduce dissolved As(V), but strain DJ-4 could release the most As from goethite. Microorganisms with a powerful ability to reduce dissolved As do not necessarily have a great capacity to

release As from iron (hydr)oxides. One of the reasons may be attributed to strain DJ-4 carrying the *arrA* gene. The respiratory As(V) reductase, ArrA (encoded by the *arrA* gene) is mainly located at the periplasm or membrane, and this location will increase its possibility to contact the adsorbed As(V) (Tian et al., 2015). Another

Table 3
Comparison of As(V)-reduction capacity for different As(V)-reducing bacteria.

Strain	Genus	Initial As(V) concentration (mM)	Reduction rate (%)	Reduction time	References
M17-15	<i>Bacillus</i> sp.	0.1	100	6 h	(Guo et al., 2015)
M17-1	<i>Pseudomonas</i> sp.	0.1	100	24 h	(Guo et al., 2015)
DJ-3	<i>Desulfotobacter</i> sp.	5	100	24 h	This study
JQ	<i>Bacillus</i> sp.	5	100	30 h	This study
DJ-4	<i>Exiguobacter</i> sp.	5	100	30 h	This study
WB3	<i>Desulfuromonas</i> sp.	5	More than 90	30 h	(Osborne et al., 2015)
PSR-1	<i>Deltaproteobacteria</i> .	5	Near 100	4 d	(Kudo et al., 2013; Yamamura et al., 2005)
DAR-403	<i>Nitrocola</i> sp.	5	Near 86	22 d	(Hamamura et al., 2014)
OR-1	<i>Geobacter</i> sp.	6	Near 100	72 h	(Ohtsuka et al., 2013)
GBFH	<i>Desulfotobacter</i> sp.	10	100	90 h	(Niggemyer et al., 2001)
NKSG1T	<i>Proteobacteria</i>	12	100	10 d	(Handley et al., 2009)
DJ-3	<i>Desulfotobacter</i> sp.	15	100	3 d	This study
DJ-3	<i>Desulfotobacter</i> sp.	60	53	5 d	This study
NC-1	<i>Citrobacter</i> sp.	60	33.3	48 h	(Chang et al., 2012)

Table 4
The concentrations of dissolved As(III) and As(V) after incubation for 4 d (mg L⁻¹).

Treatments	As(III)	As(V)	Total As
Control	0.29	11.03	11.32
Strain JQ	12.66	1.00	13.66
Strain DJ-3	12.12	7.24	19.36
Strain DJ-4	21.42	1.17	22.59

reason is that the As(V)-reducing bacteria have different As release mechanisms. Some bacteria could directly reduce As(V) adsorbed on iron (hydr)oxides, while others could reduce Fe(III) and cause the dissolution of the iron (oxy)hydroxides that will result in As release (Cerkez et al., 2015; Dia et al., 2015; Ehler et al., 2014; Vaxevanidou et al., 2015). Different As release mechanisms will lead to disparate As release capacities. The mechanism through which strains JQ, DJ-3 and DJ-4 released As from As(V)-adsorbed iron (oxy) hydroxides still needs further investigation.

4. Conclusions

In this study, three anaerobic As(V)-reducing bacterial strains were isolated from the soil with a high ratio of As(III). Strains JQ, DJ-3 and DJ-4 are all able to resist high concentrations of As(III) and As(V). All three strains have a very strong As(V) reducing capacity, and they could reduce As(V) at a very fast speed. Strain DJ-3 could not only reduce 5 mM As(V) in only 20 h, but strain DJ-3 also obtained the highest As(V) reduction rate (53%) in the treatment of 60 mM As(V). In addition, the three strains all could promote the release of As(V) adsorbed on goethite into solutions. In consideration of the common existence of the isolated strains, strains JQ, DJ-3 and DJ-4 may have played a very important part in the mobilization and transformation of As in soil.

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