Assessing the mechanisms controlling the mobilization of arsenic in the arsenic contaminated shallow alluvial aquifer in the blackfoot disease endemic area

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ABSTRACT

High levels of arsenic in groundwater and drinking water represent a major health problem worldwide. Drinking arsenic-contaminated groundwater is a likely cause of blackfoot disease (BFD) in Taiwan, but mechanisms controlling the mobilization of arsenic present at elevated concentrations within aquifers remain understudied. Microcosm experiments using sediments from arsenic contaminated shallow alluvial aquifers in the blackfoot disease endemic area showed simultaneous microbial reduction of Fe(III) and As(V). Significant soluble Fe(II) (0.23 ± 0.03 mM) in pore waters and mobilization of As(III) (206.7 ± 21.2 nM) occurred during the first week. Aqueous Fe(II) and As(III) respectively reached concentrations of 0.27 ± 0.01 mM and 571.4 ± 63.3 nM after 8 weeks. We also showed that the addition of acetate caused a further increase in aqueous Fe(II) but the dissolved arsenic did not increase. We further isolated an As(V)-reducing bacterium native to aquifer sediments which showed that the direct enzymatic reduction of As(V) to the potentially more-soluble As(III) in pore water is possible in this aquifer. Our results provide evidence that microorganisms can mediate the release of sedimentary arsenic to groundwater in this region and the capacity for arsenic release was not limited by the availability of electron donors in the sediments.

1. Introduction

Arsenic (As), a known human carcinogen, is widely distributed in food, water, soils, and air [1,2]. It is a ubiquitous element of both natural and anthropogenic origin and is often responsible for contaminating water supplies [3]. In most cases, arsenic contamination of groundwater is derived naturally from arsenic-rich aquifer sediments [4]. Natural arsenic in groundwater used as a source for drinking, household, and agricultural purposes represents a major health problem for humans in many places around the world, and particularly in South Asia (West Bengal, Bangladesh) where tens of millions of people in the Bengal Delta are at risk from drinking arsenic-contaminated water [4–6]. Numerous other locations worldwide have been reported, and some of these, such as those in Taiwan, have been recognized for several decades [4].

In Taiwan, arsenic-contaminated aquifers typically exist in the coastal plain sediments where groundwater is reducing [7–10]. The southwestern coast area is historically associated with endemic cases of blackfoot disease (BFD), a peripheral vascular disease caused by long-term ingestion of arsenic-contaminated groundwater [11]. In addition to skin pigmentation, a very high incidence of keratosis, and skin, lung, bladder and other cancers were found among people who lived in southwestern coast area and drank arsenic-contaminated well water prior to the 1990s [11,12]. At present, although well water is not directly ingested by most residents in this region, it is still extensively provided to meet domestic, irrigation, aquacultural, and industrial needs. High bioaccumulated arsenic concentrations were found in farmed fish that were associated with the arsenic found in pond water in this area [12]. However, there is still intense debate on the etiology of BFD as well as the sources of arsenic. Despite considerable research over the past 30 years into the occurrence and distribution of arsenic in the region's groundwater, few studies have been conducted to investigate the geochemical and microbiological processes that influence the element's speciation and mobility in the aquifers of the BFD endemic area. To this end, it is important to understand all relevant abiotic and biotic factors that contribute to arsenic mobilization and sequestration in such environments.

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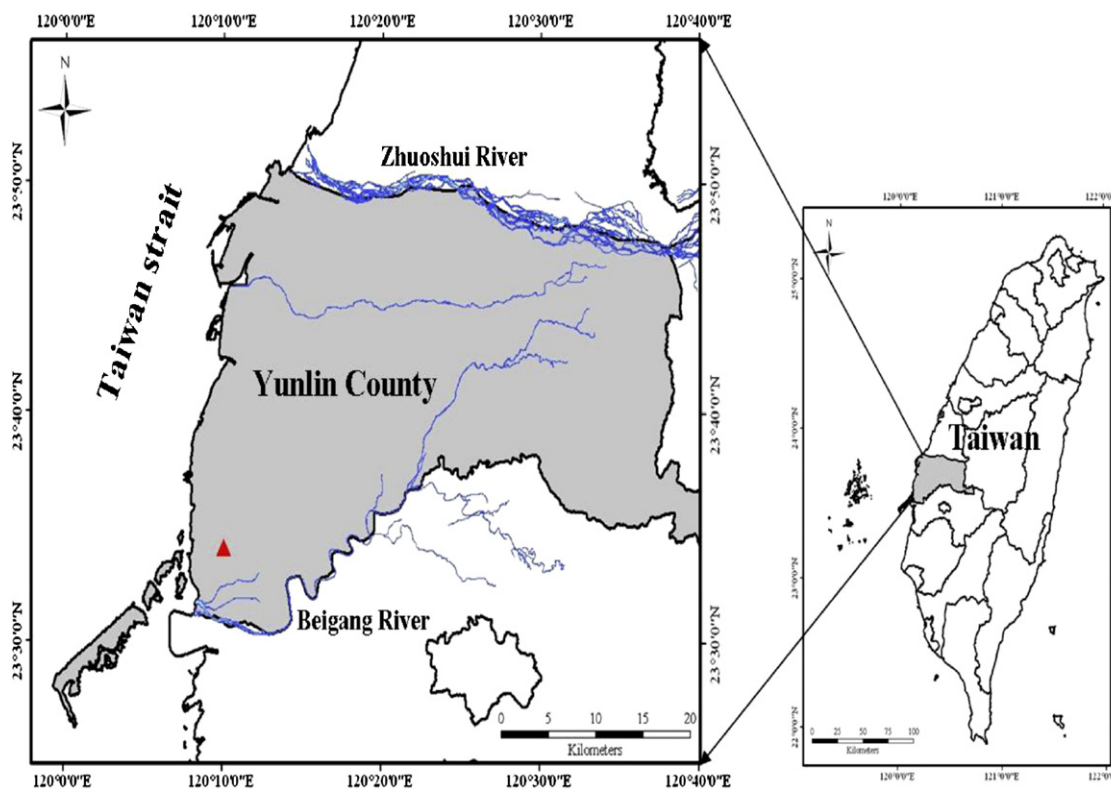[1] These authors contributed equally to this work.

Fig. 1. Site map of the study area and approximate sediments sampling site (▲).

The processes controlling the release of arsenic into groundwater systems are complex and have been extensively studied over the past decade, but still remain a subject of intense debate. Various mobilization mechanisms have been proposed, including oxidation of arsenic-rich pyrite [13], reductive dissolution of iron oxyhydroxide phases [14,15], competition of solutes for sorption sites on iron oxides [16], mineral weathering [17,18], and microbial reduction of sorbed As(V) to the potentially more-mobile As(III) under anoxic conditions [19,20]. Among the proposed mechanisms, reductive dissolution of arsenic-bearing ferric oxyhydroxides has gained particular attention, with several studies showing that indigenous anaerobic metal-reducing bacteria can play a significant role in controlling arsenic mobilization [20–22]. However, the precise role of these anaerobic bacteria in arsenic mobilization remains largely uncharacterized [23]. Most studies that described metal-reducing bacteria in mediating arsenic mobilization have been based on molecular approaches with the sequences of 16S rRNA gene clone libraries [21,24–26]. Using a culture-dependent technique to identify and isolate the indigenous bacteria which is native to the aquifer sediment that might play a role in mediating the release of arsenic into groundwater systems has seldom been described.

In this study, we examine the role that indigenous microbial communities play in arsenic mobilization in an arsenic-contaminated aquifer in the BFD endemic area. Herein, we present data on the concentration and speciation of arsenic and iron associated with sediments from a well drilled in the BFD endemic area. In order to test whether arsenic-contaminated groundwater was attributable to microbiological processes in this aquifer, we conducted sediment incubation experiments under a range of biogeochemical conditions. A microcosm-based study was further carried out to elucidate the role of indigenous anaerobic bacteria in the biogeochemical cycling of arsenic and iron in aquifer sediments.

2. Materials and methods

2.1. Site description and sample collection

The sampling site was located in the southern Zhuoshui River alluvial fan of Taiwan (23°34′07″N, 120°10′04″E) (Fig. 1), which is close to southwestern coast in the blackfoot disease endemic area. The southern Zhuoshui River alluvial fan has an area of around 1000 km^2. It is bounded by two major rivers that flow through the area, the Zhuoshui River to the north and the Beigang River to the south, partitioned into the proximal-fan, the mid-fan, and the distal-fan areas [27].

A borehole was drilled about 10 m away from a well that has been routinely monitored for arsenic contamination by the Taiwan Industrial Development Bureau of the Ministry of Economic Affairs. The well had elevated levels of arsenic ranging from 0.30 to 0.78 mg/L [28]. To collect sediment samples, briefly, a borehole was drilled without using drilling mud. A drill rig and a split-tube sampler with PVC liner (50 mm outside diameter) which had been pre-cleaned were used to collect sediment cores. Cores were extruded and segmented by depth in an anoxic, sterilized glove box containing N_2 gas. Groundwater collected from wells nearby the drilled borehole with similar depth has been found to be reducing (−144 mV to −178 mV), indicating that the sediments were under reducing conditions. Therefore, after sampling, sediment samples were stored in sterile airtight polyethylene bags in an anaerobic tank and preserved at 4 °C in the dark to minimize microbial activity. Further sediment manipulations were performed only under strict anoxic conditions.

2.2. Microcosm sediment cultures

About 15 g of sediments (from 20 m in depth, dark gray fine sandy loam) was mixed with 30 mL of artificial

groundwater (AGW) (1.97 mM $MgCl_2$, 0.33 mM $MgSO_4$, 0.51 mM $NaHCO_3$, 0.01 mM $NaNO_3$, 0.01 mM K_2HPO_4, and 5.50 mM $CaCO_3$, with the pH adjusted to 7.0 with concentrated HCl) based on the constituents of groundwater samples near the study site. A sediment/water slurry was placed in 100-mL serum bottles, fitted with a butyl rubber stopper, sealed with an aluminum clamp under an N_2–CO_2–H_2 atmosphere and incubated at room temperature (24–26 °C) in the dark. The experimental conditions included: (i) aerobic; (ii) anaerobic; (iii) anaerobic amended with 50 mM acetate; and (iv) anaerobic autoclaved sterile (the control), amended with 50 mM acetate. Aerobic conditions were maintained by piercing the butyl stopper with a hypodermic syringe to allow the free passage of oxygen into the microcosm. For microcosm incubation with the ARS-3 bacterial strain (isolation procedures for ARS-3 see Section 2.7), the sterilized sedimentary slurry was inoculated with ARS-3 (1.2×10^7 cells mL^{-1}) and incubated under anaerobic conditions.

Microcosms were subsampled at discrete time points, taking care to maintain the same sediment/water ratio throughout the experiment and to prevent microbial contamination. Samples at $t = 0$ referred to samples that were withdrawn immediately after sediments and AGW were mixed together. Samples were immediately analyzed for iron species, and the aqueous-phase arsenic species were analyzed within 12 h. Each microcosm was prepared in triplicate. The limited sample volumes available precluded the determination of other key analytes in the microcosms.

2.3. Chemical analyses

For microcosm sediment cultures, about 1.5 mL of sample was withdrawn from the microcosm culture at each time point in an anaerobic chamber. One hundred microliters of sample slurry was immediately digested with 4.9 mL 0.5 M HCl at room temperature for 1 h anaerobically and analyzed for iron species using a modified ferrozine-based method [29]. The remaining samples were passed through a 0.45-μm filter. Filtered aqueous samples were analyzed for iron species by the ferrozine-based method and for arsenic species by liquid chromatography/inductive coupled plasma/mass spectrometry (LC–ICP–MS) (Agilent 1100 series; Agilent 7500C, Santa Clara, CA, USA). Before incubation (samples at $t = 0$), all microcosms (including autoclave-"killed" controls) had similar arsenic concentrations (typically < 26.7 nM, approximately 2 μg/L) and no detectable concentrations of soluble Fe(II) in the aqueous phases.

For sediments prior to incubation, arsenic associated with poorly crystalline Fe oxyhydroxides (adsorbed arsenic) and iron oxides were extracted from 1 g of sediment with 0.2 M NH_4-oxalate buffer (pH 3.0) as previously described [30,31]. Subsequently, arsenic concentrations were determined by LC–ICP–MS, and iron species were determined using a ferrozine-based method. Total organic carbon (TOC) in sediments was determined by a chromic acid digestion procedure [32].

2.4. 16S rRNA amplification

After 8 weeks of anaerobic incubation, microbial communities present in non-amended and acetate-amended sediments were analyzed. Sediment DNA was extracted from approximately 1 g of sediment using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The 16S rRNA fragment (645 bp) was amplified by a polymerase chain reaction (PCR) from extracted DNA samples using universal bacterial primers [33]. PCR amplifications were performed in a 50-μL reaction volume containing 0.2 μM of each primer, 1.5 mM of Mg^{2+}, 0.2 mM of dNTPs, and 2 U of Taq DNA polymerase (GeneTeks, Taipei, Taiwan). The purity of the amplified product was determined by gel electrophoresis and the amplified
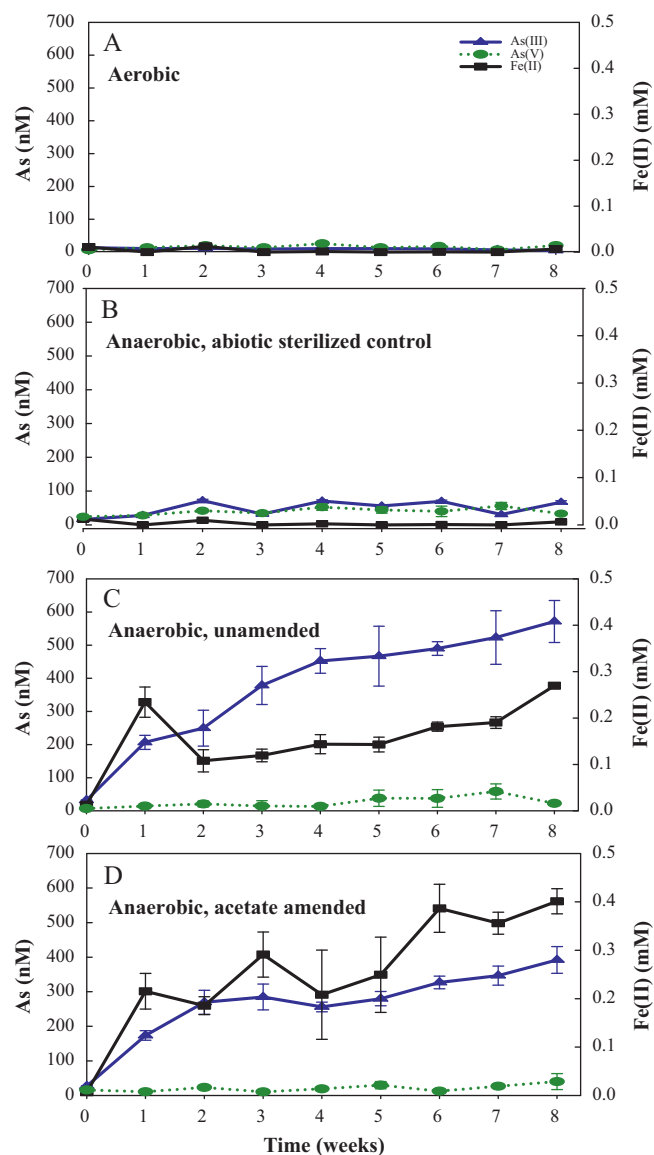

Fig. 2. Mobilization of arsenic, and reduction of Fe(III) in microcosms containing sediments incubated under a range of biogeochemical regimes: (A) aerobically; (B) anaerobically, 'abiotic' sterilized control; (C) anaerobically; and (D) anaerobically with 50 mM sodium acetate as a proxy for organic matter. Black squares, aqueous Fe(II); blue triangles, aqueous As(III); green circles, aqueous As(V). Each point and error bar represent the mean and standard deviation of three replicate experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

PCR products were purified using a BioMan kit (GeneTeks, Taipei, Taiwan). The purified PCR product was cloned into a yT&A vector using a TA cloning kit and competent *Escherichia coli* cells (Yeastern Biotech, Taipei, Taiwan) according to the manufacturer's instructions.

2.5. DNA sequence analysis

DNA from 35 randomly selected recombinant clones was purified using the BioMan kit, and nucleotide sequences were determined by the dideoxynucleotide method by cycle sequencing of the purified PCR products with an ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Nucleotide sequences were checked for the presence of chimaeras using the Pintail program [34] for the 16S rRNA genes. Any

Table 1
Percentage distribution of 16S rRNA gene sequences in clone libraries obtained from arsenic-contaminated sediments after incubation under anaerobic conditions, showing closest known microorganism and its percentage match to reference DNA sequence.

Phylogenetic class	Closest matching microorganism	Unamended[a]	Amended with acetate[a]
Proteobacteria	*Caulobacter segnis* strain MBIC2835	20 (99)	14.3 (99)
α	*Rhizobium* sp. strain TCK	2.9 (99)	
	Rhodobium orientis strain JA263		11.4 (92)
β	*Brachymonas denitrificans* strain a119	2.9 (97)	
γ	*Escherichia hermannii* strain st6		2.9 (98)
	Natronocella acetinitrilica strain ANL 1		2.9 (94)
	Pseudomonas sp. strain BBK3	31.4 (99)	31.4 (99)
	Pseudomonas stutzeri	2.9 (97)	
	Shewanella sp. ANA-3	2.9 (100)	11.4 (99)
δ	*Desulfuromonas michiganensis* strain BB1	5.7 (97)	
Firmicutes			
Lactobacillales	*Alkalibacterium* sp. 12A2	25.7 (94)	11.4 (94)
Clostridia	*Clostridium jejuense*	2.9 (90)	
	Clostridium sp. enrichment culture clone MB2_34		2.9 (97)
	Gracilibacter thermotolerans strain JW/YJL-S1 clone 4	2.9 (96)	
Uncultured environmental sample	Iron-reducing bacterium enrichment culture clone HN-HFO4		11.4 (96)

[a] Numbers in the parentheses indicate the percentage of 16S rRNA identity to the closest relative in database. For each clone library, a total number of 35 clones were analyzed.

sequence suspected to be a chimaera was removed from the analyses. Sequences were analyzed against the NCBI database using the BLAST program and matched to known 16S rRNA gene sequences. In addition to BLAST searches, the bacterial 16S rRNA gene sequences were analyzed using the RDP (Ribosomal Database Project) classifier (http://rdp.cme.msu.edu/). The bacterial 16S rRNA clones were then assigned to a genus, a family or an order based on the confidence of the combined results obtained.

2.6. Nucleotide sequence accession numbers

Sequences obtained in this study were deposited in the GenBank database. The accession numbers for the 16S rRNA nucleotide sequences are: HQ007254–HQ007287, and GU372946 (strain ARS-3).

2.7. Indigenous bacteria isolation procedures

Strain ARS-3 was isolated from sediment collected in the aquifer. Briefly, after 8 weeks of anaerobic incubation, the microcosm sample slurry was plated onto the agar plates containing AGW with 0.1% yeast extract under anaerobic condition and incubated at room temperature (24–26 °C) in the dark. After 1 week, single colonies were picked and streaked onto the solid medium twice in succession and incubated anaerobically to insure purity. The pure culture was then analyzed for its ability to reduce As(V) and its 16S rRNA sequences for bacterial identification and strain ARS-3 was identified.

3. Results and discussion

3.1. Groundwater and sediment characteristics

Total arsenic concentrations in the groundwater from black-foot disease endemic area occur predominantly as As(III), and range from <1.0 to 562.7 μg/L [7]. The sediments collected at 20 m depth were dark gray fine sandy loam. The sediments contained As(III) (0.06 ± 0.02 mg/kg) and As(V) (7.06 ± 0.95 mg/kg). The sediments also contained Fe(II) (5850 ± 700 mg/kg) and Fe(III) (1630 ± 300 mg/kg). The total organic carbon contents in the sediments were very low with an average of 0.05%.

3.2. Microbially mediated As(III) release and Fe(III) reduction

Sediments incubated under aerobic conditions showed negligible reduction of Fe(III) and release of arsenic with time from the sediments (Fig. 2A), suggesting the minimal importance of other potential release routes for arsenic to the groundwater, such as the oxidative breakdown of small amounts of sedimentary arsenic-bearing sulfides [35]. The anaerobic sterile controls did not exhibit arsenic mobilization (Fig. 2B), suggesting that the release of arsenic through desorption from the surface or the re-equilibrium after re-suspension of sediment during the experimental period was not an important factor.

During incubation under anaerobic conditions, however, soluble Fe(II) was released into the pore water concomitant with arsenic mobilization from non-sterilized sediments, predominantly as As(III) (Fig. 2C), demonstrating that this process is biologically mediated. Significant soluble Fe(II) (0.23 ± 0.03 mM) in pore waters and mobilization of As(III) (206.7 ± 21.2 nM, approximately 15.5 ± 1.6 μg/L) occurred during the first week. Aqueous Fe(II) and As(III) respectively reached concentrations of 0.27 ± 0.01 mM and 571.4 ± 63.3 nM (42.9 ± 4.7 μg/L) after 8 weeks (Fig. 2C). This implies the potential connection between concentrations of As(III) and Fe(II) in the aqueous phase of the sediment suspensions.

3.3. Roles of carbon source in As(III) release and Fe(III) reduction

Several studies suggested that arsenic release into groundwater is coupled to the microbial oxidation of organic matter [36–38]. Furthermore, microcosm-based studies showed that metal-reducing activities of microorganisms potentially involved in arsenic release can be stimulated by an input of organic matter such as acetate [21,39]. Our data showed that the addition of acetate as a potential electron donor for metal reduction and a proxy for organic matter caused a further increase in aqueous Fe(II) but the dissolved arsenic did not increase (Fig. 2D), suggesting that As(III) was released by sedimentary bacteria using only endogenous electron donors that are present in the sedimentary inoculants. This also indicates that the population of As(V)-reducing bacteria in the aquifer is well suited to use endogenous heterotrophic electron donors that are naturally present in the aquifer sediments (*i.e.*, natural organic matter) and that this process is not electron-donor limited under *in situ* conditions.

It is noted that the very low total organic carbon content of the sediments in current study (0.05 ± 0.02 wt%) is consistent with several previous studies, suggesting that sediments associated with arsenic-rich groundwaters in Southeast Asia contain low organic carbon (*i.e.*, <1%) [23,36,40,41]. However, several studies showed that arsenic release was stimulated by an input of organic matter such as acetate [21,39]. In the present study, acetate addition had no effect on arsenic release. This is consistent with previous studies using West Bengal and Cambodian sediments in which low carbon contents were present [23,25]. Studies showed that arsenic release was associated with microbially mediated As(V) and Fe(III) reduction and demonstrated that very low concentrations of organic matter are able to support this process [23,25]. However, small but significant amounts of naturally occurring petroleum-derived organic carbon were present in these sediments, suggesting that this might support metal reduction leading to As(III) release [25]. In the present study, the potential role of organic matter in the sediment associated with arsenic release and iron reduction requires further study.

3.4. Characterization of the microbial communities and their role in arsenic release

Decoupling of the reduction of Fe(III) and the release of As(III) from the sediments was not unexpected. Our results support the findings of other studies using sediments from West Bengal [21,42], that decoupling of the reduction of Fe(III) and As(V) may reflect an adaption of respiratory pathways by microorganisms in the sediments. With potentially important roles of microorganisms that promote arsenic mobilization, the microbial communities present in the unamended and acetate-amended conditions after 8 weeks of anaerobic incubation were analyzed, using both cultivation-dependent and molecular (PCR) techniques. PCR-based amplification of 16S rRNA gene fragments provided a reliable snapshot of the dominant microbes present in the sediments in the microcosm study. Differences in the Fe(III) reduction and As(III) release profiles for sediments could be explained in terms of the contrasting microbial communities, and their interactions with the distinct geochemical environments in the microcosms.

An approximately 650-base pair (bp) region of the 16S rRNA gene was amplified from sediments after 8 weeks of anaerobic incubation using broad-specificity bacterial primers. Subsequently, the PCR-amplified 16S rRNA fragment was cloned to create a bacterial library. Analysis of the microbial communities in the unamended microcosm from a library of 35 clones showed that the majority of these clones were affiliated with known members of α- and γ-proteobacteria, and firmicutes (Table 1). Among the α-proteobacteria, the majority of clones corresponded to sequences derived from *Caulobacter* species (20% of the total clone library), which are bacteria that often survive in nutrient-poor environments and were shown to reduce As(V) in aerated serum bottles [43], whereas their metal-reducing properties in anaerobic conditions were not implicated. γ-Proteobacteria were the most prominent group (37.1%), represented mainly by organisms related to *Pseudomonas* (34.3%) and *Shewanella* sp. ANA-3 (2.9%). *Pseudomonas* species are facultative anaerobes able to respire using alternative electron acceptors including nitrate, but not Fe(III) or As(V) [19]. Shewanella sp. strain ANA-3 is a metal-reducing bacterium possessing the ability to reduce As(V) and Fe(III) [44]. Firmicutes (31.4%) were also well represented in the clone library, with the numerically dominant clone identified to be most closely related to *Alkalibacterium* sp. (25.7%); and other species present related to *Clostridium* and *Gracilibacter*. *Clostridium* species are involved in the fermentation of organic matter, and can reduce a range of metals including As(V) [19]. *Alkalibacterium* and *Gracilibacter* species have not been implicated in anaerobic metal

reduction. The only δ-proteobacteria detected in the clone library were *Desulfuromonas* species which are involved in dissimilatory Fe(III) reduction [45]. Surprisingly, no representatives of the Geobacteraceae of the δ-proteobacteria, a phylogenic group known to be dominant in many Fe(III)-reducing sediments [46], were present in the clone library.

When acetate was added, sequence analysis from a library of 35 clones showed that the percentage of γ-proteobacteria increased from 37.1% to 48.6% (Table 1), which is similar to that reported by Islam et al. [21]. The percentages of *Pseudomonas* species were similar to those in the unamended microcosm whereas *Shewanella* sp. ANA-3 increased to 11% (Table 1). This implies that *Shewanella* sp were responsible for the observed increase of Fe(II) in the acetate-amended microcosm (Fig. 2D). No significant change in the α-proteobacteria occurred, whereas the population of firmicutes decreased from 31% to 14% (Table 1), suggesting that fermentation became less important when acetate was added.

Although most of the cloned sequences could be assigned to key phylogenetic groups by comparison with 16S rRNA gene sequences of previously characterized organisms, many were either poor matches (*i.e.*, < 97% identity) or matched with (as yet) uncultured bacteria (Table 1). Furthermore, some of the phylogenetic classifications implicated in the reduction of Fe(III) or As(V) in these microcosms have not, as of yet, yielded Fe(III)- or As(V)-reducing bacteria. However, the 16S rRNA gene sequences based methods can provide information on the identity of the dominant bacterial populations present or the structure of the bacterial communities in the microcosms. The potential functions associated with these microorganisms require further study.

3.5. Characterization of the indigenous As(V)-reducing prokaryote ARS-3 and its role in arsenic release

A stable culture of As(V)-reducing bacteria was obtained from an unamended microcosm, and the ARS-3 bacterium was isolated. Strain ARS-3 is a gram-negative, facultatively anaerobic, rod-shaped bacterium that grows in minimal salt medium (MSM) [47] amended with pyruvate, citrate, glucose, or sucrose. Analysis of 16S rRNA sequence showed that the ARS-3 strain belongs to the *Enterobacter* genus (99% identity of 16S rRNA sequence) in the γ-proteobacteria. Under anaerobic conditions, strain ARS-3 completely reduced $250\,\mu M$ (~18.75 mg/L) As(V) within 17 h (Fig. 3A). Moreover, ARS-3 shares 100% sequence identity to that strain AR-8. AR-8 was previously isolated from arsenic-contaminated groundwater [28] that is close to the drilled borehole in this study.

We further examined the potential of strain ARS-3 to mobilize arsenic via direct enzymatic reduction or indirect mechanisms linked to Fe(III) reduction. We conducted microcosm experiments containing strain ARS-3 and autoclaved sediments under anaerobic conditions. Significant arsenic mobilization from the sterilized sediments, predominantly as As(III) (199.0 ± 5.9 nM), occurred during the first 2 weeks and remained at this level throughout the incubation period (Fig. 3B), which might have been due to a lack of a sufficient carbon source for ARS-3 during the latter part of the incubation period. A negligible reduction in Fe(III) with time from the sediments was observed (Fig. 3B), suggesting that strain ARS-3 mobilized arsenic via the direct enzymatic reduction of As(V) to the potentially more-soluble As(III), but not by indirect mechanisms linked to Fe(III) reduction, thereby indicating that the direct enzymatic reduction of As(V) to the potentially more-soluble As(III) in the pore water is possible in this aquifer. The sterile controls did not mobilize arsenic (Fig. 3C). This is the first study showing that the As(V)-reducing bacterium native to aquifer sediments is capable of directly mediating the release of arsenic into groundwater systems.
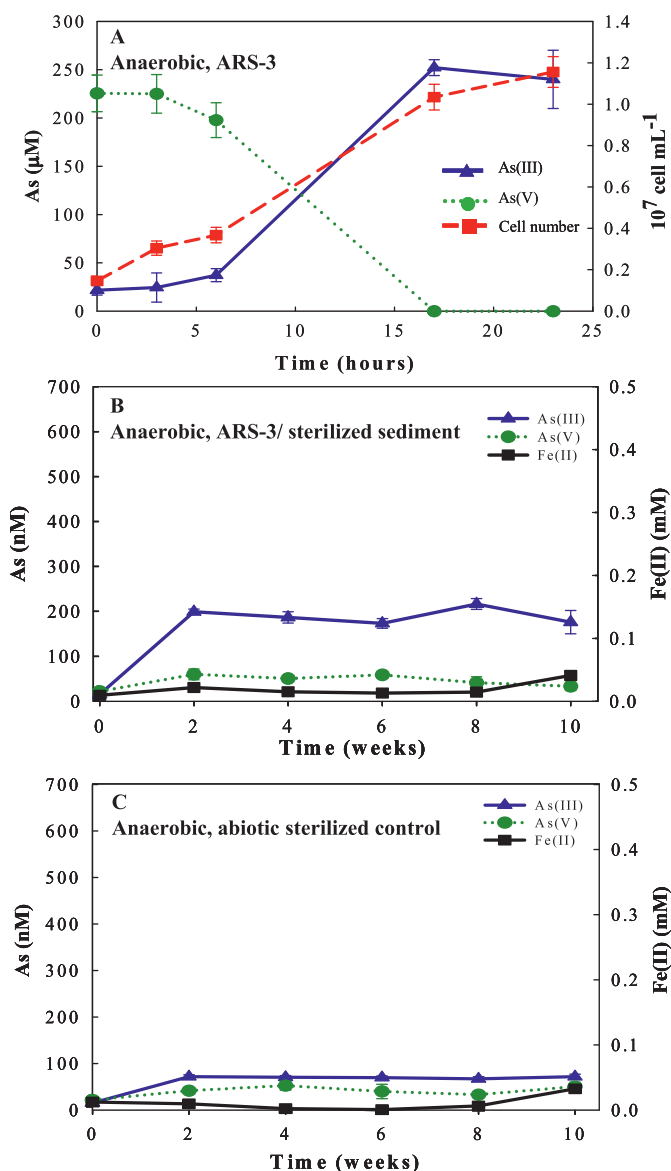
Fig. 3. (A) Arsenic transformation of bacterial strain ARS-3 in minimal salt medium (MSM) containing 10 mM glucose plus 250 μM As(V) under anaerobic condition. Mobilization of arsenic and reduction of Fe(III) in microcosms containing (B) bacterial strain ARS-3 incubated with sterilized sediments under anaerobic conditions; and (C) abiotic sterilized control sediments incubated under anaerobic conditions. Black squares, aqueous Fe(II); blue triangles, aqueous As(III); green circles, aqueous As(V). Each point and error bar represent the mean and standard deviation of three replicate experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

4. Conclusion

This is the first study focused on mechanisms controlling the mobilization of arsenic present in elevated concentrations within aquifers in the blackfoot disease endemic area. Our studies offer direct evidence that microbes play key roles in mediating the release of toxic, mobile As(III) from a shallow aquifer in the BFD endemic area. Our results also suggest that the capacity for arsenic release is not limited by the availability of electron donors in the sediments from our study site, implying that the bacteria are using only endogenous electron donors that are naturally present in the aquifer sediments (*i.e.*, natural organic matter). We isolated the *Enterobacter* bacterium (ARS-3) native to aquifer sediments that can account for the release of arsenic from this arsenic

contaminated shallow reducing aquifer to the pore water via the direct enzymatic reduction of As(V) but not through indirect mechanisms linked to Fe(III) reduction. Moreover, we showed that the As(V)-reducing bacterium native to aquifer sediments is capable of directly mediating the release of arsenic into groundwater systems. These microcosm-based experiments can potentially aid interpretations of processes affecting arsenic solubility and partitioning in arsenic contaminated aquifers in the BFD endemic area of Taiwan. Importantly, such investigations are crucial for understanding the mobilization mechanisms of arsenic in drinking water aquifers.

Competing interests

The authors declare that no competing interests exist.

Acknowledgments

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