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SHORT RESEARCH AND DISCUSSION ARTICLE

Culture-dependent study of arsenic-reducing bacteria in deep aquatic sediments of Bengal Delta

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Abstract

Biogeochemical release of soil-bound arsenic (As) governs mobilization of the toxic metalloid into the groundwater. The present study has examined As^V-reduction ability of bacteria from anoxic aquatic sediments that might contribute to arsenic mobilization in the Bengal Delta. Arsenic-reducing bacteria from deep layers of pond sediment were enriched and isolated in anaerobic environments and As^V reduction was assessed in culture medium. The pond sediment enrichments harboured As^V-reducing bacteria belonging to the phyla Firmicutes and Proteobacteria with dominance of *Paraclostridium benzoelyticum* and *P. bifermentans*. Among total 17 isolates, the respiratory reductase genes were not detected by the most common primers and only 3 strains had arsenic reductase *ArsC* gene suggesting involvement of resistance and some unknown mechanisms in As^V reduction. Presence of high levels of organic matter, As, and As-reducing bacteria might make deep aquatic sediments a hot spot of As mobilization and aquifer contamination.

Keywords Pond · Anoxic sediment · As^V-reducing enrichments · As^V-reducing strains · *Arr* · *ArsC*

Arsenic (As) contamination of groundwater is a serious public health problem in Bangladesh and eastern part of India exposing over 100 million people to unsafe levels of As in drinking water (Raessler 2018). The region geologically contains high levels of As sorbed onto soil particles. Dissimilatory microbial reduction of soil-bound arsenate (As^V) (Malasarn et al. 2004; van Geen et al. 2004; Farooq et al. 2012) and/or reductive dissolution of arsenic-bearing minerals such as Fe, Mn and Al (Islam et al. 2004; Hery et al. 2010; Paul et al. 2015) followed by As reduction in deep soils majorly govern the As release in alluvial aquifers. The reduced form of As (arsenite, As^{III}), having less affinity for soil minerals and clay, is released and transported to the groundwater through subsurface hydrologic gradient (van Geen et al. 2004; Harvey et al. 2006).

Besides biogeochemical cycling of As in deep aquifer soils, microbial As cycling also occurs in surface aquatic environments (Oremland and Stolz 2003; Bandopadhyay et al. 2017; Sultana et al. 2011; Goswami et al. 2015). In Mekong River floodplains of Cambodia, there is >60–90-fold increase in dissolved As concentration within a few metres depth of water-sediment interface of wetlands; this is coupled with organic matter oxidation suggesting role of the wetland sediment microbiota in the mobilization process (Polizzotto et al. 2008). Bengal Delta has thousands of such ponds and wetlands that significantly contribute to the groundwater arsenic contamination in the area by providing labile organic matter for microbial arsenic reduction in subsurface soil (Fendorf et al. 2010; Mladenov et al. 2010; Lawson et al. 2013), as well as hydrologic gradient for transport of the solutes to the groundwater (Harvey et al. 2006). Besides, ponds in the area also contain a significant amount of As (Chowdhury et al. 2015) and might act as active sites of As mobilization similar to that in the Mekong River wetlands. Farooq et al. (2012) observed significant As mobilization from the upper 2.6 m of pond sediment, under influence of organic matter loading from jute retting. We had earlier observed As reduction by microbiota

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present in top layers of pond sediment in partial to true anoxic conditions (Bandopadhyay et al. 2017). Interestingly, the As-reducing bacteria in top layers of pond sediments were distinct from those observed in deep aquifers (Sultana et al. 2011; Paul et al. 2015; Bandopadhyay et al. 2017) suggesting possible role of diverse groups of microbiota or even different biogeochemical processes in As cycling. This paper has isolated As^V-reducing anaerobic bacteria by enrichment method and examined their As-reduction ability in deep layers of pond sediments. The observation emphasizes the role of aquatic sediments in As reduction and mobilization and fills a void in information on bacteria involved in As transformation between top layers of aquatic sediments and deep aquifer soils.

Sediment samples were collected, with help of a sediment sampler (*Eijkelkamp*), from the middle and two sides of four numbers of ponds used for aquaculture and household purposes (Bandopadhyay et al. 2017) in arsenic-affected villages of Chakdaha and Haringhata blocks of West Bengal, India. Samples from 150–170 cm sediment depth were incised out with help of a scalpel-blade, quickly filled up to the brim in sterile bottles avoiding air pockets, tightly capped and brought to laboratory under ice cover. For estimation of total As contents, sediment samples were air dried, grounded and sieved through 100 mesh sieve. These were digested with tri-acid mixture (HNO_3:$HClO_4$:H_2SO_4::10:4:1), filtered and As content measured by atomic absorption spectrometer (GBC Avanta Sigma) coupled with a hydride generator following method described in detail by Chowdhury et al. (2015). Total As level ranged between 13.58 and 34.72 mg kg^{-1} (mean 21.73 ± 2.48 mg kg^{-1}) and was significantly higher than that at top layers of the sediment (10.34 ± 0.4 mg kg^{-1}) (Bandopadhyay et al. 2017) indicating higher As levels in deep sediment layer either as geological deposition, or use of contaminated groundwater for re-filling of ponds during dry season (Chowdhury et al. 2015) and downward mobilization of the metalloid. This was similar to the increasingly higher As level with sediment depth in wetlands of the Mekong River (Polizzotto et al. 2008).

Anaerobic As-reducing bacteria in sediment enrichments

Bacterial isolation and As-reduction studies by the isolates were conducted in gas mixture (80% N_2:10% CO_2:10% H_2) environment in an anaerobic workstation (Ruskinn Bugbox). Ten percent (w/v) of suspensions of freshly collected sediment samples were made in 0.85% saline solution, serially diluted (10^{-1} to 10^{-3}) and 500 μL volume from different dilutions were enriched in 20 mL culture medium [K_2HPO_4 0.225g, KH_2PO_4 0.225g, NaCl 0.46g, $(NH_4)_2SO_4$ 0.225g, $MgSO_4$ 0.117g, yeast extract 0.5g, distilled water to 1000 mL, pH 7.5] supplemented with 27.3 mM sodium acetate and 10 mM sodium arsenate ($Na_2HAsO_4{\cdot}7H_2O$) as electron donor and acceptor, respectively. As-transforming prokaryotes use a wide range of carbon substrates such as glucose, lactate, acetate, butyrate and citrate as electron donor (Oremland and Stolz 2003; Paul et al. 2015). Among these, acetate has been widely used to stimulate growth of As-transforming microbes and As reduction/release from deep soil or pond sediment (van Geen et al. 2004; Lear et al. 2007; Hery et al. 2010; Bandopadhyay et al. 2017) and was used as electron donor in present experiment. Unlike As-resistant bacteria which can tolerate very high concentrations of the metalloid, As-respiring prokaryotes have low level of resistance, generally up to 10 mM As: as such, 10 mM As^V concentration was used to favour growth of As-respiring microbes. The enrichment broths were incubated at 30 °C for 15 days when cultures were transferred to fresh medium containing 10 mM As^V. After 15 days of further incubation, the cultures were streaked several times on to the As^V-containing medium solidified with 1.5% agar for pure culture isolation.

A total of 17 presumptive arsenic-reducing bacteria were isolated from sediment enrichments. Identification of the bacterial isolates was done through PCR amplification and sequencing of 16S rRNA gene using 8F and 1492R universal bacterial primers. Bacteria were tentatively identified by finding similarity of the sequences with SSU sequences in the NCBI GenBank, Greengenes (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi) and RPD (http://rdp.cme.msu.edu) databases. The evolutionary phylogenetic tree was prepared by minimum evolution method (Rzhetsky and Nei 1992) with 1000 bootstrap using MEGA7 (Kumar et al. 2016) bioinformatics software. *Shigella* strain was further identified through a battery of biochemical tests following Bergey's manual (Strockbine and Maurelli 2005). About 41% of the strains were identified as *Paraclostridium benzoelyticum*, 23.6% strains as *P. bifermentans*, 17.6% strains as *Clostridium* and *Paraclostridium* sp.; only one strain was identified as *Shigella boydii* and 2 strains belonged to the genus *Exiguobacterium* (Fig. 1). *Clostridium* has earlier been detected in anaerobic enrichments of polluted river sediment in a gold-mine area (Suhadolnik et al. 2017) as well as As-affected Bengal Basin soil (Islam et al. 2004) signifying role of *Clostridium* in As transformation in diverse environments. *Paraclostridium* is a newly coined genus with reclassification of *Clostridium bifermentans* (Sasi Jyothsna et al. 2016). *Paraclostridium benzoelyticum* gen. nov was reported from marine sediments (Sasi Jyothsna et al. 2016); we detected the species in freshwater environments suggesting its wide habitat distribution. Members of *Clostridium* avidly ferment organic matter and are known to reduce a number of metals and metalloids including arsenic (Oremland and Stolz 2003; Suhadolnik et al. 2017). Total organic carbon of dried sediment samples, measured in TOC analyser (OI Analytical), was 1.65 ± 0.12% and might have favoured growth of *Clostridium* and

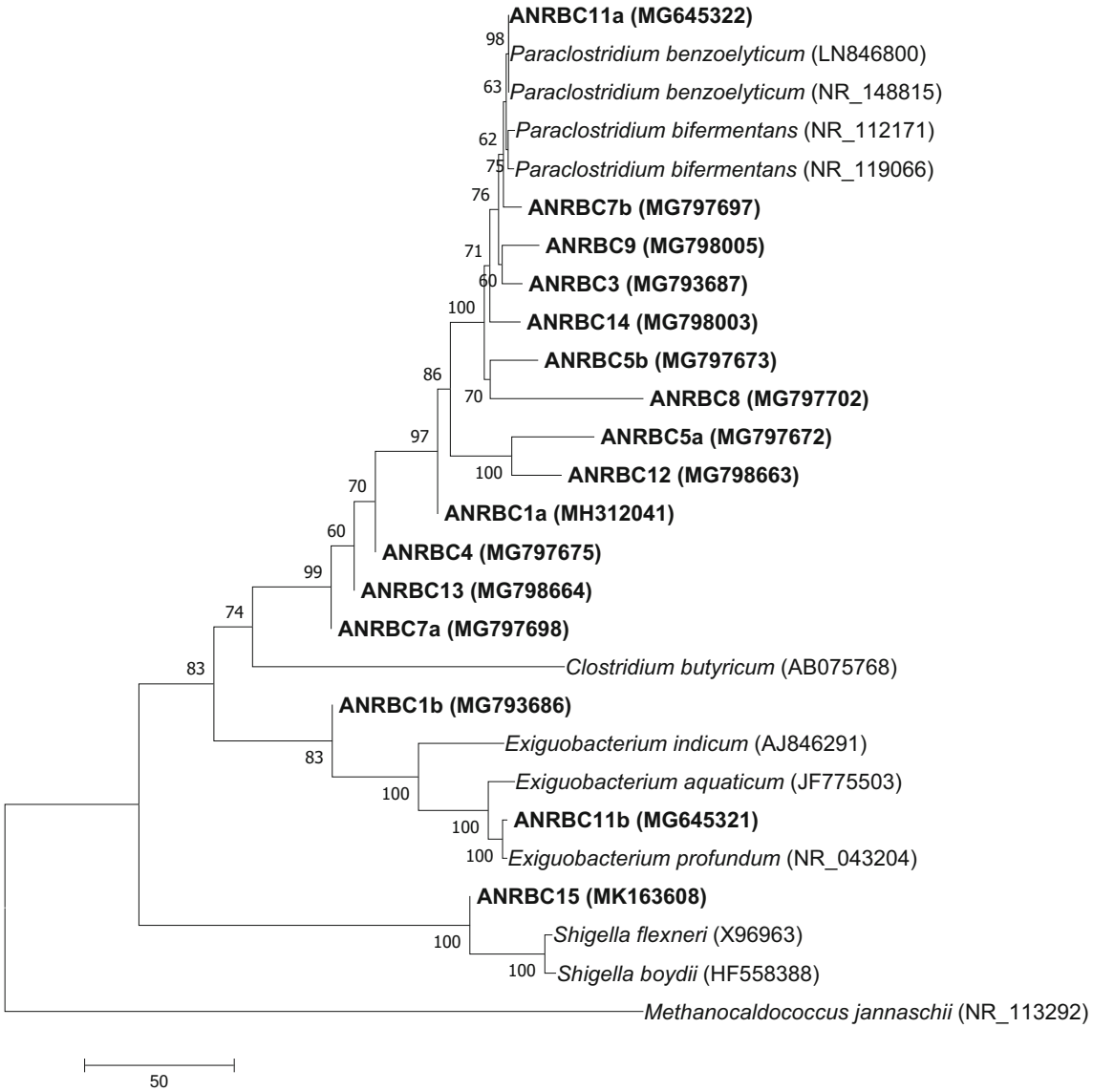

Fig. 1 Phylogenetic relationships of arsenic-reducing bacteria from deep pond sediment, inferred from 16S rDNA sequences. Strains in the present study are indicated in bold

Paraclostridium in pond environments. *Exiguobacterium* is a well-known arsenic-resistant bacterium and has been reported from As-rich soil of Chhattisgarh, India (Pandey and Bhatt 2016).

No true chemolithotroph was isolated in present study probably due to limitation of the culture protocol followed. However, some other studies have also observed absence of chemolithotrophs and abundant presence of aerobic, facultative anaerobic or denitrifying populations of Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria suggesting involvement of diverse groups of heterotrophic bacteria in As reduction in shallow and deep aquifers of Bengal Delta (Islam et al. 2004; Sutton et al. 2009; Sultana et al. 2011; Paul et al. 2015). The bacteria identified from deep pond sediment enrichments were different from those isolated from pond water and superficial sediment (*Chryseobacterium* sp., *Pseudomonas* spp., *Acinetobacter* spp., *Microbacterium oleivorans* and *Comamonas aquatica*) (Goswami et al. 2015; Bandopadhyay et al. 2017) suggesting a likely shift in As-reducing bacterial community with sediment depth. However, culture-dependent study can identify only a small fraction of the total microbiota and metagenomic approach might reveal the wider spectrum of microbial assemblages along the sediment depths.

Arsenic reduction by bacterial strains

Arsenic reduction by different bacterial strains was estimated in culture medium supplemented with 10 mM arsenate and

10 mM sodium acetate following method described before (Bandopadhyay et al. 2017). Briefly, 50 μL of individual bacterial culture (8.83×10^9–1.12×10^{10} CFU) was added to 20 mL culture medium and incubated at 30 °C. Thirty microlitre volume of culture was taken in duplicate tubes at different time intervals (0, 1, 2, 7, 10, 15 and 20 days of incubation) for As estimation: one set of tubes was treated with reducing agent (1.4% $Na_2S_2O_3$:14% $Na_2S_2O_5$:3.5 N H_2SO_4::2:2:1) for 12 min for complete reduction of As^V ("reduced") and the other set was acidified with 25 mM HCl ("unreduced"). Both the "reduced" and "unreduced" samples were treated with mixed reagent (containing ammonium molybdate, ascorbic acid and antimony potassium tartrate) for 10 min at 78 °C, centrifuged at 10,000 × g for 3 min and supernatant read at 865 nm. Both As^V and phosphate form coloured complexes with molybdenum, while As^{III} does not: so only phosphate is detected in "reduced" samples while As^V and phosphate are detected in "unreduced" samples. As^V remaining in the sample was calculated by subtracting the "reduced" sample value from that of the "unreduced". The amount of As^V reduction was obtained by subtracting the amount of As^V remaining in culture medium from 10 mM As^V added to the culture medium. Sterile culture medium amended with As^V and acetate was kept as negative control to assess chemical As reduction in anaerobic culture environment. Arsenic reduction by each bacterial strain was examined in two occasions keeping 5 replicate tubes every time. Bacterial growth was measured photometrically at 600 nm and correlated with As reduction. Data are presented as mean ± S.E. Pearson's correlation test was applied to examine relationships between bacterial growth and As reduction. One-way ANOVA was applied to find significant difference in growth or As reduction among strains.

All the bacterial isolates caused ≥ 5 mM As^V reduction (Fig. 2). Among these, AnRBC1a and AnRBC3 strains caused 8.05–9.22-mM reduction in 7–10-day period; other strains took 15–20 days to reduce 5.14–8.19 mM As. Species-wise, majority of the *Paraclostridium benzoelyticum* strains were more efficient ($p < 0.05$), reducing 5.95–9.22 mM As^V in 7–10 days. Strains belonging to *Paraclostridium bifermentans*, *Paraclostridium* sp. and *Clostridium* sp. transformed 5.2–7.75 mM As in 15–20 days. *Exiguobacterium* and *Shigella boydii* caused about 6-mM reduction in 10 and 20 days, respectively. All the strains took 10–15 days to reach highest density of growth, with comparatively higher growth achieved by *P. benzoelyticum* strains. Bacterial growth was significantly correlated with As^V reduction ($p < 0.05$) with minor strain to strain variations: less number of cells of *P. benzoelyticum* strain AnRBC1a but more number of cells of *Shigella boydii* strain AnRBC15 were required for sufficient reduction. The rate of reduction was comparable to that observed in Cambodian sediment (Lear et al. 2007) but slower as compared that observed by Paul et al. (2015) in Bengal Delta sediment or by As-respiring or resistant strains from other geographical region (Li and Krumholz 2007), probably because our medium had limited energy supply. However, As^V reduction rates of the present bacteria were higher than that caused by isolates from superficial sediments and water (Bandopadhyay et al. 2017) showing higher As transformation ability of bacteria at sediment depths.

Identification of the arsenic reduction gene(s)

Microbes reduce As^V by two distinct mechanisms: arsenic respiration facilitating growth in anoxic environments with help of periplasmic/membrane associated respiratory arsenate reductase (Arr) or cytoplasmic As-reduction and expulsion mechanism (Ars) (Silver and Phung 2005). While the former is considered to be the principle mechanism of As release in aquifer soil, the latter is a part of As resistance strategy found in a wide diversity of microbes from contaminated environments (Oremland and Stolz 2003). Presence of As-reducing genes in the genome and plasmid was examined through PCR amplification of the dissimilatory As^V-respiring genes (*ArrA* and *ArrB*) and cytoplasmic As^V-reducing gene (*ArsC*) of *Ars* operon using primers described in Table 1. The genomic DNA and plasmid DNA of individual bacteria were extracted separately using commercially available kits (Sigma-Aldrich and Invitrogen) and taken as template in PCR amplification following the reaction conditions given in references in the Table 1. Bacteria, viz., *Pseudomonas* sp., *Comamonas aquatica*, *Acinetobacter junii* and *Chryseobacterium* sp., isolated earlier from superficial sediments (Bandopadhyay et al. 2017) were also included in the study. The PCR amplicons were gel-purified and sequenced. Some of the PCR products were cloned in *Escherichia coli* DH5α using pGEM®-T vector system (Promega) and sequenced following standard protocol (Sambrook and Russell 2001). The gene sequences were edited by Sequence Scanner v1.0 software (Applied Biosystems, Inc.) and BLAST searched in NCBI database: Blastn programme was used for finding the closest nucleotide matches, blastp for protein blast and blastx for protein databases using a translated nucleotide query. Aligned nucleotide sequences were also transformed in to protein sequences by ExPASy (https://web.expasy.org/translate) translation tool to obtain the converted protein sequences and similarity of the gene. The phylogenetic relationships of the As reduction genes with those from other bacteria was derived using MEGA7 software by maximum likelihood method based on the JTT matrix-based model and 1000 bootstrap replications (Jones et al. 1992).

Earlier studies have identified respiratory arsenate reductase, coded by the *arr* operon, as a principal mechanism As reduction in deep soils of South and SE Asia (Krafft and Macy 1998; Afkar et al. 2003). However, despite use of 6 sets of

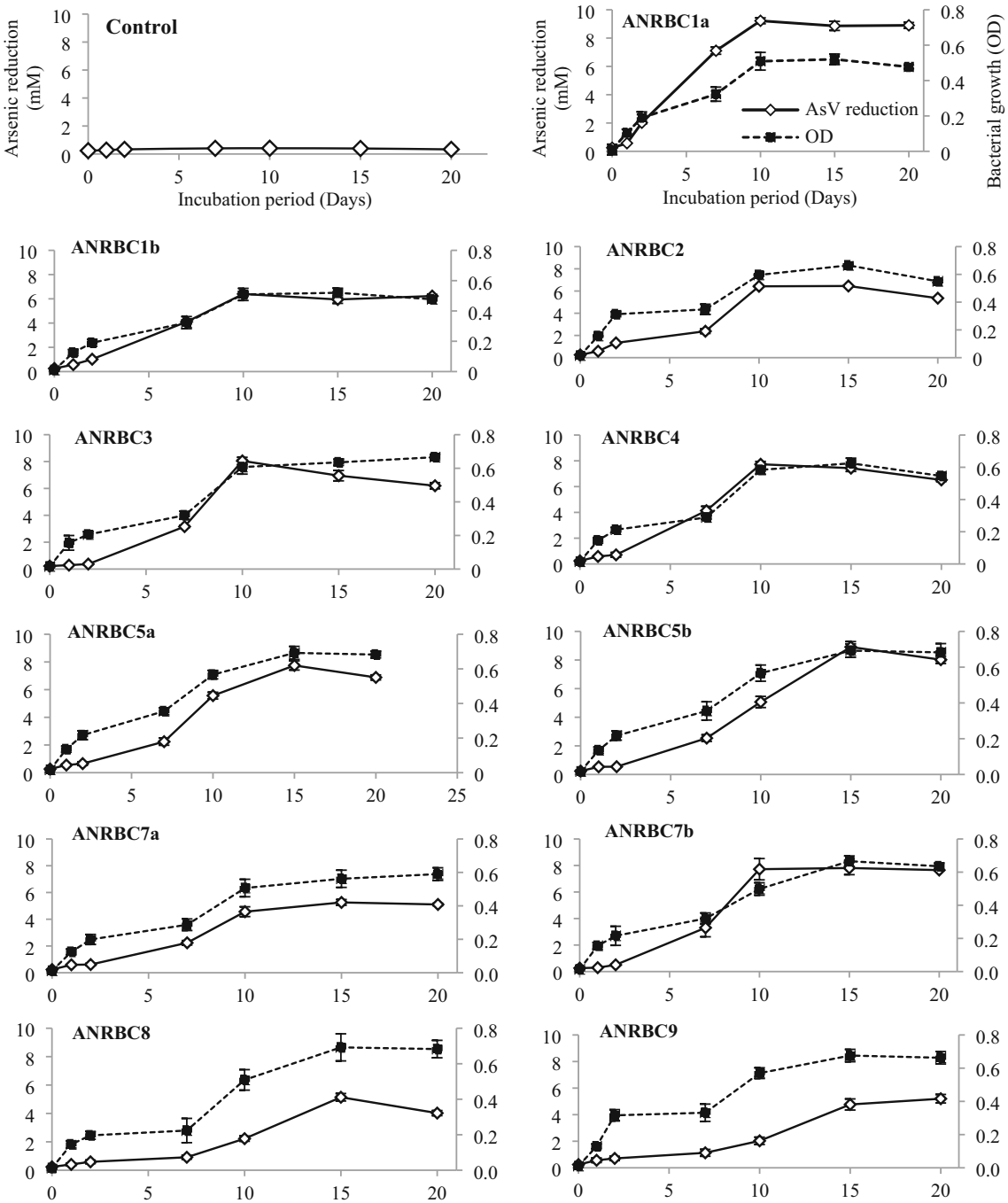

Fig. 2 Arsenate reduction and growth curve of bacterial strains from deep pond sediments. Figure title indicates control or strain ID. Horizontal axis represents incubation period in days. Extent of arsenate reduction (out of initial 10 mM arsenate) is in primary Y-axis, and bacterial growth (OD) in secondary Y-axis. Solid line indicates As^V reduction; broken line indicates bacterial growth (OD)

commonly used primers, we did not detect presence of *Arr* genes in any of the sediment isolates. Although As^V reduction was positively correlated with bacterial growth, absence of *Arr* genes suggests that these bacteria were not using As^V for respiration. Degenerate primers of *Arr* genes yielded non-specific amplifications but none of those was identified as *ArrA* or *ArrB* gene following cloning and sequencing (raw data not given). The study, however, found presence of the *ArsC* gene. Among 3 sets of ArsC primers used, only *amlt* successfully amplified the *ArsC* gene with amplicon length of 390 bp, which is partial sequence of *ArsC* gene. Among all the strains, only 3, viz., *P. benzoelyticum* (strain AnRBC1a, AnRBC5b) and *Shigella boydii* strain AnRBC15, carried the *ArsC* gene in their chromosome and plasmid respectively (Table 2, Fig. 3) suggesting that neither all the As-reducing bacteria nor all the members of a species carried the gene.

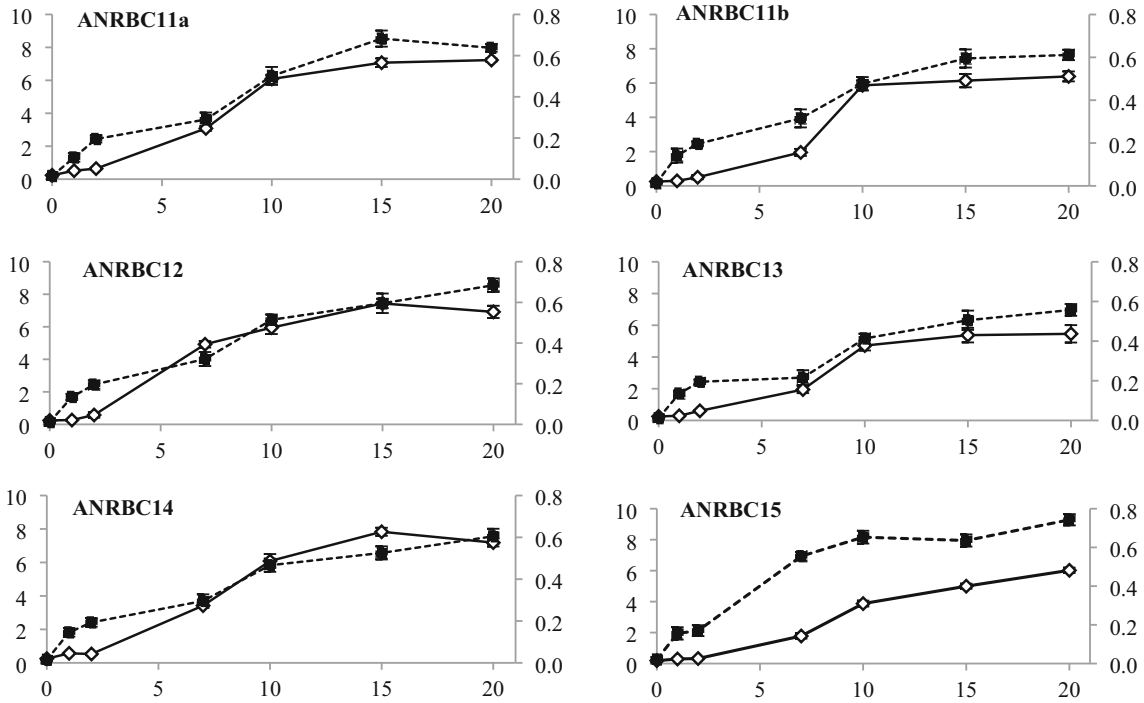

Fig. 2 continued.

None of the other strains belonging to *Paraclostridium*, *Clostridium* sp. and *Exiguobacterium* sp. carried the gene and their As-reduction mechanism is presently undefined. However, the *amlt* primers were originally designed from *Enterobacteriaceae* (Sun et al. 2004) which might be a reason why only a few strains of Firmicutes harboured the gene. This is also a likely reason of phylogenetic closeness of the identified *ArsC* genes with those from the family *Enterobacteriaceae*, but distant from Firmicutes (Fig. 3). *ArsC* is one of the most common detoxification mechanisms among diverse groups of prokaryotes with early origin and rapid evolution (Fekih et al. 2018) that demands use of a wide

Table 1 Primers used for detection of *ArrA*, *ArrB* and *ArsC* genes

Gene	Primer sequence	Reference
ArrA	as1-F: (5′-CGAAGTTCGTCCCGATHACNTGG-3′)	Song et al. 2009
	as1-R: (5′-GGGGTGCGGTCYTTNARYTC-3′)	
ArrA	XhoarrA3-2: (5′-CATGCCATGGGTGCTGAATTACCCGCGCCTCT A-3′)	Malasarn et al. 2008
	NcoarrA5-2: (5′-CCGCTGAGTCACACTTTCTCAACACGAAC-3′)	
ArrB	XhoarrB5: (5′–CCATGGGTATGAGATTAGGAATGGTGATT-3′)	Malasarn et al. 2008
	NcoarrB3: (5′–CCGCTCGAGTTAATAAGCGGTTTTAACACC-3′)	
ArrA	Fwd: (5′-AAGGTGTATGGAATAAAGCGTTTgtbgghgaytt-3′)	Malasarn et al. 2004
	Rev: (5′-CCT GTGATTTCAGGTGCCcaytyvggngt-3′)	
ArrA	Q-arsC: (5′-AATGGTCAGATACCTCACCGCAC-3′)	Saltikov et al. 2005
	Q-arrA: (5′-GCTATTCCACACCCCTTTTTGC-3′)	
ArrA, *ArrB*	JS1: (5′-GARCARGGNGARTGGATHGC-3′)	Afkar et al. 2003
	JS2: (5′-TTYTCRTTYTTRCANGTNAC-3′)	
ArsC	Parsc1F: 5′-GGTTCATGTTCGCCTCTGCC-3′	Li and Krumholz 2007
	Parsc1R: 5′-GGCCGTCTCTTTCGCGCTG-3′	
ArsC	Inverse: 5′-GCGCGTTGTAGACCTGTTCG-3′	Wang et al. 2004
	Reverse: 5′-AACAGACACCACCGGAACGC-3′	
ArsC	amlt-42 F: 5′-TCGCGTAATACGCTGGAGAT-3′	Sun et al. 2004
	amlt-376 R: 5′-ACTTTCTCGCCGTCTTCCTT-3′	

Table 2 Arsenic reduction by the bacterial isolates from deep pond sediments and comparison with bacteria from pond water and superficial layers of the sediment

Source of the isolate	Strain	Identification	Maximum As^V reduction (%)*	As^V reduction rate ($\mu M\ h^{-1}$)**	Presence of *Arr* and *ArsC* genes#	Reference
Deep pond sediment	ANRBC1a	*Paraclostridium benzoelyticum*	92.28	38.42	*ArsC*	Present study
	ANRBC1b	*Exiguobacterium* sp.	64.73	26.67	Nil	
	ANRBC2	*P. bifermentans*	64.50	26.67	Nil	
	ANRBC3	*P. benzoelyticum*	80.56	33.54	Nil	
	ANRBC4	*Paraclostridium* sp.	77.32	32.21	Nil	
	ANRBC5a	*P. bifermentans*	77.52	23.17	Nil	
	ANRBC5b	*P. benzoelyticum*	89.18	21.13	*ArsC*	
	ANRBC7a	*Clostridium* sp.	52.53	19.03	Nil	
	ANRBC7b	*P. benzoelyticum*	78.14	32.17	Nil	
	ANRBC8	*Clostridium* sp.	51.46	9.17	Nil	
	ANRBC9	*P. bifermentans*	52.44	8.46	Nil	
	ANRBC11a	*P. benzoelyticum*	72.39	25.33	Nil	
	ANRBC11b	*Exiguobacterium profundum*	63.81	24.42	Nil	
	ANRBC12	*P. benzoelyticum*	74.31	24.79	Nil	
	ANRBC13	*P. bifermentans*	54.64	19.67	Nil	
	ANRBC14	*P. benzoelyticum*	78.36	25.33	Nil	
	ANRBC15	*Shigella boydii*	60.28	16.13	*ArsC*	
Superficial pond sediment	AASRB1	*Chryseobacterium* sp.	29.85	21.25	Nil	Bandopadhyay et al. (2017)¥
	AASRB5	*Chryseobacterium* sp.	25.2	17.92	Nil	
	AASRB2	*Pseudomonas* sp.	16.35	12.83	Nil	
	AASRB6	*Pseudomonas* sp.	19.95	16.67	Nil	
	AnASRB32	*Comamonas aquatica*	15.15	13.25	Nil	
	AnASRB41	*Acinetobacter junii*	22.1	17.37	Nil	
Pond water	Several strains	Unidentified	1.15-18.55	0.83-15.41	Not studied	

* The highest level of As reduction out of initial 10 mM As^V concentration in the medium. ** As^V reduction rate was calculated from extent of reduction of initial 10 mM As^V on 10th day of incubation. # Nil indicates absence of *Arr* and *ArsC* genes. ¥ Bandopadhyay et al. (2017) used 20 mM As^V to study As reduction rate

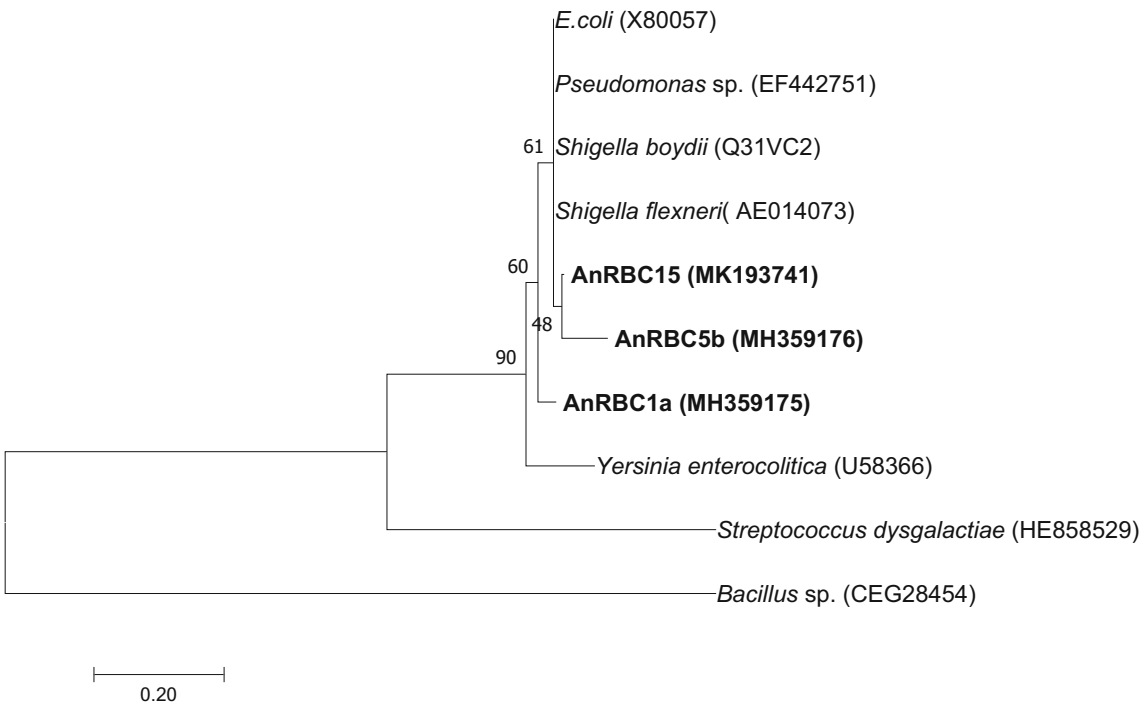

Fig. 3 Phylogenetic relationships of *ArsC* in this study with that of other bacteria. Strains in present study are indicated in bold

range of primers for their detection. While glutaredoxin reductase-dependent *ArsC* is common among *Enterobacteriaceae*, thioredoxin-coupled *ArsC* is found in Gram-positive bacteria and in a wide range of soil arsenic concentrations (Escudero et al. 2013). As such, our study based on enterobacterial related primers does not rule out a possibility of presence of thioredoxin-*ArsC* among the members of Firmicutes.

An earlier study of contaminated groundwater has also detected dominance of arsenic reductase (*ArsC*) over dissimilatory arsenic reductase (*Arr*) gene (Paul et al. 2015). They detected the *Arr* gene in few strains only which also harboured *Ars* genes, and the extent of As release in sediment microcosm was independent of *Arr* gene; *ArsC*, rather than the *Arr*, was more closely correlated with As release. Goswami et al. (2015) also observed absence of *Arr* gene in arsenic-reducing *Acinetobacter* and *Microbacterium* from surface waters in Bengal Delta. Our study, in association of others (Paul et al. 2015; Goswami et al. 2015), indicates cytosolic arsenate reductase as a possible As-reduction mechanism in the Bengal Basin.

As summarized in Table 2, the level of As^V reduction by bacteria having *ArsC* gene was not significantly higher than by those lacking the gene. Thus, neither the presence of the identified *ArsC* was stringently associated with As reduction, nor an absence of *Arr* and *ArsC* genes disables an organism from As reduction suggesting non-exclusive role of these reductases in As transformations. Interestingly, *Clostridium*, the major group of anaerobes found here, has been shown to methylate As (Wang et al. 2015: Zeng et al. 2018) using thiols such as GSH as a reductant (Bentley and Chasteen 2002). Microbial methylation in anaerobic sediments is an important As detoxification mechanism with significance in the global biogeochemical cycles of the metalloid (Zeng et al. 2018). Whether these sediment microbiota cause significant As methylation as a part of their resistance strategies using thiols and other reducing agents in labile organic matter-rich aquatic environments needs further study.

Conclusion

Paraclostridium and *Clostridium* isolated from the anaerobic pond sediment enrichments showed strong As^V reduction activity. Such As reduction by aquatic bacteria might contribute significantly to the groundwater arsenic contamination in Bengal Basin inundated with thousands of ponds and wetlands.

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Author contribution Chinmay Bandopadhyay—bacterial isolation, identification, arsenic reduction study and gene identification.

Sanjib Kumar Manna—guidance to C. Bandopadhyay, experimental design, analysis of data, Ms. preparation.

Srikanta Samanta—guidance in setting of experiments, analysis of arsenic data, Ms. preparation.

Atalanta Narayan Chowdhury—arsenic estimation.

Nilanjan Maitra—analysis of molecular data and identification, gene information submission to NCBI.

Bimal Prasanna Mohanty—site selection, overall guidance.

Keka Sarkar—guidance to C. Bandopadhyay, data analysis and interpretation.

Samir Kumar Mukherjee—guidance to C. Bandopadhyay, data analysis and interpretation.

Availability of data and materials

All data generated and analysed during this study are included in this article. Gene sequence information generated during this study is deposited with NCBI.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

Afkar E, Lisak J, Saltikov C, Basu P, Oremland RS, Stolz JF (2003) The respiratory arsenate reductase from *Bacillus selenitireducens* strain MLS10. FEMS Microbiol Lett 226:107–112

Bandopadhyay C, Manna SK, Naitra N, Samanta S, Chowdhury AN (2017) Significant arsenic reduction by pond sediment microflora in the arsenic-affected Bengal Delta. Soil Sedi Contam 26:471–485

Bentley R, Chasteen TG (2002) Microbial methylation of metalloids: arsenic, antimony, and bismuth. Microbiol Mol Biol Rev 66:250–271

Chowdhury AN, Samanta S, Manna SK, Mohanty BP, Sharma AP, Bandopadhyay C, Pramanik K, Sarkar S (2015) Arsenic in freshwater ecosystems of Bengal Delta: status, sources and seasonal variability. Toxicol Environ Chem 97:538–551

Escudero LV, Casamayor EO, Chong G, Pedros-Alio C, demergasso C (2013) Distribution of microbial arsenic reduction, oxidation and extrusion genes along a wide range of environmental arsenic concentrations. PLoS ONE 8: e78890. doi:https://doi.org/10.1371/journal.pone.0078890

Farooq SH, Chandrasekharam D, Abbt-Braun G, Berner ZA, Norra S, Stüben D (2012) Dissolved organic carbon from the traditional jute processing technique and its potential influence on arsenic enrichment in the Bengal Delta. Appl Geochem 27:292–303

Fekih IB, Zhang C, Li YP, Zhao Y, Alwathnani HA, Saquib Q, Rensing C, Cervantes C (2018) Distribution of arsenic resistance genes in prokaryotes. Front Microbiol 9:2473. https://doi.org/10.3389/fmicb.2018.02473

Fendorf S, Michael HA, van Geen A (2010) Spatial and temporal variations of groundwater arsenic in South and Southeast Asia. Science 328:1123–1127

Goswami R, Mukherjee S, Rana VS, Saha DR, Raman R, Padhy PK, Mazumder S (2015) Isolation and characterization of arsenic-resistant bacteria from contaminated water-bodies in West Bengal, India. Geomicrobiol J 32:17–26

Harvey CF, Ashfaque KN, Yu W, Badruzzaman ABM, Ashraf Ali M, Oates PM, Michael HA, Neumann RB, Beckie R, Islam S, Feroze AM (2006) Groundwater dynamics and arsenic contamination in Bangladesh. Chem Geol 228:112–136

Hery M, van Dongen BE, Gill F, Mondal D, Vaughan DJ, Pancost RD, Polya DA, Lloyd JR (2010) Arsenic release and attenuation in low organic carbon aquifer sediments from West Bengal. Geobiology 8: 155–168

Islam FS, Gault AG, Bootham C, Polya DA, Charnock JM, Chatterjee D, Lloyd JR (2004) Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. Nature 430:68–71

Jones DT, Taylor WR, Thornton JM (1992) The rapid generation of mutation data matrices from protein sequences. Comput Appl Biosci 8:275–282

Krafft T, Macy JM (1998) Purification and characterization of the respiratory arsenate reductase of *Chrysiogenes arsenatis*. FEBS J 255: 647–653

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870–1874

Lawson M, Polya DA, Boyce AJ, Bryant C, Mondal D, Shantz A, Ballentine CJ (2013) Pond-derived organic carbon driving changes in arsenic hazard found in Asian ground waters. Environ Sci Technol 47:7085–7094

Lear G, Song B, Gault AG, Polya DA, Lloyd JR (2007) Molecular analysis of arsenate-reducing bacteria within Cambodian sediments following amendment with acetate. Appl Environ Microbiol 73:1041–1048

Li X, Krumholz LR (2007) Regulation of arsenate resistance in *Desulfovibrio desulfuricans* G20 by an *arsRBCC* operon and an *arsC* gene. J Bact 189:3705–3711

Malasarn D, Saltikov CW, Campbell K, Santini JM, Hering JG, Newman DK (2004) arrA is a reliable marker for As(V) respiration. Science 306:455

Malasarn D, Keefle JR, Newman DK (2008) Characterization of the arsenate respiratory reductase from *Shewanella* sp. Strain ANA-3. J Bact 190:135–142

Mladenov N, Zheng Y, Miller MP, Nemergut DR, Legg T, Simone B, Hageman C, Moshiur Rahman M, Matin Ahmed K, McKnight DM (2010) Dissolved organic matter sources and consequences for iron and arsenic mobilization in Bangladesh aquifers. Environ Sci Technol 44:123–128

Oremland RS, Stolz JF (2003) The ecology of arsenic. Science 300:939–944

Pandey N, Bhatt R (2016) Arsenic removal and biotransformation potential of *Exiguobacterium* isolated from an arsenic-rich soil of Chhattisgarh, India. Clean Soil Air Water 44:211–218

Paul D, Kazy SK, Gupta AK, Pal T, Sar P (2015) Diversity, metabolic properties and arsenic mobilization potential of indigenous bacteria in arsenic contaminated groundwater of West Bengal, India. PLoS ONE 10:e0118735

Polizzotto ML, Kocar BD, Benner SG, Sampson M, Fendorf S (2008) Near-surface wetland sediments as a source of arsenic release to ground water in Asia. Nature 454:505–509

Raessler M (2018) The arsenic contamination of drinking and groundwaters in Bangladesh: featuring biogeochemical aspects and implications on public health. Arch Environ Contam Toxicol 75:1–7

Rzhetsky A, Nei M (1992) A simple method for estimating and testing minimum evolution trees. Mol Biol Evol 9:945–967

Saltikov CW, Wildman RA Jr, Newman DK (2005) Expression dynamics of arsenic respiration and detoxification in *Shewanella* sp. strain ANA-3. J Bact 187:7390–7396

Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Coldspring-Harbour Laboratory Press, UK

Sasi Jyothsna TS, Tushar L, Sasikala C, Ramana CV (2016) *Paraclostridium benzoelyticum* gen. nov., sp. nov., isolated from marine sediment and reclassification of *Clostridium bifermentans* as *Paraclostridium bifermentans* comb. nov. Proposal of a new genus *Paeniclostridium* gen. nov.to accommodate *Clostridium sordellii* and *Clostridium ghonii*. Int J Syst Evol Microbiol 66: 1268–1274

Silver S, Phung LT (2005) Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. Appl Environ Microbiol 71:599–608

Song B, Chyun E, Jaffe PR, Ward BB (2009) Molecular methods to detect and monitor dissimilatory arsenate-respiring bacteria (DARB) in sediments. FEMS Microbiol Ecol 68:108–117

Strockbine NA, Maurelli AT (2005) *Shigella*. In: Brenner DJ, Krieg NR, Staley JT (eds) Bergey's manual of systematic bacteriology, volume 2, part B. Springer, US, pp 811–823

Suhadolnik MLS, Salgado APC, Scholte LLS, Bleicher L, Costa PS, Reis MP, Dias MF, Avila MP, Barbosa FAR, Chartone-Souza E, Nascimento AMA (2017) Novel arsenic-transforming bacteria and the diversity of their arsenic-related genes and enzymes arising from arsenic-polluted freshwater sediment. Sci Rep 7:11231. https://doi.org/10.1038/s41598-017-11548-8

Sultana M, Hartig C, Planer-Friedrich B, Seifert J, Schlomann M (2011) Bacterial communities in Bangladesh aquifers differing in aqueous arsenic concentration. Geomicrobiol J 28:198–211

Sun Y, Polishchuk EA, Radoja U, Cullen WR (2004) Identification and quantification of *arsC* genes in environmental samples by using real-time PCR. J Microbiol Method 58:335–349

Sutton NB, van der Kraan GM, van Loosdrecht MC, Muyzer G, Bruining J, Schotting RJ (2009) Characterization of geochemical constituents and bacterial populations associated with As mobilization in deep and shallow tube wells in Bangladesh. Water Res 43:1720–1730

van Geen A, Rose J, Thoral S, Garnier JM, Zheng Y, Bottero JY (2004) Decoupling of As and Fe release to Bangladesh groundwater under reducing conditions. Part II: evidence from sediment incubations. Geochim Cosmochim Acta 68:3475–3486

Wang G, Kennedy SP, Fasiludeen S, Rensing C, Das Sarma S (2004) Arsenic resistance in *Halobacterium* sp. Strain NRC-1 examined by using an improved gene knockout system. J Bact 186:3187–3194

Wang P-P, Bao P, Sun G-X (2015) Identification and catalytic residues of the arsenite methyltransferase from a sulfate-reducing bacterium, Clostridium sp. BXM. FEMS Microbiol Lett 362:1–8

Zeng XC, Yang Y, Shi W, Peng Z, Chen X, Zhu X, Wang Y (2018) Microbially mediated methylation of arsenic in the arsenic rich soils and sediments of Jianghan Plain. Front Microbiol 9:1389. https://doi.org/10.3389/fmicb.2018.01389