

Abstract

Long term exposure to elevated levels of geogenic arsenic in the groundwater ($> 10 \mu\text{g As/L}$ of permissible safe limit) of Bengal basin (covering the alluvial landscapes of India and Bangladesh) has created chronic health risks to millions of inhabitants. The problem is endemic to other countries around the world including many Southern and South-Eastern Asiatic counterparts. Biogeochemical functions of diverse microbial populations inhabiting the alluvial groundwater along with hydro-geological factors are attributed towards the mobilization of toxic As species (As^{3+} and As^{5+}) from sub-surface sediment into the groundwater. Characterization of microbial communities and metabolic properties of few cultivable members have been reported from parts of the As affected states; although investigation on thorough eco-physiological provenances of predominated organisms, analysis of their genomic repertoire (through whole genome sequencing) and their role in As-biogeochemistry in subsurface conditions remain incomplete. The present study encompasses detailed eco-physiological and genomic characterization of As-transforming groundwater bacteria to gain better insights into the role of microorganisms in sub-surface As release processes. A comprehensive suite of state-of-the-art geo-microbiological techniques (including culturomics, whole genome sequencing, proteomics, and sediment microcosm experiments) is used. Four As-resistant (minimum tolerance values: 5-15 mM As^{3+} , 10-100 mM As^{5+}), As^{5+} -reducing, facultative anaerobic bacterial strains initially designated as KAs 5-22^T, KAs 5-3^T, KAs 3-5^T, and Cont-1 isolated from As-contaminated groundwater of West Bengal are characterized. All the strains are able to utilize multiple substrates as terminal electron acceptors (TEA) with following order of preference: $\text{NO}_3^- > \text{NO}_2^- > \text{Fe}^{3+} > \text{SO}_4^{2-}$. Anaerobic respiration with As^{5+} as TEA is observed only by the strain KAs 5-22^T. Among the carbon sources, preference towards organic acid, sugar, fatty acids, and most interestingly aromatics and low chain alkanes like catechol, benzene, dodecane, pentadecane, and nonadecane is noted for all the strains. Functional gene-based analysis validates the observed eco-physiological attributes of all these strains. Based on polyphasic taxonomy, all three strains (KAs 5-22^T, KAs 5-3^T, KAs 3-5^T) are proposed to be novel species of the genus *Rhizobium* (*R. arsenicireducens*), *Pseudoxanthomonas* (*P. arseniciresistens*), and *Achromobacter* (*A. arsenitransformans*).

Whole genome sequencing and comparative genomics corroborate the eco-physiological attributes of the test organisms and establish coherence to their respective type species

members. Moderate to high genome size (4-6 Mb), fewer copies (1-2 copies/genomes) of 16S rRNAs genes, high number of transmembrane helices, and higher G+C content (49-66 %) describe the genomic features highlighting the strains' adaptive capabilities to survive within the As-rich, low nutrient groundwater. Open pan genomic structures (asymptotic curve) with substantial evidence of possible horizontal gene transfer events, as evident from genomic islands are observed for all the studied strains. It is interesting to note that the test bacteria are differentially enriched with a number of genes for hydrocarbon utilization (*bphA*, *nph*, *catAB*, *bssA*), NO₃⁻ reduction (*narGHJ*), and As⁵⁺ reduction (*arsCBHR*, *arrA*). Presence of these genes and other properties highlight the genomic capabilities of these organisms to survive in the alluvial groundwater environment, particularly the niche colonization and As transformation abilities of these bacteria.

Sediment groundwater-microbiome (obtained from one of the high As contaminated groundwater samples of West Bengal) incubation within laboratory microcosms shows significant role of groundwater bacteria in bringing out changes in both sediment as well as aqueous geochemistry. Considerable reduction ($r = 0.91$, $p < 0.05$) of pH (7.2 to pH 5.9), dissolved oxygen (2.2 to 0.11), E_h (from 11 mV to -58 mV) and increased release of Fe (mainly as Fe²⁺, 138 mg/L, 137 fold) and As (mainly as As³⁺, 43.2 mg/L, 42.2 fold) from sediment to groundwater are noted. Effect of added organic carbon (OC) in enhancing the changes is observed. The native groundwater-microbiome is mainly composed of *Acinetobacter*, *Brevundimonas*, *Sideroxydans*, *Alkanindiges*, *Nocardiodes*, *Rhizobacter*, *Janibacter*, *Enhydrobacter*, etc. Following sediment incubation with added OC, a shift in microbiome composition with predominance of *Rhizobium*, *Escherichia*, *Clostridium sensu stricto* 1, *Acidiphilium*, *Bacillus*, *Shewanella*, *Salinicoccus*, *Halomonas* is observed. 16S rRNA gene sequences of these enriched organisms highlight close relatedness to organisms with known abilities towards As⁵⁺/Fe³⁺ reduction/respiration, alkane utilization, fermentation, and elemental dissolution. Quantitative PCR with cDNA obtained from the total RNA extracted from sediment microcosm slurry confirmed the high abundance of *Alphaproteobacteria* as the most active population along with enhanced transcript expression of both respiratory As⁵⁺ reductase (*arrA*) as well as cytosolic As⁵⁺ reductase (*arsC*). Simultaneous presence of *arsC* and *arrA* genes; predominance of *Rhizobium*, *Bacillus*, *Shewanella*, and *Clostridium* members suggest a complex role of bacterial members in high As release. Anaerobic isolation of cultivable bacteria from the sediment-groundwater microbiome confirms presence of As⁵⁺ respiring, hydrocarbon metabolizing *Rhizobium* spp. and *Bacillus* spp. X-ray diffraction (XRD) and fluorescence (XRF) analyses of sediment

samples indicate alteration of Fe-oxide minerals, while X-ray photoelectron spectroscopy (XPS) data show relative abundance of Fe²⁺ (53 relative % abundance) over the abiotic control. The data strongly suggest a definite role of groundwater bacteria in metabolising the As and Fe bearing minerals, facilitating relevant geochemical changes leading towards As release. A second microcosm containing aquifer-sand incubated anaerobically with *Rhizobium arsenicireducens* KAs 5-22^T confirms the simultaneous role of both cytosolic and respiratory As⁵⁺ reductases in As metabolism. X-ray absorption of near edge structures (XANES) analysis validates preferential transformation of solid phase As⁵⁺, while dissolution of other elements like Ca, K, Si is observed through ion chromatography. Overall data highlight a multipartite As release process by the bacterial members affecting strong interaction with Fe and As minerals of the sediment, utilizing the organic substrates available as the main mechanism for solid phase As release. Our study confirms that dissimilatory As or Fe reduction through respiratory metabolism although plays an important role, synergistic involvement of fermentative metal reducing organisms allowing indirect release of As through non-respiratory Fe transformations including mineral weathering facilitate As mobilization. Further study involving transcriptome, metabolome, and interactome based responses of the groundwater microbiome might help in deciphering more detailed mechanisms of As release in the groundwater and strategies for sustainable groundwater management.

Keywords: Arsenic, Groundwater, Geomicrobiology, Polyphasic taxonomy, Whole genome sequencing, Comparative genomics, Microbial diversity, Sediment-microcosm