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Arsenic biotransformation and release by bacteria indigenous to arsenic contaminated groundwater

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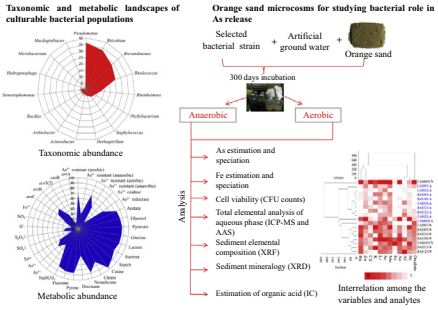
HIGHLIGHTS

- As-rich groundwater harbor bacteria with broad taxonomic and metabolic diversity.
- As- and Fe-reducing bacteria catalyze higher As release at anaerobic condition.
- As^{3+} oxidizing bacteria/oxic condition have an antagonistic role in As release.
- Fostering As^{3+} oxidizing bacteria/oxic state would control geogenic As contamination.

GRAPHICAL ABSTRACT



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ABSTRACT

Arsenic (As) biotransformation and release by indigenous bacteria from As rich groundwater was investigated. Metabolic landscape of 173 bacterial isolates indicated broad catabolic repertoire including abundance of As^{5+} reductase activity and abilities in utilizing wide ranges of organic and inorganic respiratory substrates. Abundance of As homeostasis genes and utilization of hydrocarbon as carbon/electron donor and As^{5+} as electron acceptor were noted within the isolates. Sediment microcosm study (for 300 days) showed a pivotal role of metal reducing facultative anaerobic bacteria in toxic As^{3+} release in aqueous phase. Inhabitant bacteria catalyze As transformation and facilitate its release through a cascade of reactions including mineral bioweathering and As^{5+} and/or Fe^{3+} reduction activities. Compared to anaerobic incubation with As^{5+} reducing strains, oxic state and/or incubation with As^{3+} oxidizing bacteria resulted in reduced As release, thus indicating a strong role of such condition or biocatalytic mechanism in controlling *in situ* As contamination.

1. Introduction

Geogenic arsenic (As) contamination in groundwater of alluvial aquifers has been creating severe health problems to millions of people worldwide (Oremland and Stolz, 2005). Bengal Delta Plain (BDP) spreading large areas of Bangladesh and West Bengal (India) is considered the worst affected where more than 40 million people have been known to be exposed to drinking water containing >10 μg L^{-1} As (WHO, 2001). Understanding the processes that regulate biogeochemical cycling of this metalloid in subsurface environment including the mechanisms underlying its release into groundwater from the host minerals is a subject of high interest for developing sustainable strategies for drinking water supply in the affected regions. A number of plausible mechanisms including reductive dissolution of iron oxyhydroxide, replacement of As^{5+}

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with PO_4^{3-}, mineral weathering and oxidation of pyrites have been proposed to explain the subsurface release of As. Each of these mechanisms contain both abiotic and biotic components; but in all, role of microbially mediated processes are established beyond doubt (Islam et al., 2004; Mailloux et al., 2009). Inhabiting bacteria in contaminated groundwater have been shown to regulate the toxicity and solubility of As, as well as their release from host minerals by catalyzing redox transformations and other reactions and thereby could play critical roles in controlling the subsurface contamination and/or developing any remediation system (Gault et al., 2005; Hery et al., 2010; Islam et al., 2004; Lloyd and Oremland, 2006).

In contaminated groundwater soluble As is mostly represented by its two forms; arsenate (As^{5+}) and arsenite (As^{3+}). Abundance and distribution of these species are mainly dependent on redox condition, pH and microbial activity. In oxic condition As^{5+} is the predominant form that tends to adsorbed strongly onto the solid mineral phases (i.e., ferrioxyhydroxide, ferrihydrite, apatite, alumina, etc.). Conversely, As^{3+} which is adsorbed poorly onto such minerals and is more toxic (and more mobile) is prevalent under anoxic environment (Oremland and Stolz, 2005; Yang et al., 2014). In recent years, although considerable effort has been given to elucidate the microbial role in understanding their metabolic potential but in controlling the level of toxic metalloids in BDP remained less studied. It is imperative to know the exact role of inhabitant bacteria in controlling the release of As and its transformation to develop any mitigation strategies including long term application in bioremediation. Taxonomic identity and metabolic properties of bacteria indigenous to As contaminated groundwater and sediment have been documented (Ghosh and Sar, 2013; Liao et al., 2011; Sarkar et al., 2013). Some bacteria can reduce As^{5+} to As^{3+} during their anaerobic respiration or as a means of As detoxification, while others oxidize As^{3+} to As^{5+} during their chemolithoautotropic/heterotrophic metabolism. Recent geomicrobiological studies within As rich groundwater have revealed simultaneous presence of both As-oxidizing and -reducing abilities in the resident bacteria (Sarkar et al., 2014; Yang et al., 2014). Presence and abundance of As^{3+} oxidase and As^{5+} reductases enzymes and corresponding genes are found to be ubiquitous within the bacterial populations obtained from various As rich aquifer samples (Escudero et al., 2013). With respect to the microbial role in As mobilization, interaction of autochthonous bacteria with As, As bearing minerals and other elements present in the aquifer environment have been noted. Bacteria mobilize As either by reducing labile Fe^{3+}/As^{5+} of sediment minerals using them as alternative electron acceptors under anoxic and reducing environment or by releasing non labile Fe^{3+}/As^{5+} during nutrient acquisition or other metabolic processes and reduce the resultant As^{5+} (Hery et al., 2010; Islam et al., 2004; Zhang et al., 2012).

The present study describes the broad metabolic repertoire of indigenous groundwater bacterial community, and their involvement in regulation the level of toxic As species in the groundwater. Overall 170 pure culture bacterial isolated from different groundwater were used to ascertain their metabolic landscape of the groundwater community. Selected bacterial strains (nine) were used in sediment based microcosm studies for getting better insight into the underlying mechanism of As -transformation and -release in aquifer system.

2. Methods

2.1. Isolation, identification and metabolic characterization of bacterial isolates

Six arsenic contaminated groundwater samples were collected from existing household tubewells of different sites of Barasat and Chakdaha of West Bengal, India. Range of the As level in groundwater is 15–1364 ppb. From each of the groundwater samples, cultivable bacterial populations were isolated. The samples were serially diluted in normal sterile saline (0.9%) and 100 μL of suspension from each dilution was plated on R2A (Reasoner and Geldreich, 1985) and minimal salt medium (MSM) (Kazy et al., 1999) agar plates in triplicate and incubated at 30 °C for 7 days. Morphologically distinct colonies were selected and purified by repeated subculturing in R2A and MSM medium, respectively. Each purified bacterial strain was stored at −80 °C with 15% glycerol. Identification and taxonomic characterization of the isolated bacteria were done by 16S rRNA gene sequencing (detailed methods are given in Supplementary information). All the strains were characterized with respect to their ability to resist As under aerobic and anaerobic conditions, As^{3+} oxidase and As^{5+} reductase activities, utilization of different carbon and inorganic electron acceptors (during anaerobic growth) and presence of As resistant and transformation genes (*ars*C, *ars*B, *acr*3(2), *aio*B and *arr*A) (detailed methods are given in Supplementary information).

2.2. Microcosm analysis

For microcosm study nine selected bacterial strains [*Acinetobacter* BAS123i (KF442760.1), *Arthrobacter* CAS4101i (KF442753.1), *Brevundimonas* CAS4005i (KF442756.1), *Pseudomonas* BAS323i (KF442756.1), *Pseudomonas* CAS907i, *Phyllobacterium* BAS224i (KF442766.1), *Rhizobium* BAS305i (KF442754.1), *Rhodococcus* CAS922i (KF442755.1) and *Staphylococcus* BAS108i (KJ493796.1)] were used. These strains were selected based on their taxonomic and metabolic representativeness. Microcosm studies were conducted by incubating As bearing orange colored Pleistocene sand (referred as orange sand) with the test bacteria under aerobic and anaerobic conditions. Orange sand was obtained from subsurface sedimentary cores recovered from drillings conducted at highly As contaminated site of Chakdaha, West Bengal (location 23°01′049.00″N/88°35′07.05″E) by Geological Survey of India (GSI), Kolkata (Pal and Mukherjee, 2009). The sand was recovered from the drill-cores under aseptic condition (inside sterilized laminar Air flow hood) using sterile spatula. Arsenic bearing "orange sand" was incubated aerobically (set A) and anaerobically (set B) in artificial groundwater with either of the nine selected strains. Prior to its use in microcosm, the orange sand was thoroughly homogenized and sterilized by autoclaving at 120 °C, 15 psi for 40 min to remove the viable cells present within it. For each set (A or B), a total of 10 microcosms, nine bioaugmented by nine individual strains and one control set devoid of any bioaugmentation was prepared. All microcosms were prepared in triplicate and incubated aerobically and anaerobically. In each set, 10 g of sterilized sand was added in serum bottle (Sigma–Aldrich, St. Louis, USA) having 20 mL artificial groundwater (composition g L^{-1}: $CaCl_2$. $2H_2O$ 0.148; NaCl 0.315; KCl 0.1067; $MgSO_4$. H_2O 0.9399; $NaNO_3$ 0.2488 and $NaHCO_3$ 0.11; to pH 7) and amended with carbon source (i.e., 10 mM glucose + 10 mM acetate). In all biotic microcosms initial bacterial cell density (added as inoculum) was maintained as 10^5–10^6 CFU mL^{-1}. For anaerobic microcosms, artificial groundwater medium was autoclaved for 45 min at 121 °C and amended with 1% (w/v) cysteine hydrochloride as a reducing agent. Bottles were capped by airtight butyl stopper, crimp sealed and bottle headspace was replaced with deoxygenated ultrapure N_2. For aerobic microcosms, except purging of N_2 and addition of reducing agent other steps remained same. Head space was filled with sterile air and bottles were closed by cotton plug. All bottles were set horizontally on a rotary shaker, gently rotating at 50 rpm at 26 °C over the entire period. Microcosms were subsampled at discrete time points with

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