Abstract

Microbial diversity of arsenic (As) contaminated groundwater of North Eastern state (Assam) in the Brahmaputra river basin (BRB) was studied using culture dependent approach. Diversity of cultivable bacterial populations has revealed predominance of *Brevundimonas* (35%) and *Acidovorax* (23%) along with *Acinetobacter* (10%), *Pseudomonas* (9%), *Undibacterium, Herbaspirillum, Rhodococcus, Staphylococcus, Bosea, Bacillus, Ralstonia, Caulobacter* and *Rhizobiales* (<5%). The microbial diversity obtained exhibited high resistance to As, diverse metabolism related to their growth utilizing various C-sources and alternate inorganic electron acceptors [As^{5+}, Se^{6+}, Fe^{3+}, NO_3^-, SO_4^{2-}, $S_2O_3^{2-}$] during anaerobic growth. Enrichment study to explore the predominating anaerobic microbial community was performed through PCR-DGGE technique. Microbial enrichment microcosm study from As rich aquifer sediments revealed highest anaerobic microbial diversity in absence of added organic carbon. Phylogenetic analysis revealed the dominance of strict to facultative anaerobic bacterial members of *Clostridiaceae, Lachnospiraceae, Peptostreptococcaceae, Desulfotomaculum, Bacillus, Anaerostipes* etc. in the enrichment cultures. Addition of As^{5+} to the enrichment cultures had a profound impact on selective bacterial enrichment as well as elemental release in the supernatant. Geological analysis including XRD, SEM and EDX revealed sediment weathering due to microbial activity. Sulphate reduction in absence of added As^{5+} by potent sulphate reducers appears to be limited upon addition of As^{5+}. Among the various genera detected through cultivable (aerobic) as well as enrichment (anaerobic) studies, *Bacillus* was found to be a facultative anaerobic bacterium which could be a potent As metaboliser in varying redox environments. Therefore, strain IIIJ3-1 a member of genus *Bacillus* was selected for further study. The bacterium was found to be gram positive, endospore forming, non-capsulated, catalase and oxidase positive, moderately alkaliphilic which exhibited facultative anaerobic growth with As^{5+} as TEA. Anaerobic growth kinetics and electron acceptor reduction profile revealed preference of As^{5+} followed by Fe^{3+}, Se^{6+}, NO_3^-, SO_4^{2-}. Phylogenetic analysis, biochemical properties, metabolic profiling, chemotaxonomic characters and molecular properties confirmed the isolate to be a novel member of *B. cereus sensu lato* for which the type strain is *Bacillus inferioriaquae* IIIJ3-1(T) (=MCC2980T =BCCM LMG 29433T =JCM 31241T). Deduced amino acid sequences of As homeostasis genes *arr*, *acr3(1*) and *arsB* obtained from strain IIIJ3-1 revealed phylogenetic relatedness with those reported from other As resistant *Bacillus* sp. whereas *aioB* showed phylogenetic incongruency. Microcosm studies showed enhanced ability of the strain IIIJ3-1 to release As from As rich sand in presence of lactate as electron donor. EDX analysis and XRD data corroborated this observation. Addition of NO^- in aerobic or anaerobic condition further enhanced the release of As from sand by strain IIIJ3-1. Decoupled release of As with that of Fe was found. Arsenic sequestration occurred in the secondary mineral phases of the sediment coupled with release of Fe. Minimal concentrations of organic matter in the aqueous phase supports the growth of this strain under reducing conditions with NO_3^- as a favourable TEA. Addition of As^{5+} as TEA showed selective growth of the dominant bacterial species and abolished the sparse population. The study reports for the first time the identity and metabolic abilities of bacteria in As contaminated ground water of BRB, useful to elucidate the microbial role in influencing mobilization of As in the region.

Keywords: Microbial diversity, arsenic contaminated groundwater, *Bacillus cereus,* dissimilatory arsenate reducing prokaryote, microcosm study, subsurface As release.