Analyzing differential distribution Of Dissimilatory Arsenate Reducing Bacterial Community along depths of Aquifers in Bengal Delta Plain.

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Abstract

Groundwater contamination with geogenic arsenic poses a major health risk to millions of people throughout the world. Among various group of microbes, dissimilatory arsenate reducing bacteria (DARB) are considered to be primarily responsible for arsenic mobilization in anaerobic environments of deep underground aquifer sediments. This group of microbes carries out enzyme catalyzed conversion of more immobilized and less toxic arsenate [As (V)] to more soluble and more toxic arsenite [As (III)]. Aquifers are deep subsurface layers of rocks, sand or soil capable of storing and transmitting water. These are potential environments for arsenic mobilization by anaerobic dissimilatory arsenate reducing bacteria (DARB). Study of these bacteria has been restricted to culture based microcosm studies, which suffers from several drawbacks like inappropriate simulation of ecological factors, exclusion of unculturable members, inappropriate elucidation of community behavior etc. With the recent advent of culture independent molecular analysis, more wholesome analysis of microbial community in diverse ecological habitats has become possible. Anaerobic dissimilatory As(V) reduction is catalyzed by the periplasmic arsenate respiratory reductase (Arr) complex, which consists of a large catalytic subunit (ArrA) and a small subunit (ArrB). arrA gene encoding large subunit of the reductase can be used as a reliable marker for arsenate respiration. Our study is a preliminary attempt to isolate community DNA from aquifer sediments collected from various depths and study the differential distribution of arrA in community genome at various depths. We had successfully isolated humic contaminant free community DNA from aquifer sediments and subjected them to PCR amplification with arrA gene specific primers. The amplicons obtained from community DNA of various depths were subsequently sublected to RFLP analysis by HaeIII and the restriction patterns was compared. The study revealed differential distribution of arrA containing DARB population at various depths of aquifer sediments.

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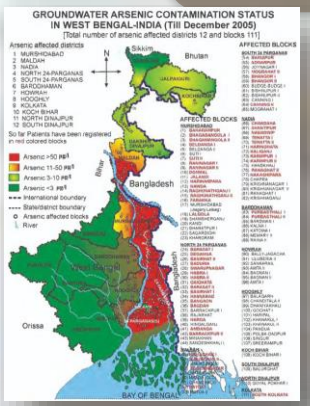
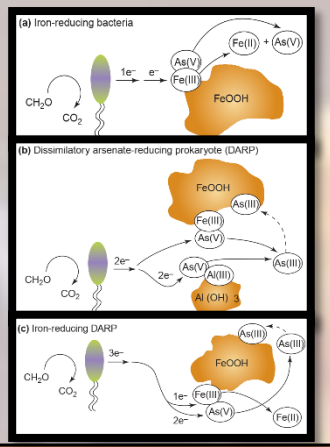


- Geogenic Arsenic (As) is a toxic metalloid pollutant detrimentally affecting population in Bengal Delta plain
- 125 million inhabitants of Bangladesh and more than 40 million people in West Bengal are at risk of Arsenic poisoning for consuming waters contaminated with arsenic.
- Aquifers are the source of arsenic contaminated groundwaters.
- Microbial communities in aquifer sediments has often been associated with release of arsenic from sediments into ground water.

In environment, Arsenic predominantly exists as:

- AsIII: More Toxic and soluble form, predominant in groundwater
- AsV: Less Toxic and predominantly remains bound to sediments

The anaerobic Aquifer ecosystem is best suited for microbial arsenic reduction and subsequent mobilization from sediment into ground water.



- Dissimilatory arsenate reducing prokaryotes (DARPs) are one of the physiological groups responsible for arsenate reducation and mobilization at deeper anaerobic aquifer sediments.
- *arrA* gene is the molecular determionants for DARPs

Objectives

- To assess the arsenate reduction potential of native microbial community of aquifer sediments of various depth
- To analyze the effect of DARP diversity in aquifer sediments on arsenate reduction potential of the native microbial community.

Aquifer sediments from various depths were collected from Chakdah, West Bengal India

↓

Microcosm systems with sediments were prepared in SeFR media spiked with 25mM arsenate and incubated under anaerobic condition with nitrogen head

↓

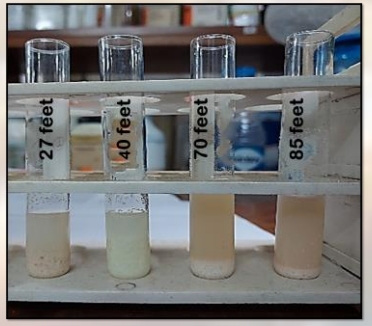
Following incubation, cells and sediments were washed and incubated with 1.33mM arsenite (AsV) under anaerobic condition for 24 hours

↓

Presence of Arsenite (AsIII) was determined by reaction with $AgNO_3$

$3Ag + AsO_3^{3-} \rightarrow Ag_2AsO_3\downarrow$ (Yellow)

$3Ag^+ + AsO_4^{-} \rightarrow Ag_3AsO_4\downarrow$ red-brown



Community DNA was directly isolated from sediment samples

↓

Subjected to PCR amplification with
arrA-CVF1 (5'-CACAGCGCCATCTGCGCCGA-3')
arrA-CVR1 (5'-CCGACGAACTCCYTGYTCCA-3')

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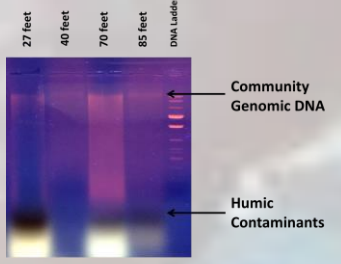
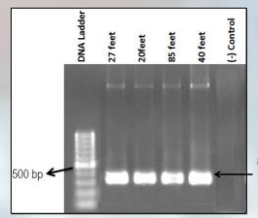
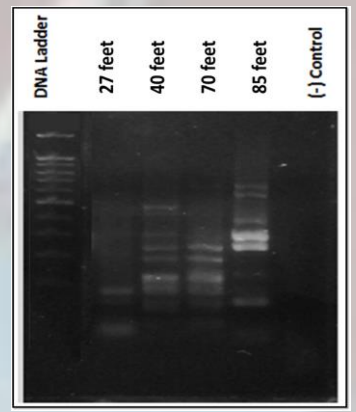
Amplicons were subjected to RFLP analysis using restriction enzyme *Hae* III







RFLP Analysis

CONCLUSION More the diverse the DARP community in aquifer sediments, higher is their arsenate reduction potential