ABSTRACT
Many microorganisms show resistance to arsenic especially to the arsenate while there are only few bacterial isolates which exhibit tolerance to arsenite. As low as 200 μM As(III) concentration has been proved lethal for many bacteria. Low concentration of arsenite directly can be methylated or prior to methylation, oxidation to arsenate may take place. Oxidation of arsenite to arsenate attenuates toxicity, provided re-conversion of arsenate in cell does not happen. Recent studies, specially a large fragment clone (~74 kb) of Alcaligenes faecalis has been modeled for arsenical resistance. It possesses a transport protein for both the arsenic valencies (3 and 5) as well as an enzyme to catalyze the conversion for both the species. Now it is generally believed that arsenite oxidizing bacteria can harbor both of the enzymes, i.e. arsenite oxidase as well as arsenate reductase.

Here we report a detail study of a isolate from contaminated site, possessing high As(III)-oxidizing activity that can grow without organic nutrient supply. Cultures were setup for isolation of arsenite oxidizing bacteria from municipal sewage by an enrichment culture technique. The arsenite oxidizing gene (aox) responsible for arsenite oxidation was cloned and sequenced. Biochemical properties of the isolate was studied. Purification and characterization of the enzyme from the isolate was carried out in detail. An attempt was made to develop a microbially assisted process for the removal of arsenic from the standard (AsIII and AsV) solution using immobilization technique.

Those bacteria that are highly resistant to arsenite hypothesized to be over expressing those proteins which bind to arsenite, before converting it or facilitating its transport. This ability of binding of proteins to arsenite was seen as a promising process for removal of metal from the potable water system. Aim of this study was to look for bacteria which had high tolerance to arsenite and thus possessing an enzyme arsenite oxidase. Screening of potent arsenite oxidizing bacteria and employing directly (whole cell) or its protein content for metal sequestering was the central theme of this study. In present study using Bio-informatics tool, it was observed that Molybdenum containing Large subunit of arsenite oxidase (~90kD), binds to substrate (AsIII) and was subsequently selected for screening of the bacteria for the first time.
Abstract

We designed a gene specific primer from highly conserved region of large subunit of arsenite oxidase using multiple gene alignment (ClustalW), to amplify partial arsenite oxidase gene. This conserved region is the region where binding of arsenite occurs. Selecting this region for the study was solving our dual purposes-
The conserved marker region must be occurring in every arsenite oxidase gene, therefore employing this conserved region as a probe for hybridization to select new isolates which harbor genes for arsenite oxidase is validated.

In our study Chemolithoautotroph was chosen as bacteria of interest which can thrive over higher concentration of sodium arsenite while drawing carbon source from bicarbonates. A bacteria capable of tolerating high sodium arsenite concentration and able to grow over wide range of temperature and pH was the bacteria of our interest. In the present we have isolated and characterized a potential bacteria, which could transform the highly toxic trivalent form of arsenic (AsIII) into less toxic pentavalent As(V) form. Organism was isolated from Okhla waste water treatment plant Delhi. The applicability of this bacterial biomass or enzyme derived from it has been looked for treatment of arsenic infested water.

In order to assess specific microbial population resistant to arsenic, an attempt was made to isolate nutritionally two different types of bacteria. Bacteria were cultivated either in (1) chemically defined minimal media supplemented with 7.5 mM NaAsO₂ or in (2) Nutrient media supplemented with 500μM of NaAsO₂. Using the first medium, we have been able to isolate a bacterium which grows without utilizing organic carbon. They are chemolithoautotrophs because source of carbon in media was HCO₃⁻. First enrichment was done without adding any Yeast extract, later on to enhance the growth and biomass we added 0.04% YE. The bacterium identified as Arthrobacter sp.15 b was a facultative chemolithoautotroph because it was growing both in minimal media and nutrient media. Bacteria accumulated high arsenic in its biomass as has been shown by EDX. Isolate 15b cultured in minimal media containing 7.5mM sodium arsenite was subjected to XRD analysis. The XRD data shows that sodium arsenite has been transformed in to Cadmium arsenic oxide Hydrate (CdAs₄O₁₁.5H₂O) and incorporated into cell biomass. The Enzyme arsenite oxidase form isolate Arthrobacter sp.15b is purified though different steps of column chromatography and identified using MALDI-TOF MS. Arsenite oxidase from
this bacteria is heterodimer showing native molecular mass ~100 kDa which appeared ~80 kDa LSU + 14 kDa SSU on SDS PAGE. $V_{\text{max}}$ of enzyme was 2.45$\mu$m (As(III))/min/mg) and $K_m$ value 26$\mu$m. Enzyme shows inhibition due to metal ions like cobalt, zinc and DEPC. Minimum inhibitory concentration (MIC) of different metal salt is determined in case of isolate 15b. Apart from many other metal salts *Arthrobacter* could tolerate 85mM of As(III) which is a highest concentration for a bacteria to tolerate As(III) as per reports available.

Secondly bacteria isolated in nutrient broth were phenotypically different since we kept NaAsO$_2$ concentration deliberately low (500$\mu$M). The presence and absence of arsenic related genes like arsenite oxidase and arsenate reductase was tested by dot blot hybridization and PCR mediated technique. The clone pUM3 harboring set of arsenate reductase gene arsC, arsB and arsA was amplified and used as a probe for screening purposes. Partial arsenite oxidase gene (656bp) cloned by us was used for screening organism possessing arsenite oxidase gene. Both dot blot hybridization and PCR with gene specific primer were used for the same. Dot blot results shows differential hybridization with 46 isolate. PCR was carried out using gene specific primer for amplifying aoxB, arsA arsB and arsC genes using genomic DNA of isolate as a template. PCR results did not match completely with dot blot results perhaps due to sequence divergence of these genes as reported earlier.

Whole cell and cell free extract from this isolate was immobilized on to calcium alginate beads to investigate if As(III) and As(V) is binding to the protein. This experiment shows that immobilized protein eliminate 80% of arsenate and 65% of arsenite from 10mg/l standard sodium arsenite and arsenate solution.