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Phylogeny and diversity of genes for poorly characterized type of arsenite oxidase involved in anaerobic arsenic oxidation [an abstract of dissertation and a summary of dissertation review]

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Phylogeny and diversity of genes for poorly characterized type of arsenite oxidase involved in anaerobic arsenic oxidation

The biological arsenic cycle is mainly governed by microbial processes to transform two main inorganic forms of arsenic, arsenite oxidation and arsenate reduction. Arsenite is more abundant in anoxic environments than oxic environments and thus anaerobic arsenite oxidation may be an essential process in the arsenic cycle. Anaerobic arsenite oxidation have been described in bacteria using an enzyme coded by the recently discovered arx genes. They were described in 2010, and have been primarily detected in anaerobic arsenite-oxidizing bacteria within the class Gammaproteobacteria, isolated from extreme arsenic-rich environments. Detection of a homologous arx gene cluster in a betaproteobacterium Sulfuricella denitrificans skB26T, isolated from a freshwater lake, opened the question about the distribution and phylogenetic diversity of these genes. However, until now, the progress to answer this question had been slow for its recent identification and low numbers of arx-harboring cultivated strains.

To contribute to better understanding of the phylogeny and diversity of arx genes, the following analyses were conducted. The first analysis explored the presence and distribution of the arxA gene in three samples from non-extreme environments. The exploration was made by clone library approach, analyzing fragments of the arxA gene amplified by PCR. For this purpose, a PCR primer set was newly designed based on the sequences available in the public database. Partial arxA-like sequences were recovered in all samples, and they were phylogenetically distinct from those of strain isolated in extreme environments. The majority of them formed a unique cluster within the ArxA clade along with the sequences of Sulfuricella denitrificans skB26T. The remaining sequences were clustered with the ArxA sequence of another isolated strain, Azoarcus sp. CIB. These results show evidence of a wider distribution of arxA-like sequences in non-extreme environments.
The purpose of the second analysis was to explore the taxonomic and environmental distribution of arx gene cluster in prokaryotic genomes. All the genomes harboring a homologous arx gene cluster were affiliated to the phylum Proteobacteria, and the majority belonged to the classes Gammaproteobacteria and Betaproteobacteria. The arxB’ABCD genes, encoding the putative arsenite oxidase, were homogeneous in almost all the genomes, regarding both their genetic content and organization. The presence of the regulatory genes, arxXSR predicted to control the expression of the enzyme, varied between genomes even in those of closely related species. It was also found that the arxA gene sequences in some genomes hold a long or short insertion at the same position. In the phylogenetic tree of ArxA, sequences with the insertion exclusively belonged to the cluster which includes Sulfuricella denitrificans skB26T. Clone library exploration of arxA sequences with long insert was made in two environmental samples to confirm presence of diverse sequences with insertion. These results show evidence of the genetic diversity of arxA genes and conservation of arxB’ABCD gene cluster within the Proteobacteria.

Lastly, a novel arsenite-oxidizing betaproteobacterium strain M52 was isolated from a hot spring microbial mat. Strain M52 can transform arsenite to arsenate in the presence of nitrate or low concentration of oxygen. Detection of nap genes encoding nitrate reductase and ccoNOQP and cydAB genes coding for two microaerophilic-related cytochrome oxidases may suggest the use of nitrate and oxygen as electron acceptors. Lack of aio genetic signature and presence of a complete arx gene cluster are suggestive of an arx-mediated arsenite oxidation. The arxA gene of this strain has the long insertion, as is the case with other two strains isolated from the same microbial mat. The nearest relatives of strain M52 are Georgfuchsia toluolica G5G6 and Denitratisoma oestradiolicum AcBE2-1 with 94% of similarity, based on comparison of nearly full length (~1400 bp) 16S rRNA gene sequences. The distinct phylogenetic identity suggests that it is a novel species, from a still undefined genus within the family Sterolibacteriaceae. Further study is needed to genetically validate the arsenite oxidation by the arx genes, and confirm the chemical species used as electron acceptor.