学 位 論 文 内 容 の 要 旨

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Phylogeny and diversity of genes for poorly characterized type of arsenite oxidase involved in anaerobic arsenic oxidation
(嫌気的ヒ素酸化に関わる亜ヒ酸酸化酵素遺伝子の系統及び多様性)

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* skB26^{T}, 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* skB26^{T}. 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* skB26^{T}. 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.