# 学位論文の要旨

Abstract of Thesis



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**学位論文題目** Title of Thesis（学位論文題目が英語の場合は和訳を付記）

Analysis of thiosulfate metabolism in a marine acidophilic sulfur-oxidizing bacterium, *Acidithiobacillus thiooxidans* strain SH.
海洋性好酸性硫黄酸化細菌 *Acidithiobacillus thiooxidans* SH 株のチオ硫酸代謝の解析

**学位論文の要旨** Abstract of Thesis


Bacterial leaching is used to recover metals from low-grade of sulfide ores. Acidophilic iron- and sulfur-oxidizing bacteria are used in the process. The technique can also be applied for bioremediation of sediments and soils polluted with heavy metals. Since acidophilic sulfur-oxidizing bacteria that are tolerant to NaCl or require NaCl for growth are useful in developing remedial technologies for salt-containing environments, an acidophilic sulfur-oxidizing bacterium strain SH was isolated, which requires NaCl for growth. Strain SH autotrophically grew on elemental sulfur, thiosulfate and tetrathionate as growth substrates, and was identified as *Acidithiobacillus thiooxidans* based on the 16S rRNA gene sequence.

For bacterial leaching, thiosulfate metabolism is a key pathway, because a sulfur moiety of sulfide is thought to be metabolized via thiosulfate as the intermediate. Thiosulfate has been suggested to be metabolized by thiosulfate dehydrogenase, TSD. The oxidation pathway for elemental sulfur and reduced inorganic sulfur compounds (RISCs) in strain SH has been previously proposed. However, little information on the biochemical and genetic properties of enzymes involved in elemental sulfur and RISCs is now available. To get a better understanding of sulfur metabolism in strain SH, the purification of TSD from strain SH was carried out. TSD activity measured using ferricyanide as the electron acceptor was detected in the cell-free extract of strain SH cells grown on thiosulfate. The enzyme was localized in membrane fraction and solubilized by n-dodecyl-β-D-maltopyranoside. A three-step procedure resulted in approximately 71-fold purification of the enzyme. An SDS-PAGE analysis of the final preparation revealed a major protein with an apparent molecular mass of 44 kDa. Heme was not detected in the preparation, according to the result of spectroscopic analyses. The maximum enzyme activity (45 U·mg-1) was observed at pH 4.0, 40°C, and 200 mM NaCl. Ubiquinone could be used as an electron acceptor, while horse heart cytochrome *c* could not be used. The activity was strongly inhibited with 2-n-heptyl-4-hydroxy-quinolone-*N*-oxide and sulfite.

To identify the gene encoding TSD, the determination of the N-terminal amino acid sequence was carried out. However the N-terminus was blocked and the sequence could not be determined. Therefore, the analysis of peptide fragments produced by in-gel digestion with trypsin has been done using HPLC-chip/QTOF and Mascot Server search. Unfortunately, we could not found any homologous proteins with a high score and coverage in the database. Therefore, the gene was determined by analyzing a draft genome sequence of strain SH.

The draft genome of *At. thiooxidans* SH contains a total of 2.91 Mbp distributed in 73 contigs with a G+C content of 54.3%. The annotation results revealed one 5S-16S-23S operon, 45 tRNAs, and 2,986 CDSs. Draft genome sequences of three *At. thiooxidans* strains, A01, ATCC19377 and Licanantay, have been already released. Taking advantage of the availability of the other three *At. thiooxidans* draft genome sequences, the genomic comparison analysis of strain SH was carried out. Total length of genomes from strains Licanantay and A01 (3.94 Mb and 3.82 Mb respectively) showed similar size, and larger than that from strain SH. Total number of CDS in strain SH genome was also smaller than those in strain Licanantay and A01 (3,898 and 3660 respectively). Sulfur oxidizing complex (*sox*), tetrathionate hydrolase (*tet*), sulfide quinone reductase (*sqr)*, and heterodisuifide reductase (*hdr*), found in strain SH were also found in other genomes with the similar gene arrangement. Two *sqr* genes, 2 *sox* gene clusters, 2 cytochrome *c* oxidase (*cox*) gene clusters, and 4 *bd*-type ubiquinol oxidase (*cyd*) gene clusters were found in SH genome.

Based on the documented models in other *Acidithiobacillus* species, such as *A. ferrooxidans, At. caldus and At. thiooxidans*, a hypothetical model for sulfur oxidation pathway in strain SH was developed from the genome sequence analysis. In this model, thiosulfate spontaneously generated from sulfite and elemental sulfur in periplasm is metabolized by thiosufate:quinone oxidoreductase (TQO) or incomplete SOX system (without SoxCD). TQO catalyzes the conversion of two molecules of thiosulfate to tetrathionate by using a quinone as the electron acceptor. SOX catalyzes the conversion of thiosulfate to sulfate and elemental sulfur. Thiosulfate in cytoplasm may be metabolized to sulfite and elemental sulfur by thiosulfate sulfurtransferase. *At. thiooxidans* SH draft genome sequence provides new insights into the genomic diversity of members of the genus *Acidithiobacillus* and reveals a closer functional relatedness of *At. thiooxidans* to sulfur-oxidizing-bacterium, *Acidithiobacillus caldus* than to iron-oxidizing bacteria, such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus ferrivorans*. It also provides new opportunities for experimental research and contributes to a better understanding of the ecophysiology of extreme acidic environments.

Using sequence data obtained by HPLC-chip/QTOF analysis of peptide fragments produced in-gel trypsin digestion of TSD, a Mascot Server search was carried out to determine a gene encoding TSD in strain SH genome. The protein was encoded in gene 739 in contig 2 of strain SH genome. The gene encoded 444 amino acids with the signal peptide of 29 amino acids, indicating the periplasmic localization. The molecular mass without the signal peptide was calculated to be 45,971, which was similar to the apparent molecular mass (44 kDa) estimated by SDS-PAGE.

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In the sulfur oxidation pathway proposed on the basis of genome analyses of strain SH and other *At. thiooxidans* strains, thiosulfate is suggested to be oxidized by TQO or SOX system. However, a gene encoding TSD was different from the *tqo* or the *sox* gene in strain SH genome. A BLAST search revealed some homologous proteins from sulfur-oxidizing bacteria, such as *At. caldus, At. ferroxidans* and *At. ferrivorans*, but the homologies were relatively low. The *tqo* (*doxDA*) gene was initially determined in *Acidianus ambivalens*. TSD found in strain SH showed a low homology to the TQO from *Ac. ambivalens*. Surprisingly, any homologous proteins with TSD from strain SH were not found in other *At. thiooxidans* strains. To clarify the reason why genes homologous to *tsd* of strain SH was not found in genomes of other *At. thiooxidans* strains, the gene cluster containing *tsd* of strain SH was compared with those of other three *At. thioxidans* strains. The *tsd* gene cluster of strain SH involved genes encoding one hypothetical protein, two transposes and DoxD. DoxD is a homolog of the subunit of the putative thiosulfate:quinone oxidoreductase (TQO). This gene cluster was not found in the corresponding region of the other *At. thiooxidans* genomes.

Sequence analysis also showed that strain SH contained about 110 transposase genes which showed relatively high sequence similarity to *At. caldus* or *At. ferrooxidans*. *tsd* gene newly determined in strain SH was found in a genomic island with 4.8 kb containing transposases showing the similarity to IS4 or Lferr from *At. ferrooxidans,* suggesting that strain SH acquired this gene through an horizontal gene transfer. This is one of the reasons why homologous proteins were not found in other three *At. thiooxidans* strains than strain SH.

In this research, TSD is purified for the first time from a marine sulfur-oxidizing bacterium. Comparison with thiosulfate metabolizing enzymes from the other microorganisms showed that TSD from strain SH was structurally different from the thiosulfate-oxidizing enzymes reported so far. In addition, this is the first report of thiosulfate dehydrogenase whose activity is stimulated by NaCl. TSD from strain SH was able to use quinone ($Q_2$) as the electron acceptor, suggesting that the TSD had TQO activity. The TQO from *Ac. ambivalens* is the only one TQO consisting of two subunits (DoxAD) and characterized on the molecular genetic level. The TSD from strain SH showed a low homology to the TQO from *Ac. ambivalens*. Although further detailed investigations are necessary to clarify the kinetic mechanism, the TSD from strain SH may be a novel thiosulfate:quinone oxidoreductase.