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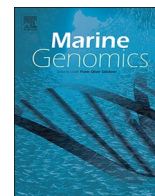
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Genome analysis of *Thermosulfuriphilus ammonigenes* ST65^T, an anaerobic thermophilic chemolithoautotrophic bacterium isolated from a deep-sea hydrothermal vent

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ABSTRACT

Thermosulfuriphilus ammonigenes ST65^T is an anaerobic thermophilic bacterium isolated from a deep-sea hydrothermal vent chimney. *T. ammonigenes* is an obligate chemolithoautotroph utilizing elemental sulfur as an electron donor and nitrate as an electron acceptor with sulfate and ammonium formation. It also is able to grow by disproportionation of elemental sulfur, thiosulfate and sulfite. Here, we present the complete genome sequence of strain ST65^T. The genome consists of a single chromosome of 2,287,345 base pairs in size and has a G + C content of 51.9 mol%. The genome encodes 2172 proteins, 48 tRNA genes, and 3 rRNA genes. Genome analysis revealed a complete set of genes essential to CO₂ fixation and gluconeogenesis. Homologs of genes encoding known enzyme systems for nitrate ammonification are absent in the genome of *T. ammonigenes* assuming unique mechanism for this pathway. The genome of strain ST65^T encodes a complete set of genes necessary for dissimilatory sulfate reduction, which are probably involved in sulfur disproportionation and anaerobic oxidation. This is the first reported genome of a bacterium from the genus *Thermosulfuriphilus*, providing insights into the microbial contribution into carbon, sulfur and nitrogen cycles in the deep-sea hydrothermal vent environment.

1. Introduction

The deep-sea hydrothermal systems are characterized by darkness, high pressures and steep gradients of physical and chemical parameters formed in mixing zones between hot reduced hydrothermal fluid and cold oxidized ocean water. As water column prevents the penetration of the sunlight, the trophic chains in these ecosystems are based primarily on chemosynthesis with chemolithoautotrophic microorganisms as primary producers. A number of studies have shown the predominance of *Proteobacteria*, especially *Epsilonproteobacteria* in deep sea hydrothermal systems, while other bacterial groups like the *Aquificales*, *Thermales*, *Thermotogales*, *Deltaproteobacteria*, and *Thermodesulfobacteriaceae* are also often detected (Nakagawa and Takai, 2008; Orcutt et al., 2011; Flores et al., 2012). Most of the cultivated representatives of these taxa are associated with utilization of inorganic sulfur compounds.

The genus *Thermosulfuriphilus* belongs to a deep lineage in the phylum *Thermodesulfobacteria* (Slobodkina et al., 2017). Currently, the

genus comprises a sole species, *T. ammonigenes* ST65^T, an extremely thermophilic, anaerobic, obligately chemolithoautotrophic bacterium that was isolated from a deep-sea hydrothermal vent at the Eastern Lau Spreading Centre in the Pacific Ocean, at a depth of 1870 m. The energy metabolism of *T. ammonigenes* ST65^T is mandatorily dependent on the transformations of sulfur compounds. The strain grows due to oxidation of elemental sulfur or thiosulfate coupled to reduction of nitrate with ammonium production. It also grows by disproportionation of sulfur, thiosulfate and sulfite (Slobodkina et al., 2017).

In this study, we analyzed the genome of *T. ammonigenes* ST65^T, the first sequenced complete genome within the species of genus *Thermosulfuriphilus*, and highlighted its general metabolic pathways. The availability of the genome sequence will promote the better understanding of the metabolic traits of prokaryotes participating in sulfur, nitrogen and carbon cycles in such unique types of ecosystems as deep-sea hydrothermal vents.

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2. Data description

For genomic DNA extraction, the strain was cultivated anaerobically at 65 °C with elemental sulfur (5 g/L) and potassium nitrate (10 mM) as an electron donor and an electron acceptor, respectively. Cells were harvested in the late exponential phase of growth. The DNA was extracted using a FastDNA™ Spin Kit (MP Biomedicals, USA) according to the manufacturer's instructions. The whole genome was sequenced using the Illumina nanoMiSeq technology (Fasteris, Switzerland) (2 × 150 bp paired-reads, Nano V2 chemistry) generating more than 200 Mb clean data, and using the PacBio Sequel technology (MrDNA, USA). Clean reads from long-reads and short reads sequencing were assembled and circularized using Unicycler v0.4.8-beta assembly pipeline (<https://github.com/rrwick/Unicycler>) (Wick et al., 2017). Average coverage was calculated using BBtools (BBMap – Bushnell B. – sourceforge.net/projects/bbmap/) and was about 88 x. Gene search and annotation were performed by means of the Rapid Annotation using Subsystem Technology (RAST/SEED v2.0) pipeline (Overbeek et al., 2014), the Integrated Microbial genomes IMG/M v.5.0 analysis system (Chen et al., 2019) and NCBI's (National Center of Biotechnology Information) Prokaryotic Genome Annotation Pipeline (PAGP) (Tatusova et al., 2016). The search and analysis of transposase families was performed by ISSaga web server (Varani et al., 2011). Identification and classification of the CRISPR-Cas system was performed by the CRISPRCas Finder web server (Couvin et al., 2018). The prediction of laterally transferred gene clusters (genomic islands) was performed with the IslandViewer4 web server (Bertelli et al., 2017). Genome visualization was made with the CGView program (Grant and Stothard, 2008).

The general features of *T. ammonigenes* strain ST65^T and the genome sequencing information are summarized in Table 1.

The complete genome of strain ST65^T consisted of a single circular chromosome with a total length of 2,287,345 bp and a G + C content of 51.9 mol%. No plasmids were detected (Fig. 1). CheckmM v1.1.2 estimated the genome to be 99.5935% complete based on the presence of default single-copy marker genes (1 marker was missing) and hypothetical contamination to be 1.6260%. Annotation with PGAP resulted in prediction of 2236 genes, 2172 of which are protein-coding sequences (CDSs) that cover about 97% of the entire genome. Genome also contained one operon of 5S, 16S and 23S rRNA genes and 48 tRNA genes for all 20 standard amino acids. No CRISPR loci were found.

Analysis of the COG (Clusters of Orthologous Genes) functional categories was performed with the eggNOG-Mapper (v.5.0) (Huerta-Cepas et al., 2019). Majority of the CDSs (95.3%) could be assigned to at least one COG group. The main predicted COG categories (encompassing more than 100 CDSs) were energy production and conversion (11.9%); translation, ribosomal structure and biogenesis (7.97%); cell wall/membrane/envelope biogenesis (7.23%); amino acid transport and metabolism (6.92%); signal transduction mechanisms (6.81%); replication, recombination and repair (5.97%) and coenzyme transport and metabolism (5.56%).

A large number of various transposases and integrases could facilitate the transfer of adaptive genes between different microbial species, and thus contribute to the diversity of deep-sea bacteria. The search and analysis of transposases revealed the presence of 10 insertion sequences (IS) belonging to 4 different IS families (IS3_ssr_IS407, IS256, IS3_ssr_IS150 and IS630). These sequences contained putative genes of 4 transposases and 10 integrases that according to Pfam database belonged to diverse protein families (Table 2).

The prediction of laterally transferred genes showed that the *T. ammonigenes* ST65^T genome possesses 5 genomic islands (GI) of 124.3 kb total length. The vast majority of the genes located on the genomic islands encode proteins annotated as hypothetical proteins. Meanwhile, one of genomic islands carried genes encoding ribosomal proteins and also genes involved in dissimilatory sulfate reduction (*sat*, *aprAB* and *qmoABC*) (Fig. 1).

Table 1

General features and genome sequencing information for *Thermosulfuriphilus ammonigenes* ST65^T according to MIGS recommendations.

Item	Description
Investigation	
Submitted to INSDC	GenBank: CP048877
Investigation type	Bacteria
Project name	<i>Thermosulfuriphilus ammonigenes</i> type strain ST65 genome sequencing
NCBI BioProject	Accession: PRJNA606893 ID: 606893
NCBI BioSample	Accession: SAMN14116118 ID: 14116118
Geographic location (latitude and longitude)	21° 59°35'S, 176° 34'06"W
Geographic location (country and/or sea, region)	Eastern Lau Spreading Centre, south-west Pacific Ocean
Collection date	June 2009
Environment (biome)	marine hydrothermal vent biome ENVO:01000030
Environment (feature)	marine hydrothermal vent ENVO:01000122
Environment (material)	marine hydrothermal vent chimney ENVO:01000129
Depth	1870 m
General features	
Classification	Domain <i>Bacteria</i> Phylum <i>Thermodesulfobacteria</i> Class <i>Thermodesulfobacteria</i> Order <i>Thermodesulfobacteriales</i> Family <i>Thermodesulfobacteriaceae</i> Genus <i>Thermosulfuriphilus</i> Species: <i>Thermosulfuriphilus ammonigenes</i>
Gram stain	Negative
Cell shape	Rod
Motility	non-motile
Growth temperature range	Thermophilic, optimum at 65° C
Relationship to oxygen	Obligate anaerobe
Trophic level	Chemolithoautotroph
Biotic relationship	free-living
Isolation and growth conditions	DOI https://doi.org/10.1099/ijsem.0.002142
Sequencing	
Sequencing platform	Illumina MiSeq + PacBio Sequel (hybrid)
Assembler	Unicycler v 0.4.8-beta
Finishing strategy	complete
Method reads	Hybrid
Contig number	1
N50	2,287,345
Genome coverage	88 x
Genomic features	
Genome size (bp)	2,287,345
G + C content (mol %)	51.9
Number of genes	2236
Protein coding genes	2172
Genes with COGs	1908
Number of RNAs	55
rRNAs	1, 1, 1 (5S, 16S, 23S)
tRNAs	48

The whole-genome sequence data were generally consistent with the main metabolic features experimentally demonstrated in strain ST65^T (Slobodkina et al., 2017). The genome of strain ST65^T possessed the genes essential for the Wood–Ljungdahl (the reductive acetyl-CoA) pathway for the fixation of CO₂. In consistence with the inability of ST65^T to utilize organic substances, the tricarboxylic acid cycle (TCA) was found to be incomplete (4 enzymes out of 9 were missing). The genome contained all genes for glycolysis (Embden-Meyerhof pathway) which obviously operated in the reverse direction for gluconeogenesis. The ability to use nitrate as an electron acceptor is enabled by the presence of an operon *napMADGH* encoding periplasmic Nap-type nitrate reductase. The reduction of the produced nitrite to ammonium does not proceed via the canonical Nrf system because the gene, encoding the key enzyme of this pathway (i.e., pentaheme cytochrome c

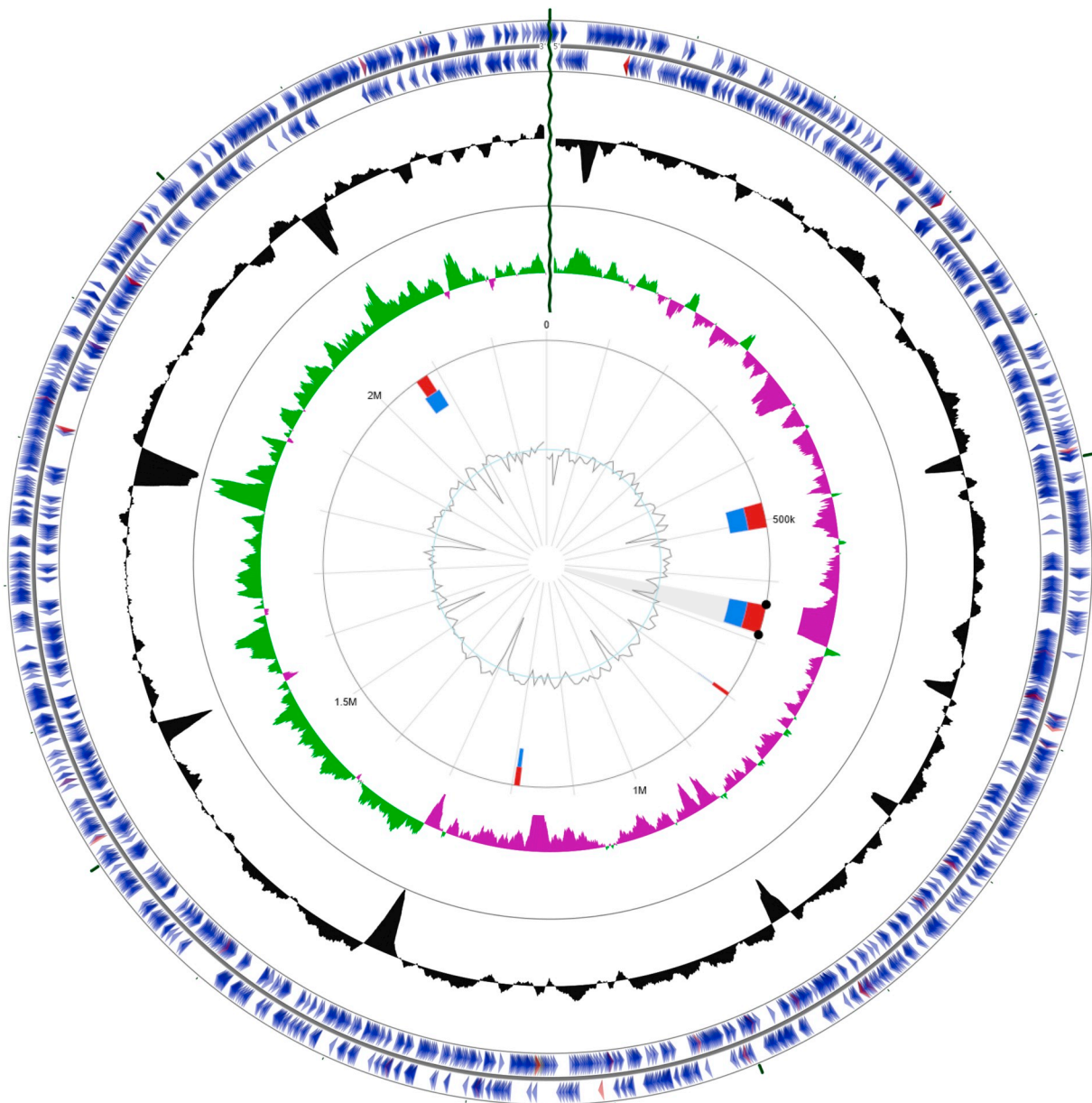


Fig. 1. Schematic representation of the *Thermosulfuriphilus ammonigenes* ST65^T genome. Labeling from the outside to the center is as follows: circle 1, genes on the forward strand; circle 2, genes on reverse strand (tRNAs pink, rRNAs lilac); circle 3, G + C content; circle 4, G + C skew. Genomic islands are shown as red-and-blue trapezes, GI carrying sulfate reduction genes highlighted in black circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

nitrite reductase NrfA), was not found in the genome. Yet, the genome of strain ST65^T contains genes of hydroxylamine oxidoreductases (Hao) and hydroxylamine reductase (Hcp). Thus, the Nrf complex in *T. ammonigenes* could be replaced by an ammonification pathway based on Hao and Hcp enzymes as it has been proposed for several other marine bacteria (Hanson et al., 2013; Slobodkina et al., 2017a; Slobodkin et al., 2019).

Sulfur cycle is of a great importance in the deep-sea environments. It is notably the case at deep-sea hydrothermal vents where the reactions of CO₂ fixation, linkage to organic matter metabolism and remineralization to CO₂ involve especially the oxidation or reduction of inorganic sulfur species of different redox states. Homologs of genes encoding known enzyme systems of reduced sulfur compounds oxidation such as sulfide: quinone oxidoreductase (SQR), sulfite oxidizing enzyme (SOE), sulfur oxidizing (Sox) enzyme complex or sulfur oxygenase/reductase (SOR) were absent in genome of *T. ammonigenes*. The genome

of strain ST65^T encodes a complete set of genes necessary for dissimilatory sulfate reduction although the ability of this microorganism to grow by sulfate respiration was not revealed in laboratory experiments (Slobodkina et al., 2017). Probably, the sulfate reduction pathway in this bacterium is involved in disproportionation of sulfur compounds as it was assumed earlier for *Desulfocapsa sulfoexigens* and *Thermosulfurimonas dismutans* (Frederiksen and Finster, 2003; Mardanov et al., 2016). Some of these genes also can be used to oxidize sulfur compounds during growth with S⁰ or thiosulfate and nitrate as it was shown for phototrophic sulfur bacteria (Frigaard and Dahl, 2009). The detection of sulfate reducing genes on GI indicates the contribution of mobile elements in the adaptation of bacteria to the environment and in active participation in the sulfur cycle.

In conclusion, the genomic analysis of *T. ammonigenes* ST65^T has revealed that despite the ability of the strain to grow by sulfur oxidation coupled to nitrate ammonification, the genes encoding the canonical

Table 2
Mobile elements in the genome of *Thermosulfuriphilus ammonigenes* ST65^T.

Gene ID	Gene product	Pfam ID	Number of genes
QIJ70900 (2 copies)	Integrase core domain	pfam13358	5
QIJ72367 (2 copies)			
QIJ72678			
QIJ71194	Phage integrase family	pfam00589	2
QIJ71591			
QIJ71463	Phage integrase	pfam13495	1
QIJ71806	Transposase, Helix-turn-helix domains	pfam01527	3
QIJ72599			
QIJ72601			
QIJ71807	Integrase core domain	pfam00665	2
QIJ72907			
QIJ71809	Transposase, Mutator family	pfam00872	1

enzyme systems for these pathways such as SQR, SOE, Sox, SOR and Nrf, are absent. Conversely, the presence of all genes necessary for dissimilatory sulfate reduction does not provide the ability to grow due to sulfate reduction. These genes are apparently involved in disproportionation and oxidation of reduced sulfur compounds. In accordance with the fact that *T. ammonigenes* is an obligate lithoautotroph its genome contains genes involved in CO₂ fixation, gluconeogenesis and incomplete set of genes participating in TCA cycle.

3. Genome sequence accession numbers

The genome sequence of *T. ammonigenes* ST65^T has been deposited in DDBJ/ENA/GenBank under the accession number CP048877. The BioSample data is available in the NCBI BioSample database (<http://www.ncbi.nlm.nih.gov/biosample/>) under the accession number SAMN14116118. The BioProject data is available in the NCBI BioProject database (<https://www.ncbi.nlm.nih.gov/bioproject/>) under the accession number PRJNA606893. The strain is available in the German Collection of Microorganisms and Cell Cultures (DSMZ) and All-Russian Collection of Microorganisms (VKM) under the accession numbers DSM 102941^T and VKM B-2855^T.

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Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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