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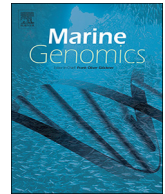
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Complete genome sequence of *Thermosulfurimonas marina* SU872^T, an anaerobic thermophilic chemolithoautotrophic bacterium isolated from a shallow marine hydrothermal vent

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ABSTRACT

Thermosulfurimonas marina strain SU872^T is a thermophilic, anaerobic, chemolithoautotrophic bacterium, isolated from a shallow-sea hydrothermal vent in the Pacific Ocean near Kunashir Island, that is able to grow by disproportionation of inorganic sulfur compounds and dissimilatory nitrate reduction to ammonium. Here we report the complete genome sequence of strain SU872^T, which presents one circular chromosome of 1,763,258 bp with a mean G + C content of 58.9 mol%. The complete genome harbors 1827 predicted protein-encoding genes, 47 tRNA genes and 3 rRNA genes. Genes involved in sulfur and nitrogen metabolism were identified. This study expands our knowledge of sulfur and nitrogen use in energy metabolism of high temperatures areas of shallow-sea hydrothermal environments. In order to highlight *Thermosulfurimonas marina* metabolic features, its genome was compared with that of *Thermosulfurimonas dismutans*, the only other species described within the *Thermosulfurimonas* genus.

1. Introduction

Thermosulfurimonas marina strain SU872^T had been discovered and characterized by Frolova et al. (2018) as a novel thermophilic, anaerobic, chemolithoautotrophic bacterium. *T. marina* was isolated from a shallow-sea hydrothermal vent located off the Kunashir Island in the Sea of Okhotsk. It is likely to be involved in the nitrogen and sulfur cycles of this ecosystem through its metabolic activities. Shallow hydrothermal vents are generally located at a water depth less than 200 m and, like deep-sea hydrothermal vents, they are characterized by wide redox, temperature and pH gradients, allowing chemotrophs to grow on chemical energy, in addition to phototrophs developing from light energy (Tarasov et al., 2005). This complex ecosystem is rich in various sulfur compounds and had been demonstrated to be inhabited by sulfur-oxidizing bacteria, and various chemotrophic microorganisms using alternative electron donors such as sulfide, thiosulfate, molecular

hydrogen and electron acceptors such as oxygen, sulfur, manganese, iron, nitrite and nitrate (Price and Giovannelli, 2017). As suggested by Price and Giovannelli (2017), nitrate reducers may represent a significant fraction of the microbial community inhabiting shallow hydrothermal vents but only few nitrate reducers have been isolated to date from this habitat. Frolova et al. (2018) demonstrated that *T. marina* grows by sulfur compounds (elemental sulfur, thiosulfate and sulfite) disproportionation (= dismutation), and by utilization of these sulfur compounds as electron donors and nitrate as an electron acceptor with CO₂/HCO₃⁻ as sole carbon source. Besides, *T. marina* is one of the few shallow vent microorganisms known to perform DNRA metabolism (Dissimilatory Nitrate Reduction to Ammonium). This energy-yielding reaction is a little more documented within deep-sea hydrothermal vent species (Slobodkina et al., 2017). *T. marina* is a member of the *Thermodesulfobacteria* phylum and the *Thermodesulfobacteriaceae* family, represented currently by five genera. Based on its 16S ribosomal RNA

Abbreviations: ANI, average nucleotide identity; CDS, coding DNA sequence; CMP-KDO, cytidine 5'-monophospho-3-deoxy-D-manno-2-octulosonic acid; COG, clusters of orthologous groups; CRISPR, clustered regularly interspaced short palindromic repeats; ENA, European nucleotide archive; KEGG, Kyoto encyclopedia of genes and genomes; ORF, open reading frame; TCA, tricarboxylic acid cycle

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Table 1

General features and genome sequencing information for *Thermosulfurimonas marina* strain SU872^T and *Thermosulfurimonas dismutans* strain S95^T, including MIGS mandatory information, based on MaGe platform.

Item	Description	
Investigation		
Strain	<i>Thermosulfurimonas marina</i> strain SU872 ^T	<i>Thermosulfurimonas dismutans</i> strain S95 ^T
Submitted to INSDC	GenBank	GenBank
Investigation type	Bacteria	Bacteria
Project name	CP042909	LWLG01
Geographic location (latitude and longitude)	44° 29.469' N, 146° 06.247' E	22° 10.82' S, 176° 36.09' W
Geographic location (country and/or sea, region)	Sea of Okhotsk, 250 m from the Kunashir Island shore (Sakhalin oblast, Russia)	Eastern Lau Spreading Center, SW Pacific Ocean
Collection date	June 2013	June 2009
Environment (biome)	marine hydrothermal vent biome ENVO:01000030	marine hydrothermal vent biome ENVO:01000030
Environment (feature)	marine hydrothermal vent ENVO:01000122	marine hydrothermal vent ENVO:01000122
Environment (material)	marine hydrothermal vent chimney ENVO:01000129	marine hydrothermal vent chimney ENVO:01000129
Depth	- 12 m	- 1910 m
General features		
Classification	Domain Bacteria Phylum <i>Thermodesulfobacteria</i> Class <i>Thermodesulfobacteria</i> Order <i>Thermodesulfobacteriales</i> Family <i>Thermodesulfobacteriaceae</i> Genus <i>Thermosulfurimonas</i> Species: <i>Thermosulfurimonas marina</i>	Domain Bacteria Phylum <i>Thermodesulfobacteria</i> Class <i>Thermodesulfobacteria</i> Order <i>Thermodesulfobacteriales</i> Family <i>Thermodesulfobacteriaceae</i> Genus <i>Thermosulfurimonas</i> Species: <i>Thermosulfurimonas dismutans</i>
Gram stain	Negative	Negative
Cell shape	Oval to short rods	Rods
Motility	Motile	Motile
Growth temperature	Thermophilic, optimum at 74 °C	Thermophilic, optimum at 74 °C
Relationship to oxygen	Anaerobic	Anaerobic
Trophic level	Chemolithoautotroph	Chemolithoautotroph
Biotic relationship	free-living	free-living
Isolation and growth conditions	doi: https://doi.org/10.1134/S0026261718040082	doi: https://doi.org/10.1099/ijms.0.034397-0
Sequencing		
Sequencing technology	Illumina MiSeq + PacBio Sequel (hybrid)	454 sequencing
Assembler	Unicycler v 0.4.8-beta	Newbler v. 2.9
Contig number	1	61
N50	1,763,258	94,683
Genome coverage	116.0086 ×	35 ×
Genome assembly NCBI	ASM1231758v1	ASM165258v1
Assembly level	Complete genome	Contig
Genomic features		
Genome size (bp)	1,763,258	2,119,932
GC content (mol %)	58.90	50.12
Protein coding genes	1827	2201
Number of RNAs	54	51
tRNAs	47	48
16S–23S–5S rRNAs	1–1–1	1–1–1

gene sequence, *T. marina* is phylogenetically closely related to *Thermosulfurimonas dismutans* S95^T, a thermophilic, anaerobic, chemolithoautotrophic bacterium isolated from a deep-sea hydrothermal vent chimney located in the Pacific Ocean. *T. dismutans* is the first described representative of the *Thermosulfurimonas* genus whose genome has been assembled, annotated and studied by Mardanov et al. (2016), and which possesses similar physiological properties (Slobodkin et al., 2012; Slobodkina et al., 2017) (Table 1).

In this study, we analyzed the genome of *T. marina* SU872^T, the second sequenced genome within the *Thermosulfurimonas* genus, and investigated its metabolic features. Genome sequence availability will promote a better understanding of metabolic traits of prokaryotes participating in sulfur, nitrogen and carbon cycles in shallow hydrothermal vents and especially sulfur compound disproportionation and DNRA metabolism. *T. marina* is the latest bacterium described to carry out sulfur compound disproportionation among thermophilic microorganisms. From evolution and adaptation perspectives, this genome sequence will also allow a better understanding of streamlined coding bacterial genomes.

2. Data description

2.1. Genome sequencing and assembly

Cultivation of *Thermosulfurimonas marina* strain SU872^T was performed as described in Frolova et al. (2018), under anaerobic conditions, at 75 °C, with elemental sulfur as an electron donor (5 g/L), nitrate (10 mM) as a terminal electron acceptor and CO₂/HCO₃⁻ as sole carbon source. Genomic DNA was extracted with a standard PCI (Phenol: Chloroform: Isoamyl Alcohol (25:24:1)) protocol, as described elsewhere (Charbonnier et al., 1995). The complete genome sequence of strain SU872^T was determined by combining short and long read sequencing. Short read DNA sequencing was performed by Fasteris SA (Plan-les Ouates, Switzerland) using the Illumina nanoMiSeq technology (2 × 150 bp paired-reads, Nano V2 chemistry). Long read DNA sequencing was done by the company Molecular Research (MrDNA Shallowater, USA), using the PacBio Sequel technology. Libraries constructions and quality controls were performed by both sequencing facilities and verified with FastQC (v0.11.8 - <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>).

All sequences were high quality score estimated and then directly assembled and circularized by using the Unicycler pipeline for *de novo* hybrid assembly (v0.4.8-beta - <https://github.com/rrwick/Unicycler>),

and its dependencies (spades.py v3.13.0; racon v1.3.3; makeblastdb v2.9.0+; tblastn v2.9.0+; bowtie2-build v2.3.5; bowtie2 v2.3.5; samtools v1.9; java v11.0.1; pilon v1.23) (Wick et al., 2017). Genome assembly statistics were obtained with Quast (v5.0.2 - <https://github.com/ablab/quast>). Genome completeness and potential contamination were controlled with CheckM (v1.1.2 - <https://ecogenomics.github.io/CheckM/>), and whole genome average coverage was calculated using BBMap (v38.70 - BBMap - Bushnell B. - sourceforge.net/projects/bbmap/).

2.2. Genome annotation

Genome was analyzed and annotated with the online version of the RAST server (v2.0 - <http://rast.theseed.org/FIG/rast.cgi>), the fast annotation software Prokka (v1.13 - <https://github.com/tseemann/prokka>), Dfast (v1.2.5 - https://github.com/nigyta/dfast_core), the MicroScope Microbial Genome Annotation and Analysis Platform (MaGe) (<https://mage.genoscope.cns.fr/microscope/home/index.php>) (supplementary material S1), using KEGG and BioCyc database, and eggNOG mapper v2 (<http://eggnog-mapper.embl.de/>), with default parameters and databases for all of the five software/pipelines (Seemann, 2014; Brettin et al., 2015; Huerta-Cepas et al., 2016; Vallenet et al., 2017; Tanizawa et al., 2018). Functional annotation of predicted CDSs was further blasted with NCBI (v2.10.0+) and UniProtKB databases (release 2020_02). To investigate sulfur oxygenase reductases, we blasted *Aquifex aeolicus* VF5 sulfur oxygenase reductase (*sor*) sequence (ENA accession: AAC06723.1) against *T. marina*'s genome.

2.3. CRISPRs and genomic islands

Identification and classification of the CRISPR-Cas systems were performed by using the CRISPRCas Finder webserver, with default parameters (<https://crispr.i2bc.paris-saclay.fr/>) (Grissa et al., 2007). The prediction of laterally transferred gene clusters (genomic islands) was performed and plotted with the IslandViewer4 webserver (<http://www.pathogenomics.sfu.ca/islandviewer/>) (Bertelli et al., 2017). Genome visualization plot was carried out with the CGView Server (http://stothard.afns.ualberta.ca/cgview_server/) merged to the IslandViewer4 plot (Grant and Stothard, 2008; Bertelli et al., 2017).

2.4. Genome properties

The complete genome sequence of *T. marina* SU872^T consisted of a single circular chromosome of 1,763,258 bp in length and a G + C content of 58.9 mol%. No plasmids were detected (Fig. 1).

CheckM estimated the genome to be 99.0398% complete (3 markers were missing) and hypothetical contamination to be 0.411523% (1 marker was duplicated). Average coverage was around 116× according to raw pair reads sequences extracted from MiSeq sequencing. Annotation with MaGe (Vallenet et al., 2017) resulted in prediction of 1881 genes, among which 1827 were protein-coding sequences (CDSs). Coding sequences are estimated to cover 95.46% of the entire genome. However, slightly different results were obtained with other annotation software: 1778 CDSs were found with RAST (1298/1778 were not integrated to subsystem categories), 1786 CDSs with Prokka, 1794 CDSs with Dfast and 1674 CDSs with eggNOG mapper.

Genome contained one operon of 5S–16S–23S rRNA genes and 47 tRNA genes for all 20 standard amino acids and selenocysteine. *T. marina* was confirmed to be a new species based on the level of its 16S rRNA gene sequence identity and ANI score with its closest relative *T. dismutans*, which were below the cut-off values for species delineation by these approaches (< 98.7% and < 95–96%, respectively) (supplementary data S2) (Richter and Rosselló-Móra, 2009). No CRISPR loci were found, while five genomic islands (GI) of a total length of 85.8 kb were detected (Fig. 1). The vast majority of genes located on these

islands encoded proteins. Based on automatic annotation, one GI region demonstrated a pattern related to NADH-quinone oxidoreductase, containing subunits A1, B2, C, D1, H, I, J, K, L, M and N. Few diverse genes encoding for carbohydrate and nucleic acid related enzymes with no precise predicted functions were found, while the majority of CDSs encoded for hypothetical proteins. Most of the CDSs obtained from the MaGe annotation pipeline (85.89%, 1560/1827 CDSs) could be assigned to at least one COG group (supplementary data S2).

2.5. Genes related to carbon metabolism

T. marina, which is capable of growing autotrophically from CO₂/HCO₃⁻ (Frolova et al., 2018), possessed a complete Wood-Ljungdahl (reductive acetyl-CoA) pathway for carbon dioxide fixation, as well as *T. dismutans* (Mardanov et al., 2016). Seven enzymes in the pathway (out of eight) were found in KEGG (Reductive acetyl-CoA pathway) and Biocyc databases. The missing enzyme, namely the 5-methyltetrahydrofolate corrinoid/iron sulfur protein methyltransferase (EC: 2.1.1.258), was however detected with Dfast and Prokka annotations. In both *Thermosulfurimonas* species, the TCA cycle seemed highly incomplete and apparently only linking citrate to succinyl-CoA, on the basis of data from KEGG and Biocyc databases. As *T. dismutans*, *T. marina* possessed also the formaldehyde oxidation V (tetrahydrofolate pathway) pathway, but we did not find any evidence for a capacity to oxidize formate into CO₂. Other known pathways for carbon fixation were lacking or partial.

T. marina possessed also a complete metabolic path for gluconeogenesis (Biocyc gluconeogenesis I), the reversal of glycolysis, and the three enzymes of the non-oxidative branch of the pentose phosphate pathway, respectively for the generation of glucose from non-sugar carbon substrates and for NADPH synthesis. *T. marina* possessed also the entire enzymatic set to synthesize one type of carbohydrate, CMP-KDO, a typical component of bacterial lipopolysaccharides.

As for *T. dismutans*, based on BioCyc database, we found several genes encoding for some amino acid biosynthesis in the genome of *T. marina*, namely fifteen amino acid complete biosynthetic pathways. Based on known amino acid biosynthetic pathways, four other pathways appear to be incomplete in this strain which is nevertheless described as autotrophic (Frolova et al., 2018). We also found few genes related to amino acid degradation, six complete degradation pathways and three partial ones.

2.6. Genes related to nitrogen metabolism

As *T. dismutans*, *T. marina* possessed the genes encoding the nitrogenase (molybdenum-iron type) (EC: 1.18.6.1) involved in nitrogen fixation. In this way, based on Prokka annotation, five grouped CDSs were found, including two CDSs related to cofactors, one to its alpha chain and one to its beta chain. Moreover, a periplasmic Nap-type nitrate reductase CDS was found and must be involved in nitrate conversion to nitrite. It has been demonstrated *in vitro* that the conversion of nitrite to ammonium may proceed *via* a non-canonical mode, potentially through the production of hydroxylamine (Hanson et al., 2013; Slobodkina et al., 2017; Slobodkin et al., 2019). The hydroxylamine reductase (EC: 1.7.99.1) found in the genome might be involved in the reduction of hydroxylamine to ammonium. In addition, we also found a hydroxylamine oxidase, a glutamine synthetase, three ammonium transporters associated CDSs and five CDSs coding for nitrogen regulatory proteins P-II with Prokka.

2.7. Genes related to sulfur metabolism

Based on the physiological work done *in vitro*, *T. dismutans* and *T. marina* are not able to reduce sulfate (Slobodkin et al., 2012; Frolova et al., 2018). Surprisingly, complete sulfate reduction pathways are present in both genomes. We found an almost complete dissimilatory

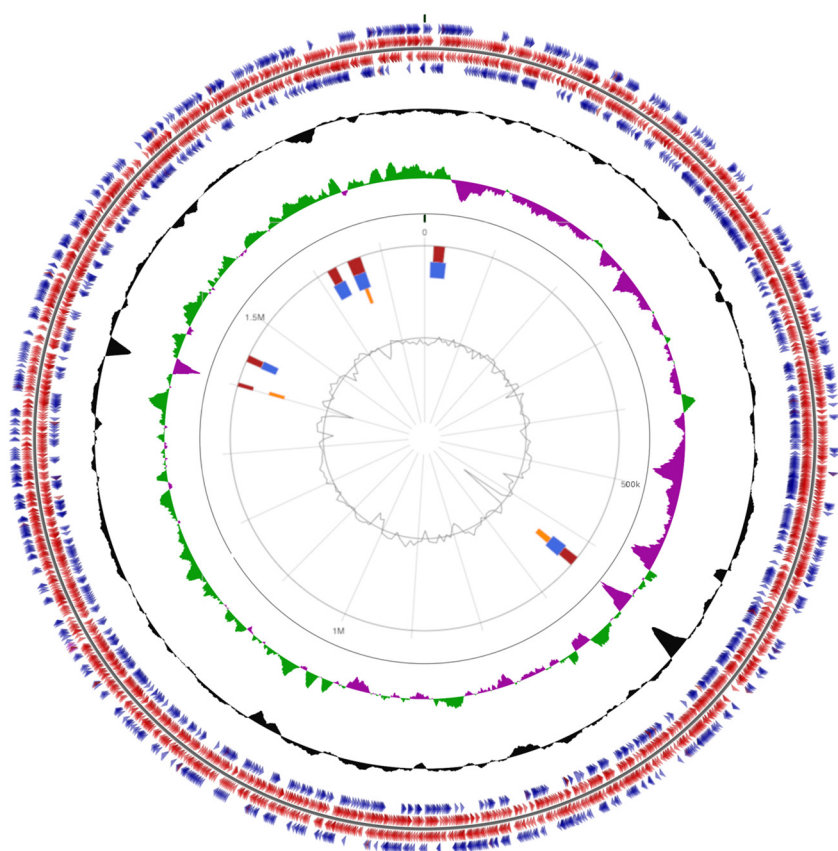


Fig. 1. Schematic representation of *Thermosulfurimonas marina* SU872^T genome. Labeling from the outside to the center is as follows: circle 1, CDSs on the forward and the reverse strand in blue, ORFs on the forward and the reverse strand in red, and tRNAs in pink, rRNAs in lilac; circle 2, G + C content; circle 3, G + C skew; circle 4, genomic islands shown as red, orange and blue rectangles attributed respectively to integrated, SIGI-HMM and IslandPath-DIMOB prediction genomic islands methods; circle 5, IslandViewer4 automatic calculated G + C content. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sulfate reduction pathway based on Prokka annotation, respectively with a sulfate adenylyltransferase (*sat*), both subunits alpha and beta of adenylyl-sulfate reductase (*aprA*, *aprB*), a manganese-dependent inorganic pyrophosphatase, and dissimilatory sulfite reductase subunits alpha, beta and gamma (*dsvA*, *dsvB*, *dsvC*, the respective homologs of DsrA, DsrB, and DsrC). The DsrC-trisulfide reductase, also known as DsrK catalytic subunit from the DsrMKJOP complex, was not detected except with the RAST annotation pipeline, in addition of all each other subunits M, K, J, O and P. A complete APS reductase-associated electron transfer complex (QmoABC) was found with Dfast according to QmoABC operon homologies annotated in Mardanov et al. (2016) study. We found furthermore a dissimilatory sulfite reductase D (DsrD) sequence which could be involved in transcription or translation of genes catalyzing dissimilatory sulfite reduction according to the literature (Mizuno et al., 2003). It can be hypothesized that those enzymes related to dissimilatory sulfate reduction are involved in the inorganic sulfur compound disproportionation pathway. This assumption is supported by the fact that these enzymes are more similar to those present in other sulfur disproportionators (Slobodkin and Slobodkina, 2019). This hypothesis is also reinforced by the finding of a complete hypothetical sulfite oxidation pathway in the genome (adenylylsulfate reductase and sulfate adenylyltransferase), as already found by Finster (2008). None of the marker genes for sulfur oxidation processes (based on the genes cited in the review of Wasmund et al., 2017) had been found in the genome, with any of the five annotation methods used. Furthermore, genes encoding for several hypothetical subunits of tetrathionate reductase were found based on Prokka annotation, namely two alpha subunits and four beta subunits, and also one gene encoding one chain of polysulfide reductase. The proteins encoded by these genes could as well be related to inorganic sulfur compounds disproportionation, or more generally to sulfur metabolism. However, in contrast to *T. dismutans* and to several sulfur disproportionators, *T. marina* did not harbor any thiosulfate reductase in its genome

(Slobodkin and Slobodkina, 2019). Moreover, *T. marina* did not harbor any sequence related to a sulfur oxygenase reductase, suggesting that the enzymes cited before are better candidates for sulfur disproportionation in this bacterial model.

2.8. Conclusion

The whole-genome annotation was generally supporting the main metabolic features demonstrated experimentally for *T. marina* SU872^T (Frolova et al., 2018). However, some pathways related to specific activities (DNRA, sulfur disproportionation) could not be retrieved precisely from genomic data and need further experimental characterization. *T. marina* is the first microorganism originating from a shallow-sea hydrothermal vent to be sequenced, that is known to be able to disproportionate sulfur inorganic compounds with another enzymatic machinery than the sulfur oxygenase reductase. Analysis of genomic data of *T. marina* shows that this bacterium is likely involved in the sulfur and nitrogen cycles in shallow-sea hydrothermal vents.

3. Nucleotide sequence accession number

The complete genome sequence of *Thermosulfurimonas marina* SU872^T has been deposited in GenBank under the accession number CP042909. The strain is available in All-Russian Collection of Microorganisms (VKM) under the accession number VKM B-3177^T.

Declaration of Competing Interest

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.margen.2020.100800>.

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