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SHORT GENOME REPORT

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Partial genome sequence of the haloalkaliphilic soda lake bacterium *Thioalkalivibrio thiocyanoxidans* ARh 2^T

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Abstract

Thioalkalivibrio thiocyanoxidans strain ARh 2^T is a sulfur-oxidizing bacterium isolated from haloalkaline soda lakes. It is a motile, Gram-negative member of the *Gammaproteobacteria*. Remarkable properties include the ability to grow on thiocyanate as the sole energy, sulfur and nitrogen source, and the capability of growth at salinities of up to 4.3 M total Na⁺. This draft genome sequence consists of 61 scaffolds comprising 2,765,337 bp, and contains 2616 protein-coding and 61 RNA-coding genes. This organism was sequenced as part of the Community Science Program of the DOE Joint Genome Institute.

Keywords: Haloalkaliphilic, Soda lakes, Sulfur-oxidizing bacteria, Thiocyanate

Introduction

Soda lakes are found in many arid zones across the world, such as the Kulunda Steppe in Russia, North-Eastern China, the Rift Valley in Africa, and in arid parts of North America, i.e. California and Nevada. The defining characteristics of these lakes are the abundance of carbonate/bicarbonate anions rather than chloride and their moderate to high salinities. This makes soda lakes a unique habitat with stable, alkaline pH values above nine and up to 11 [1]. Despite the high salinity and alkalinity, soda lakes harbor a rich microbial diversity that is responsible for highly active elemental cycles. Aside from the carbon cycle, the sulfur cycle is of great importance in these lakes [2], yet little is known about their precise biogeochemistry and dynamics [3]. A better understanding of these processes will lead to improved insights into the ecology and biogeochemical cycling in soda lakes. Additionally, sulfur-cycling extremophilic prokaryotes have important applications in bioremediation [4] and more detailed knowledge of their physiology may improve industrial waste processing. For these reasons, we have sequenced more than

70 strains belonging to the genus *Thioalkalivibrio*, a dominant cultivated group of chemolithoautotrophic haloalkaliphilic sulfur-oxidizing bacteria in soda lakes worldwide. Here we present the partial genome sequence of *Thioalkalivibrio thiocyanoxidans* ARh 2^T.

Organism information

Classification and features

T. thiocyanoxidans ARh 2^T forms motile vibrio-like cells of approximately 0.5–0.6 by 0.8–1.4 μm (basic properties are summarized in Table 1). The cells grown with thiocyanate as electron source have a remarkably extended periplasm (Fig. 1). It is a Gram-negative bacterium belonging to the *Gammaproteobacteria* (Fig. 2). The species description is based on four strains (ARh 2, ARh 3, ARh 4 and ARh 5) that were isolated from sediment samples of South-Western Siberian, Kenyan and Egyptian soda lakes. Strain ARh 2 is a type strain of the *T. thiocyanoxidans* species. As a chemolithoautotroph, ARh 2^T derives energy from the oxidation of inorganic sulfur compounds, such as sulfide, thiosulfate, thiocyanate, elemental sulfur and polysulfides. The most interesting properties are its ability to grow on thiocyanate as the sole source of energy, sulfur and nitrogen and its ability to grow in saturated soda brines with thiosulfate as energy source [5].

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Table 1 Classification and general features of *Thioalkalivibrio thiocyanoxidans* ARh 2^T [12]

| MIGS ID | Property | Term | Evidence code ^a |
|----------|---------------------|---|----------------------------|
| | Classification | Domain <i>Bacteria</i> | TAS [13] |
| | | Phylum <i>Proteobacteria</i> | TAS [14, 15] |
| | | Class <i>Gammaproteobacteria</i> | TAS [15, 16] |
| | | Order <i>Chromatiales</i> | TAS [15, 17] |
| | | Family <i>Ectothiorhodospiraceae</i> | TAS [18] |
| | | Genus <i>Thioalkalivibrio</i> | TAS [19] |
| | | Species <i>Thioalkalivibrio thiocyanoxidans</i> | TAS [5] |
| | | Type strain: ARh 2 ^T (DSM 13532) | |
| | Gram stain | Negative | TAS [5, 19] |
| | Cell shape | Vibrios | TAS [5] |
| | Motility | Motile | TAS [5] |
| | Sporulation | Non-sporulating | NAS |
| | Temperature range | Mesophilic | TAS [5] |
| | Optimum temperature | 35–37 °C | TAS [5] |
| | pH range; Optimum | 8.5–10.5 | TAS [5] |
| | Carbon source | Inorganic carbon | TAS [5] |
| MIGS-6 | Habitat | Soda lakes | TAS [5] |
| MIGS-6.3 | Salinity | 0.3–4.3 M Na ⁺ | TAS [5] |
| MIGS-22 | Oxygen requirement | Aerobe | TAS [5] |
| MIGS-15 | Biotic relationship | Free-living | NAS |
| MIGS-14 | Pathogenicity | Non-pathogenic | NAS |
| MIGS-4 | Geographic location | Kenya | TAS [5] |
| MIGS-5 | Sample collection | 1999 | TAS [5] |
| MIGS-4.1 | Latitude | Not reported | |
| MIGS-4.2 | Longitude | Not reported | |
| MIGS-4.4 | Altitude | Not reported | |

^aEvidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [20]

Genome sequencing information

Genome project history

Thioalkalivibrio thiocyanoxidans ARh 2^T was sequenced as part of a project aimed at sequencing a large number of *Thioalkalivibrio* isolates. The goal of this project is to enable the study of the genomic diversity of the dominant genus of sulfur-oxidizing bacteria in soda lakes. *T. thiocyanoxidans* ARh 2^T was selected for its ability to grow in salt-saturated brines (4.3 M Na⁺) and for its ability to grow on thiocyanate as the sole energy, sulfur and nitrogen source. The permanent draft genome we present here consists of approximately 2.8 million base-pairs divided over 61 scaffolds. Sequencing was performed at the Joint Genome Institute under project 1008667. The

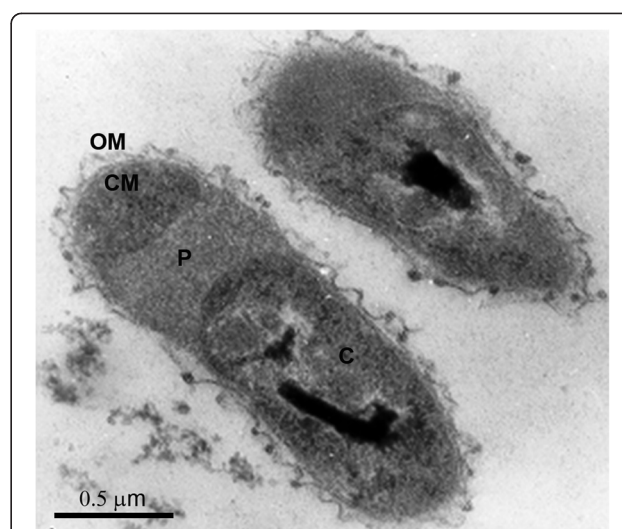


Fig. 1 Thin section electron microscopy photograph of cells of strain ARh 2^T grown with thiocyanate in batch culture at pH 9.8 and 0.6 M total Na⁺. OM - outer cell membrane; CM - cytoplasmic membrane; P - periplasm; C - cytoplasm

genome sequence was released in Genbank on December 25, 2014. An overview of the project is given in Table 2.

Growth conditions and genomic DNA extraction

T. thiocyanoxidans ARh 2^T (DSM 13532) was cultured in a standard buffer containing sodium carbonate and bicarbonate at pH 10. The total salt concentration was 0.6 M Na⁺ [6]. The energy source was thiosulfate, at a concentration of 40 mM. After harvesting, the cells were stored at –80 °C for further processing. Genomic DNA was extracted using a chloroform-phenol-isoamylalcohol mixture and precipitated with ethanol. After vacuum drying, the pellet was dissolved in water and the quantity and quality of the DNA determined using the JGI-provided Mass Standard Kit.

Genome sequencing and assembly

This strain was sequenced as part of the Community Science Program of the US Department of Energy Joint Genome Institute. The Illumina HiSeq 2000 platform was used for sequencing, with a depth of 1819X. More details regarding the library construction and sequencing are available at the JGI website. Reads were filtered using DUK and assembled using Velvet 1.1.04 [7]. Pseudoreads (1–3 Kb) were generated from the Velvet output using wgsim and reassembled using ALLPATHS-LG r42328 [8]. The final assembly consists of 61 scaffolds.

Genome annotation

Genes were predicted using Prodigal [9], followed by a round of manual curation using GenePRIMP [10] to detect pseudogenes. The resulting predicted genes were

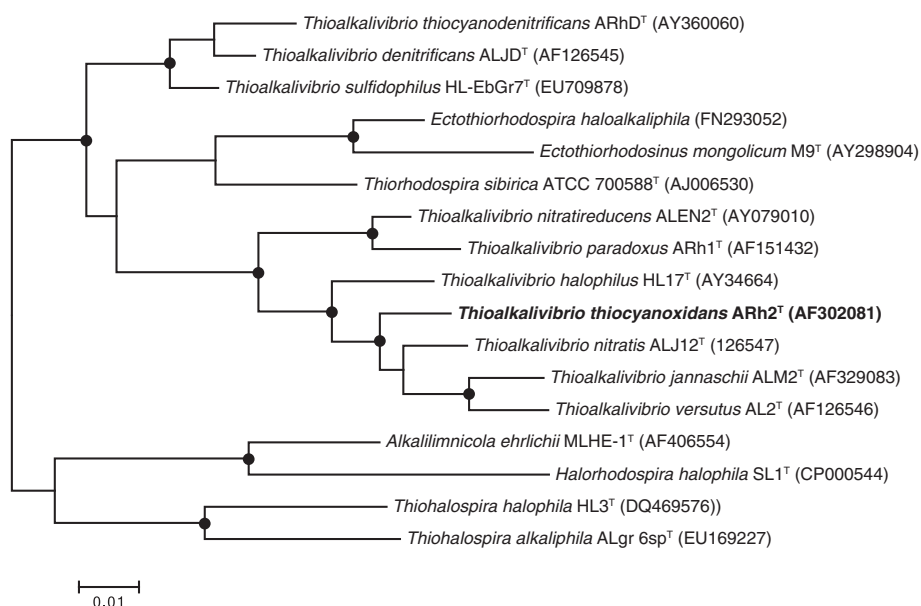


Fig. 2 Phylogenetic tree based on 16S rRNA sequences comprising the *Thioalkalivibrio* type strains and several other members of the *Ectothiorhodospiraceae* family. Black dots mark nodes with a bootstrap value between 90 and 100 %. 16S rRNA sequences of members of the *Alphaproteobacteria* were used as the outgroup, but pruned from the tree. The tree was constructed using ARB [21] and bootstrap values calculated using MEGA6 [22]

translated and annotated using the NCBI NR database in combination with the UniProt, TIGRFam, Pfam, KEGG, COG and InterPro databases and tRNAScanSE [11] for tRNA prediction. Ribosomal RNAs were detected using models built from SILVA. Further annotation was performed using the Integrated Microbial Genomes platform. All annotation data is freely available there, with IMG submission ID 12214.

Genome properties

The final draft of the genome comprises 2.8 million base pairs in 61 scaffolds, with a G + C percentage of 66.18 %. The gene calling and annotation pipeline detected 2677 genes, of which 2616 code for proteins. Basic statistics concerning the genome sequence are shown in Table 3. In total, 70 % of the genes could be assigned functional categories based on COGs (see Table 4).

Table 2 Project information

| MIGS ID | Property | Term |
|-----------|----------------------------|--|
| MIGS 31 | Finishing quality | Improved high-quality draft |
| MIGS-28 | Libraries used | Illumina standard fragment, 270 bp |
| MIGS 29 | Sequencing platforms | Illumina HiSeq 2000 |
| MIGS 31.2 | Fold coverage | 1819 |
| MIGS 30 | Assemblers | Velvet 1.1.04 [7], ALLPATHS R39750 [8] |
| MIGS 32 | Gene calling method | Prodigal [9], GenePRIMP [10] |
| | Locus Tag | G372 |
| | Genbank ID | ARQK00000000 |
| | GenBank Date of Release | 2014-12-25 |
| | GOLD ID | Gp0025980 |
| | BIOPROJECT | PRJNA185302 |
| | IMG submission ID | 12214 |
| MIGS 13 | Source Material Identifier | DSM 13532 |
| | Project relevance | Biotechnology |

Table 3 Genome statistics

| Attribute | Value | % of Total |
|----------------------------------|----------------|----------------|
| Genome size (bp) | 2,765,337 | 100.00 |
| DNA coding (bp) | 2,496,809 | 90.29 |
| DNA G + C (bp) | 1,829,984 | 66.18 |
| DNA scaffolds | 61 | 100.00 |
| Total genes | 2677 | 100.00 |
| Protein coding genes | 2616 | 97.72 |
| RNA genes | 61 | 2.28 |
| Pseudo genes | Not determined | Not determined |
| Genes in internal clusters | Not determined | Not determined |
| Genes with function prediction | 2230 | 83.30 |
| Genes assigned to COGs | 1885 | 70.41 |
| Genes with Pfam domains | 1799 | 78.94 |
| Genes with signal peptides | 217 | 8.11 |
| Genes with transmembrane helices | 655 | 24.47 |
| CRISPR repeats | 1 | 100.00 |

Table 4 Number of genes associated with the 25 general COG functional categories

| Code | Value | % age | Description |
|------|-------|-------|--|
| J | 148 | 7.09 | Translation, ribosomal structure and biogenesis |
| A | 1 | 0.05 | RNA processing and modification |
| K | 70 | 3.36 | Transcription |
| L | 98 | 4.70 | Replication, recombination and repair |
| B | 2 | 0.10 | Chromatin structure and dynamics |
| D | 32 | 1.53 | Cell cycle control, Cell division, chromosome partitioning |
| V | 29 | 1.39 | Defense mechanisms |
| T | 105 | 5.03 | Signal transduction mechanisms |
| M | 153 | 7.33 | Cell wall/membrane biogenesis |
| N | 73 | 3.50 | Cell motility |
| U | 72 | 3.45 | Intracellular trafficking and secretion |
| O | 109 | 5.23 | Posttranslational modification, protein turnover, chaperones |
| C | 148 | 7.09 | Energy production and conversion |
| G | 82 | 3.93 | Carbohydrate transport and metabolism |
| E | 145 | 6.95 | Amino acid transport and metabolism |
| F | 60 | 2.88 | Nucleotide transport and metabolism |
| H | 131 | 6.28 | Coenzyme transport and metabolism |
| I | 86 | 3.02 | Lipid transport and metabolism |
| P | 105 | 5.03 | Inorganic ion transport and metabolism |
| Q | 37 | 1.77 | Secondary metabolites biosynthesis, transport and catabolism |
| R | 228 | 10.93 | General function prediction only |
| S | 195 | 9.35 | Function unknown |
| - | 792 | 29.59 | Not in COGs |

The total is based on the total number of protein coding genes in the genome

Conclusions

Sequencing of the genome of *Thioalkalivibrio thiooxidanans* ARh 2^T is an important step towards a more comprehensive understanding of the mechanism by which this organism can adapt to extremely high salinity. In addition, it will provide important information on the role of this organism in the carbon and sulfur cycles of natural and engineered environments, in particular in the degradation of thiocyanate.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TB is responsible for drafting the manuscript and – together with GM and DS – for discussion and revisions. DS grew the organism and extracted the DNA. Sequencing and annotation were performed by NI, AP, NK, LAG and TW. All authors approve of the final version.

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