Thermodesulfatator autotrophicus sp. nov., a thermophilic sulfate-reducing bacterium from the Indian Ocean

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A novel sulfate-reducing bacterium, strain S606T, was isolated from a sulfide sample collected at a depth of 2764 m from a deep-sea vent chimney wall in the Indian Ocean. Phylogenetic 16S rRNA gene sequence analyses placed strain S606T within the genus Thermodesulfatator, with highest sequence similarity of 98.2 % to Thermodesulfatator indicus DSM 15286T, followed by Thermodesulfatator atlanticus AT1325T (97.4 %). The average nucleotide identity (ANI) values between S606T and the two other type strains (T. indicus DSM 15286T and T. atlanticus AT1325T) were 79.2 % and 71.5 %, respectively. The digital DNA–DNA hybridization estimate values between S606T and these two type strains were 22.7±2.4 % and 18.1±2.3 %, respectively. Cells were Gram-stain-negative, anaerobic, motile rods (1–1.8×0.5–0.7 µm). The novel isolate grew at NaCl concentrations ranging from 1.5 to 4.5 % (optimum 2.5–3 %), from pH 5.5 to 8 (optimum 6.5–7.0) and at temperatures between 50 and 80 °C (optimum 65–70 °C). S606T grew chemolithoautotrophically in an H2/CO2 atmosphere (80:20, v/v; 200 kPa), used sulfate as a terminal electron acceptor, but not sulfur, sulfite nor thiosulfate. The predominant fatty acids were C16:0 (24.2 %), summed feature 8 (C18:1ω6c and/or C18:1ω7c, 26.3 %), C18:0 (22.2 %) and C18:1ω9c (9.2 %). The DNA G+C content of the chromosomal DNA was 43.1 mol%. The combined genotypic, chemotaxonomic and phenotypic traits show that S606T should be described as representing a novel species of the genus Thermodesulfatator, for which the name Thermodesulfatator autotrophicus sp. nov. is proposed. The type strain is S606T (=DSM 101864T =MCCC 1A01871T).

†These authors contributed equally to this work.
The GenBank accession number for the 16S rRNA gene sequence of Thermodesulfatator autotrophicus S606T is KU681513.
Three supplementary figures and one supplementary table are available with the online Supplementary Material.
Sulfate-reducing prokaryotes (SRP) are anaerobic microorganisms, including both bacteria and archaea, that use sulfate as a terminal electron acceptor in their energy metabolism and hydrogen or various organic acids as energy sources (Heidelberg et al., 2004). Currently, the genus *Thermodesulfatator*, within the family *Thermodesulfobacteriaceae*, comprises two species, *Thermodesulfatator indicus* (Moussard et al., 2004) and *Thermodesulfatator atlanticus* (Alain et al., 2010), both of which have been isolated from deep-sea hydrothermal systems. Members of the genus *Thermodesulfatator* are sulfate-reducing chemolithoautotrophs using hydrogen as the sole electron donor and carbon dioxide as a carbon source. In addition, *T. atlanticus* has been reported to be able to use several organic compounds as carbon sources (methylamine, peptone and yeast extract) (Alain et al., 2010). In this study, we report on the isolation of a novel thermophilic sulfate-reducer, strain S606\textsuperscript{T}, belonging to the genus *Thermodesulfatator*. The characteristics of this strain indicate that it represents a novel species of the genus *Thermodesulfatator*.

In January 2015, a deep-sea sulfide sample was collected at a depth of 2771 m from a chimney wall in the Indian Ocean (37° 78′ S, 49° 65′ E; site JL-Dive90-S01), during the DY35 cruise of Xiang Yang Hong Jiu Hao. The sample was collected using a benthic seabed grab and anaerobically preserved in sterilized seawater at 4°C onboard. Once in the laboratory, a subsample was used to inoculate a SO4PNsalts medium (Alain et al., 2010), prepared with a gas phase of H\textsubscript{2}/CO\textsubscript{2} (80 : 20, v/v, 200 kPa) and incubated at 60°C. After 5 days of incubation, populations of short bacilli were observed. The enrichments were subcultured under the same conditions, and purified by six repeated dilutions-to-extinction series. One pure culture, strain S606\textsuperscript{T}, is described in this study. The purity of this isolate was checked routinely by microscopic examination, by repeated partial sequencing of the 16S rRNA gene using four different primers and by sequencing of its genome. Stock cultures were stored at −80°C with 5% (v/v) DMSO.

Genomic DNA was extracted with the QIAGEN Genomic-tip 20/G (QIAGEN) kit following the manufacturer’s standard protocol. The 16S rRNA gene sequence was determined by the Sanger method using the primers Bac8F (5′-AGA GTT TGA TCA TGG CTC AG-3′), S8dir (5′-GTA CCG GTG AAA TGC GTA GA-3′), U1492R (5′-GTT TAC CTT GTG ACT T-3′) and W34 (5′-TTA CCG CCG CTG CTG GCA C-3′). Pairwise 16S rRNA gene sequence similarity was calculated using global alignment algorithm implemented at the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; (Kim et al., 2012). Phylogenetic 16S rRNA gene sequence analysis was performed using the software MEGA version 5.0 (Tamura et al., 2011). Distances were calculated using the Kimura two-parameter model and clustering was performed with the neighbor-joining algorithm (Saitou & Nei, 1987). The robustness of the inferred topology was assessed by bootstrap analyses based on 1000 replications.

A nearly full-length 16S rRNA gene sequence (1514 bp) of strain S606\textsuperscript{T} was obtained. The 16S rRNA gene-based analysis located the novel isolate within the class *Thermodesulfobacteria*, in the bacterial domain. Comparative 16S rRNA gene sequence analysis showed that strain S606\textsuperscript{T} formed a cluster with the members of the genus *Thermodesulfatator*, within the family *Thermodesulfobacteriaceae* (Figs 1, S1 and S2, available in the online Supplementary Material). S606\textsuperscript{T} shared the highest sequence similarity of 98.2% with *Thermodesulfatator indicus* DSM 15286\textsuperscript{T}, followed by *Thermodesulfatator atlanticus* AT1325\textsuperscript{T} (97.4%) and *Thermosulfurimonas dismutans* S95\textsuperscript{T} (91.3%).

The draft genome sequence of strain S606\textsuperscript{T} was determined by Shanghai Majorbio Bio-pharm Technology (Shanghai, China), using Solexa paired-end (500 bp library) sequencing technology. The genome sequences of *T. indicus* DSM 15286\textsuperscript{T}.

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**Fig. 1.** Neighbor-joining tree showing the phylogenetic positions of strain S606\textsuperscript{T} and all species of the family *Thermodesulfobacteriaceae*, based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch nodes. Filled circles indicate nodes that were also recovered in the maximum-likelihood (Fig. S1) and minimum-evolution (Fig. S2) trees for the same sequences. Bar, 0.01 nucleotide substitution rate (K\textsubscript{sub}) units. *Thermosulfidibacter takaii* ABI70S6\textsuperscript{T} (AB282756) was used as an outgroup.
were obtained from Alain et al. (2012) and T. atlanticus AT1325T (NZ_ATXH01000001, unpublished) were downloaded from NCBI. The G+C content of the chromosomal DNA was determined from the draft genome sequence. The average nucleotide identity (ANI) was calculated using the algorithm of Goris et al. (2007) using the EZGenome web service. DNA–DNA hybridization (DDH) estimate values were analyzed using the genome-to-genome distance calculator (GGDC2.0) (Auch et al., 2010a, b; Meier-Kolthoff et al., 2013). A total of 500 Mbp clean data for each strain were generated to reach about 100-fold depth of coverage with an Illumina/ Solexa Genome Analyzer IIx (Illumina). The clean data were assembled using SOAPdenovo2 (Luo et al., 2012). The accession number for the draft genome sequence of S606 is SFI00000000. The DNA G+C content of S606 was 43.1 mol %, which was close to the contents of the other two type strains of the species of the genus Thermodesulfatator (42.4%–45.0%). Some other general features of the genomes of the three strains (genome size, number of genes, number of protein-coding genes, number of tRNA genes and number of rRNA genes) are summarized in Table 1. The ANI values between S606 and the two reference type stains (T. indicus DSM 15286T and T. atlanticus AT1325T) were, respectively, 79.2% and 71.5%, which are below the standard ANI criterion (95–96%) (Richter & Rossello-Mora, 2009). The digital DNA–DNA hybridization estimate values between strain S606 and two reference strains were 22.7 ±2.4% and 18.1±2.3%, respectively, which are far below the standard criterion (70%) for delineation of prokaryotic species (Wayne et al., 1987). These results confirmed that strain S606 represents a novel genomic species of the genus Thermodesulfatator.

Morphological characteristics of cells of strain S606 were examined by using light microscopy (BX60 and CX40, Olympus) and transmission electron microscopy (100 CXII, JEOL). Cells were motile, rods (1.0–1.8 µm in length and 0.5–0.7 µm in width, n=10, Fig. 53) that occurred singly, with a single polar flagellum. Some cells became spherical in the late-stationary growth phase. They stained Gram-negative.

Unless otherwise stated, physiological characterization was performed anaerobically in SO4PNsalts medium (Alain et al., 2010), in duplicate, using sulfate as a terminal electron acceptor, and a gas phase of H2/CO2 (80/20, v/v, 200 kPa) as energy and carbon sources. Growth experiments were generally performed as described previously (Alain et al., 2003). The utilization of organic compounds as carbon sources (methylamine, peptone and yeast extract) was tested as described previously (Alain et al., 2010). Growth of the isolate was routinely monitored by direct cell counting by using a modified Thoma chamber (depth 10 µm). Determination of the temperature range for growth was performed at 45, 50, 55, 60, 65, 70, 75, 80 and 85 °C. The isolate grew at 50–80 °C (optimum 65–70 °C). Salt tolerance was tested at 65 °C with various concentrations of NaCl (0, 0.2, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0%, w/v). Growth of S606 occurred at NaCl concentration between 1.5 and 4.5% (optimum: 2.5–3%). The pH range for growth was tested from pH 5.0 to pH 9.0 (initial pH at 20 °C) with increments of 0.5 pH units. Growth occurred between pH 5.5 and pH 8.0, the optimum being around pH 6.6–7.0.

The novel isolate was a strictly anaerobic bacterium, using hydrogen, carbon dioxide and sulfate as primary electron donor, carbon source and electron acceptor, respectively. It could not grow heterotrophically on methylamine, peptone or yeast extract. The ability to use alternative electron acceptors was tested in SO4PNsalts medium depleted of

Table 1. Differential characteristics of strain S606T and related species of the genus Thermodesulfatator

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>Length (µm)</td>
<td>1–1.8</td>
<td>1.04–6.08</td>
<td>0.8–1</td>
</tr>
<tr>
<td>Width (µm)</td>
<td>0.5–0.7</td>
<td>0.3–0.75</td>
<td>0.4–0.5</td>
</tr>
<tr>
<td>Growth temperature (°C) (optimal)</td>
<td>50–80 (65–70)</td>
<td>55–75 (65–70)</td>
<td>55–80 (70)</td>
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<tr>
<td>pH (optimal)</td>
<td>5.5–8.0 (6.5–7.0)</td>
<td>5.5–8.0 (6.5–7.5)</td>
<td>6.0–6.7 (6.25)</td>
</tr>
<tr>
<td>NaCl (%) (optimal)</td>
<td>1.5–4.5 (2.5–3.0)</td>
<td>1.5–4.5 (2.5)</td>
<td>1.0–3.5 (2.5)</td>
</tr>
<tr>
<td>Utilization of organic compounds (methylamine, peptone and yeast extract)</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Genome size (Mb)</td>
<td>2.27</td>
<td>2.30</td>
<td>2.32</td>
</tr>
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<td>Number of genes</td>
<td>225</td>
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<td>6</td>
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<tr>
<td>Number of tRNA genes</td>
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<td>45</td>
<td>49</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>43.1</td>
<td>45.6 (45.0)*</td>
<td>46 (42.4)*</td>
</tr>
</tbody>
</table>

*Data were obtained from the genome sequence.
sulfate but supplemented with elemental sulfur (12 g l⁻¹), sulfite (2 mM), thiosulfate (20 mM), nitrate (10 mM), nitrite (2 mM) or oxygen (1 % v/v). Hydrogen sulfide production was followed as described previously (Cord-Ruwisch, 1985). Sulfate was used as a terminal electron acceptor, but not sulfur, sulfite, nitrate nor thiosulfate. When using sulfate as a terminal electron acceptor, S606ᵀ grew exclusively on hydrogen as an energy source.

The whole-cell fatty acid contents of S606ᵀ were analyzed during the late-exponential phase of growth cultures grown chemolithoautotrophically with H₂, CO₂ and sulfate as respectively electron donor, carbon source and electron acceptor. The cellular fatty acids were saponified, methylated and extracted using the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0B). The fatty acids were analyzed by GC (model 6850, Agilent Technologies) and identified by using the TSBA6.0 database of the Microbial Identification System (Sasser, 1990). The principal fatty acids were C₁₆:0 (24.2 %), summed feature 8 (C₁₈:1ω₆c/C₁₈:ω₇c, 26.3 %), C₁₈:0 (22.2 %) and C₁₈:ω9c (9.2 %). The minor fatty acids are listed in Table S1 (available in the online Supplementary Material). These data indicate that the three stains of members of the genus Thermodesulfatator contain C₁₆:0 summed feature 8 and C₁₈:0 as main fatty acids, confirming the affiliation of strain S606ᵀ to the genus Thermodesulfatator.

S606ᵀ is a thermophilic, strictly anaerobic bacterium, growing exclusively chemolithoautotrophically with hydrogen as the sole electron donor and sulfate as the sole electron acceptor. Its phenotypic and genotypic properties generally meet the characteristics described for member of the genus Thermodesulfatator (Moussard et al., 2004). Based on a combination of the results of the phylogenetic, phenotypic and chemotaxonomic analyses described in this article, strain S606ᵀ should be assigned to the genus Thermodesulfatator in the family Thermodesulfobacteriaceae. Nevertheless, the novel isolate can be distinguished from the most closely related species on the basis of some phenotypic and chemotaxonomic characteristics (shown in Table 1 and Table S1), and their low ANI and DDH values. Therefore, based on the polyphasic data, we propose that S606ᵀ represents a novel species, for which the name Thermodesulfatator autotrophicus sp. nov. is proposed.

**Description of Thermodesulfatator autotrophicus sp. nov.**

*Thermodesulfatator autotrophicus* (au.to.tro’phi.cus. N.L. masc. adj. *autotrophicus* autotroph). Strictly anaerobic. Cells are Gram-stain-negative, motile rods (1.0–1.8 x 0.5–0.7 µm) with a single polar flagellum. Growth occurs at salinities from 1.5 to 4.5 % (optimum 2.5–3 %), from pH 5.5 to 8.0 (optimum 6.5–7.0), and at temperatures between 50 and 80 °C (optimum 65–70 °C). Strictly chemolithoautotrophic using sulfate as a terminal electron acceptor and hydrogen as an electron donor. The predominant fatty acids are C₁₆:0 summed feature 8 (C₁₈:1ω6c and/or C₁₈:1ω7c), C₁₈:0 and C₁₈:ω9c.

The type strain S606ᵀ (=DSM 101864ᵀ=MCC8 1A01871ᵀ) was isolated from a deep-sea hydrothermal vent sulfate sample collected at a depth of 2764 m from a chimney wall in the Indian Ocean (37°78’S, 49°65’E; site JL-Dive90-01). The DNA G+C content of the type strain is 43.1 mol %.

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**References**


