Thermodesulfitimonas autotrophica gen. nov., sp. nov., a thermophilic, obligate sulfite-reducing bacterium isolated from a terrestrial hot spring

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Abstract

A novel thermophilic, anaerobic, chemolithoautotrophic bacterium, strain SF97T, was isolated from a terrestrial hot spring (Kuril Islands, Russia). Cells of strain SF97T were rod-shaped and motile with a Gram-positive cell-wall type. The novel isolate grew at 45–72 °C (optimum 65 °C) and pH 5.5–8.5 (optimum 6.0–6.5). The strain grew chemolithoautotrophically with molecular hydrogen as an electron donor, sodium sulfite or SO₂ gas as an electron acceptor and bicarbonate/CO₂ as a carbon source. Sulfate, thiosulfate, elemental sulfur, Fe(III) or nitrate were not used as electron acceptors either with H₂ or organic electron donors. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate belonged to the family Thermoaerobacteraceae, order Thermoanaerobacterales, and was distantly related to species of the genus Ammonifex (92–93 % sequence similarity). On the basis of its physiological properties and results of phylogenetic analyses, strain SF97T is considered to represent a novel species of a new genus, for which the name Thermodesulfitimonas autotrophica gen. nov., sp. nov. is proposed. The type strain of Thermodesulfitimonas autotrophica is SF97T (=DSM 102936T =VKM B-2961T). T. autotrophica is the first reported obligate sulfite-reducing micro-organism.

The dissimilatory metabolism of sulfur compounds is considered as one of the earliest energy-yielding processes to sustain life [1]. In contrast to elemental sulfur and sulfate, which do not have a direct volcanic origin, sulfur dioxide is emitted by volcanic and hydrothermal sources and in modern geothermal environments it is one of the most typical and abundant volcanic gases. Due to its high solubility in water, in aquatic ecosystems SO₂ is present in the form of the sulfite ion. Prokaryotes capable of dissimilatory sulfite reduction are phylogenetically diverse and include a vast number of the sulfate-reducing bacteria and archaea, as well as many non-sulfate-reducing micro-organisms [2, 3]. The majority of sulfite reducers use organic compounds as electron donors for SO₂⁻ reduction and are incapable of autotrophic growth. Among non-sulfate-reducing micro-organisms, the ability to grow chemolithoautotrophically coupled with dissimilatory sulfite reduction and utilization of inorganic compounds such as molecular hydrogen or carbon monoxide has been reported for the bacteria Sulforhodobacter azorense and Calderihabitans maritimus and archaea Archaeoglobus veneficus and Pyrobaculum islandicum [4–7]. However, all these micro-organisms are not obligately dependent on sulfite and can use alternative electron acceptors. In this paper, we describe the isolation and characterization of a novel chemolithotrophic thermophilic bacterium (strain SF97T) which has very limited metabolic capabilities and is able to use only H₂ or formate as electron donors and sulfite as a sole electron acceptor. To our knowledge, strain SF97T is the first reported obligate sulfite-reducing micro-organism.

Strain SF97T was isolated from a mixed sample of sediment and water collected from a hot spring at Golovnin Caldera, Kunashir Island, Kurils, Russia. Temperature and pH values at the sampling site (43° 51.857’ N 145° 30.084’ E) were 67 °C and 6.0, respectively. Samples were taken anaerobically in tightly stopped bottles and transported to the laboratory. An enrichment culture was initiated by inoculation of the sample (10 %, w/v) into anaerobically prepared, bicarbonate-buffered liquid medium of the following composition (per litre of distilled water): 0.33 g NH₄Cl, 0.33 g KCl, 0.33 g CaCl₂·6H₂O, 0.33 g KH₂PO₄, 0.33 g MgCl₂·6H₂O, 2.0 g NaHCO₃, 1 ml trace element solution [8] and 1 ml vitamin solution [9]. The isolation medium had a pH of 6.5–6.8 (measured at 25 °C). No reducing agents were added. The medium was dispensed in 10 ml portions into
17 ml Hungate tubes. If not mentioned otherwise, the gas phase consisted of H₂/CO₂ (80:20, v/v). Sodium sulfite from a sterile stock solution was added as an electron acceptor to a final concentration of 5 mM. A sterile anoxic stock slurry of poorly crystalline Fe(III) oxide (ferrihydrite) was added to obtain final concentrations of 10 mmol Fe(III) l⁻¹, as a scavenger of sulfide generated by sulfite reduction. The ferrihydrite was synthesized by titrating a solution of FeCl₃ • 6H₂O (60 g l⁻¹) with NaOH (10 %, w/v) to pH 8.0–9.0. After incubation of the enrichment at 65°C for 5 days, ferrihydrite was converted to a black non-magnetic Fe(II)-containing precipitate, presumably FeS. After three subsequent transfers and following serial 10-fold dilutions in the same medium, only one morphological type of the bacterial cells was observed in the highest growth-positive dilution (10⁻⁷). Attempts to obtain separate colonies were unsuccessful either with 1 % Gelrite gellan gum or with 1 % agar as the solidifying agent in the medium with or without ferrihydrite. A pure culture of strain SF97T was obtained by means of multiple serial dilutions-to-extinction in the same liquid anaerobic medium. Light and electron microscopy, physiological studies on substrate and electron acceptor utilization, temperature, pH and salinity ranges for growth, analytical techniques for determination of metabolic products, determination of DNA G+C content and analysis of cellular fatty acid composition were performed as described previously [10]. Genomic DNA of the isolate was extracted using the method of Marmur [11] and purified using ‘Wizard Maxi-Preps DNA Purification Rezin’ (Promega). 16S rRNA gene amplification and sequencing were done as described previously [12]. Pairwise similarity values were calculated by means of the EzTaxon server (www.ezbiocloud.net/eztaxon; [13]). Alignment with a representative set of related 16S rRNA gene sequences, evolutionary analysis and phylogenetic tree reconstruction were performed as described previously [10].

Cells of strain SF97T were straight rods with rounded ends, 0.5–0.6 µm in diameter and 1.0–2.0 µm in length. Cultures consisted of mixtures of single cells, pairs and short chains containing 5–10 cells. The rods were motile and exhibited lateral flagellation, with up to four flagella per cell (Fig. 1a). Electron micrographs of thin sections of cells indicated a cell wall typical of Gram-positive bacteria (Fig. 1b, c). Spores were not observed for cultures that had been grown under optimal or suboptimal conditions (different pH, temperature and salinity). In addition, cultures incubated at 100°C for 20 min or at 121°C for 60 min could not be further subcultured, suggesting the absence of heat-resistant bodies such as spores. The temperature range for growth of strain SF97T was 45–72°C with an optimum at 65°C. No growth was detected at 42 or 75°C after incubation for 3 weeks. The pH range for growth was 5.5–8.5, with optimum growth at pH 6.0–6.5. No growth was detected at pH

Fig. 1. Cell morphology of strain SF97T. (a) Electron micrograph of negatively stained cells showing overall cell morphology and localization of flagella. (b) Ultrathin section showing the cell-wall structure. Bar, 0.3 µm. (c) Enlargement of (b) showing the cell-wall ultrastructure with a cytoplasmic membrane (1), an electron-dense cell-wall layer adjacent to the membrane (2) and an outer cell-wall layer composed of regular subunits (3). Bar, 0.1 µm.
5.0 or 8.8. Isolate SF97<sup>T</sup> did not require NaCl for growth. It grew in the culture medium at salt concentrations of up to 1.0 % (w/v) NaCl, but optimal growth was obtained in the absence of NaCl. No growth was observed at and above 1.5 % (w/v) NaCl.

Strain SF97<sup>T</sup> grew lithoautotrophically with molecular hydrogen as an electron donor, sodium sulfite as an electron acceptor and bicarbonate/CO<sub>2</sub> as a carbon source. The doubling time of strain SF97<sup>T</sup> under optimal growth conditions was around 3.7 h. The final cell concentration was in the range 4.0 – 6.0 × 10<sup>7</sup> cells ml<sup>–1</sup>. Sulfite could be replaced by SO<sub>2</sub> gas [SO<sub>2</sub>/CO<sub>2</sub> (15:85, v/v) in the gas phase] that resulted in growth with the same specific growth rate and final cell yield. Sulfite or SO<sub>2</sub> gas reduction was accompanied by hydrogen consumption and formation of hydrogen sulfide; the presence of ferrihydrite as a scavenger of sulfide was not essential. Among the potential electron donors tested with sulfite as electron acceptor, only formate (10 mM) was utilized, while no growth was observed with carbon monoxide (100 and 5 % in the gas phase), methyamine, dimethylamine, trimethylamine (5 mM each), acetate, propionate, butyrate, methanol, ethanol (10 mM each), glycerol, glucose, fructose (2 g l<sup>–1</sup> each), lactate, pyruvate, fumarate, malate, oxalate, succinate (10 mM each), yeast extract or peptone (2 g l<sup>–1</sup> each). In the presence of H<sub>2</sub> or formate, CO<sub>2</sub> and sulfite, the lag phase was decreased when malate or pyruvate (10 mM each) was added to the culture medium; the final cell concentration was not changed. Addition of glucose, peptone (2 g l<sup>–1</sup>) or yeast extract (0.2 g l<sup>–1</sup>) had no effect on growth. The new isolate did not grow and did not reduce sulfate (15 mM), elemental sulfur (5 g l<sup>–1</sup>) or ferrihydrite (90 mmol Fe(III) l<sup>–1</sup>) with hydrogen [H<sub>2</sub>/CO<sub>2</sub> (80:20, v/v)], formate, acetate, lactate, pyruvate, ethanol (10 mM each) and peptone (2 g l<sup>–1</sup>). It also did not grow and did not reduce nitrate (10 mM), nitrite (2.5 mM), thiosulfate (15 mM), fumarate (10 mM) or oxygen (2.0 or 20 %, v/v, in the gas phase) with hydrogen or formate as an electron acceptor.

**Fig. 2.** Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain SF97<sup>T</sup> among related Firmicutes species. The tree was reconstructed using the maximum-likelihood method. Trees reconstructed with the neighbour-joining, minimum-evolution and maximum-parsimony algorithms displayed the same topology. Each number indicates the bootstrap value from 500 trials. Bar, 0.02 substitutions per nucleotide position. GenBank accession numbers are given in parentheses.
electron donor. The isolate did not grow by oxidizing elemental sulfur (5 g l⁻¹), thiosulfate (10 mM) or sulfite (5 mM) with nitrate (10 mM) or oxygen (2.0 or 20 %, v/v, in the gas phase). Strain SF97T did not disproportionate elemental sulfur (5 g l⁻¹), thiosulfate (10 mM) or sulfite (5 mM) in the presence as well as in the absence of ferricydrate as a scavenger of sulfide.

Cells of strain SF97T for determination of cellular fatty acids were grown in the medium with formate and sulfite and harvested in the late exponential phase of growth. Cellular fatty acid analysis demonstrated a dominance of C₁₆:0 (56.2 %) with lower amounts of C₁₈:0 (14.9 %), iso-C₁₆:0 (8.2 %) and anteiso-C₁₅:0 (7.7 %). Other fatty acids were present in low or trace amounts (<5 %). The G+C content of the genomic DNA of strain SF97T was 55.8 mol% (Tm).

A comparison of a stretch of 1427 nt of the 16S rRNA gene sequence of strain SF97T with those available in GenBank and EzTaxon server databases showed that the strain belonged to the family Thermoanaerobacteraceae (Fig. 2), displaying highest sequence similarity to the type strains of Ammonifex thiophilus (93.3 %) [14] and Ammonifex degensii (92.7 %) [15] and being only distantly related (<90 % 16S rRNA gene sequence similarity) to the representatives of the other genera of the phylum Firmicutes.

Strain SF97T was isolated from a terrestrial hot spring where it participates in carbon cycling, most probably as a primary producer. The metabolic requirements of this bacterium could be fulfilled by only three volcanic gases, H₂, SO₂ and CO₂. To our knowledge, strain SF97 is the first known obligate sulfite-reducing micro-organism. The phylogenetic position of the novel isolate is within the family Thermoanaerobacteraceae of the order Thermoanaerobacterales, phylum Firmicutes. This family comprises several genera of thermophilic, strictly anaerobic, rod-shaped bacteria, which were isolated from terrestrial hot springs, deep subsurface environments such as mines, oil and gas reservoirs, and various anthropogenic habitats. The metabolism of Thermoanaerobacteraceae is variable; the family contains mainly heterotrophic, carbohydrate-fermenting members, but also several chemolithoautotrophic species [16]. Similar to representatives of the genus Ammonifex, strain SF97T is a rod-shaped, neutrophilic, thermophilic, chemolithoautotrophic bacterium isolated from a freshwater hot spring (Table 1). Like Ammonifex strains, it utilizes hydrogen or formate as an electron donor. The main physiological feature that differentiates strain SF97T from Ammonifex species is the inability to accomplish dissimilatory sulfate reduction. In addition, strain SF97T has a lower growth temperature and different pattern of utilization of electron acceptors.

On the basis of phylogenetic position and phenotypic properties, we propose to classify strain SF97T as the type strain of a novel species of a new genus in the family Thermodesulfimonas autotrophica gen. nov., sp. nov.

**DESCRIPTION OF THERMODESULFITIMONAS GEN. NOV.**

Thermodesulfimonas (Ther.mo.de.sul.fi.ti.mo’nas. Gr. adj. thermos warm, hot; L. pref. de from, off, away; N.L. n. sulfis -itis sulfite; L. fem. n. monas a unit, monad; N.L. fem. n. Thermodesulfimonas thermophilic sulfite-reducing body).

Cells are rod-shaped. Cell wall is of Gram-positive type. Thermophilic. Anaerobic. Neutrophilic. Chemolithoautotrophic growth by reduction of sulfite. Member of the family Thermoanaerobacterales. The type species is Thermodesulfimonas autotrophica.

| Table 1. Comparative characteristics between strain SF97T and its most closely related species |
|-----------------------------------------------|----------------|----------------|----------------|
| Characteristic | 1 | 2 | 3 |
| Source of isolation | Hot spring, Golovnin Caldera, Kurils, Russia | Hot spring, Kawah Candradimuka crater, Java, Indonesia | Hot spring, Uzon Caldera, Kamchatka, Russia |
| Sppores | – | – | + |
| Growth temperature (°C) | 45 – (65) – 72 | 57 – (70) – 77 | 60 – (75) – 82 |
| pH | 5.5 – (6.0 – 6.5) – 8.5 | 5.0 – (7.5) – 8.0 | 6.0 – (6.8) – 7.5 |
| Electron acceptors: | | | |
| Sulfate | – | + | +/– |
| Thiosulfate | – | – | + |
| Sulfite | + | – | – |
| Elemental sulfur | – | + | +/– |
| Nitr + | – | + | – |
| Fermentative growth | – | + | – |
| pyruvate | DNA G+C content (mol%) | 55.8 | 54 | 56.2 |

Strains: 1, SF97T (data from this study); 2, Ammonifex degensii (data from Huber et al. [15]); 3, Ammonifex thiophilus (data from Miroshnichenko et al. [14]). +, Positive; –, negative.
**DESCRIPTION OF THERMODESULFITIMONAS AUTOTROPHICA SP. NOV.**

Thermodesulfitimonas autotrophica (au.to.troph.i.ca. Gr. pron. autos self; Gr. adj. trophikos one who feeds; N.L. fem. adj. autotrophica autotrophic).

Has the following properties in addition to those given in the description of the genus. Cells are straight rods, 0.5–0.6 µm in diameter and 1.0–2.0 µm in length, growing singly, in pairs or forming short chains. Cells are motile with lateral flagellation. Spores are not observed. Growth occurs at 45–72 °C (optimum at 65 °C), pH 5.5–8.5 (optimum pH 6.0–6.5) and NaCl concentrations of 0–0.5 % (w/v) (optimum 0 %). Grows chemolithoautotrophically using hydrogen as an electron donor, sulfate or SO₂ gas as an electron acceptor and bicarbonate/CO₂ as a carbon source with sulfide formation. Does not reduce sulfate, thiosulfate, elemental sulfur, nitrate, nitrite, fumarate, ferrihydrite or oxygen with hydrogen, formate, acetate, lactate, pyruvate, succinate, ethanol and peptone. Does not oxidize elemental sulfur or sulfite with nitrate or oxygen.

The type strain, SF977T (=DSM 102936=VKM B-2961T), was isolated from a terrestrial hot spring (Kuril Islands, Russia). The DNA G+C content of the type strain is 55.8 mol% (Tm).

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**References**

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