**Dissulfurirhabdus thermomarina** gen. nov., sp. nov., a thermophilic, autotrophic, sulfite-reducing and disproportionating deltaproteobacterium isolated from a shallow-sea hydrothermal vent

Galina B. Slobodkina,¹ Tatyana V. Kolganova,² Dmitry S. Kopitsyn,³ Mikhail B. Varyasov,⁴ Elizaveta A. Bonch-Osmolovskaya¹ and Alexander I. Slobodkin¹

¹Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Leninskiy Prospect 33, bld. 2, 119071 Moscow, Russia
²Institute of Bioengineering, Research Center of Biotechnology of the Russian Academy of Sciences, Leninskiy 33, bld. 2, 119071 Moscow, Russia
³Gubkin Russian State University of Oil and Gas, Leninsskiy Prospect 65, 117485, Moscow, Russia
⁴Chemistry Department, Lomonosov Moscow State University, Leninskie Gory 1, 119899 Moscow, Russia

A thermophilic, anaerobic, chemolithoautotrophic bacterium, strain SH388ᵀ, was isolated from a shallow, submarine hydrothermal vent (Kuril Islands, Russia). Cells of strain SH388ᵀ were Gram-stain-negative short rods, 0.2–0.4 μm in diameter and 1.0–2.5 μm in length, and motile with flagella. The temperature range for growth was 25–58 °C (optimum 50 °C), and the pH range for growth was pH 5.0–7.0 (optimum pH 6.0–6.5). Growth of strain SH388ᵀ was observed in the presence of NaCl concentrations ranging from 0.5 to 4.0 % (w/v) (optimum 2.0–2.5 %). The strain grew chemolithoautotrophically with molecular hydrogen as electron donor, sodium sulfite as electron acceptor and bicarbonate/CO₂ as a carbon source. It was also able to grow by disproportionation of sulfite and elemental sulfur but not thiosulfate. Sulfate, Fe(III) and 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 class Deltaproteobacteria and was most closely related to *Dissulfuribacter thermophilus* and *Dissulfurimicrobium hydrothermale* (91.6 % and 90.4 % sequence similarity). On the basis of its physiological properties and results of phylogenetic analyses, strain SH388ᵀ is considered to represent a novel species of a new genus, for which the name *Dissulfurirhabdus thermomarina* gen. nov., sp. nov. is proposed. The type strain of the species is SH388ᵀ (=DSM 100025ᵀ=VKM B-2960ᵀ). It is the first thermophilic disproportionator of sulfur compounds isolated from a shallow-sea environment.

Chemolithoautotrophic micro-organisms can gain energy from a variety of inorganic compounds serving as electron donors and acceptors. Sulfur dioxide is one of the most typical and abundant volcanic gases. It is highly soluble in water; thus, in aquatic environments, including hydrothermal vents, SO₂ is usually present in the form of sulfite ions. Micro-organisms capable of dissimilatory sulfite reduction are phylogenetically diverse and include all sulfate-reducers as well as many nonsulfate-reducing species. Overall, the ability to use sulfite as an electron acceptor with organic or inorganic electron donors is known for representatives of the bacterial phyla *Firmicutes*, *Proteobacteria*, *Nitrospirae* and *Thermodesulfobacteria* and for archaea of the phyla *Crenarchaeota* and *Euryarchaeota* (Simon & Kroneck, 2013; Slobodkin et al., 1999). Some sulfite-reducers are also capable of sulfite disproportionation. Growth coupled to disproportionation of sulfite...
was reported for mesophilic representatives of the deltaproteobacterial genera Desulfovibrio, Desulfocapra, Desulfonatronum, Desulfonatronospira and Desulfonatronovibrio (Bak & Pfennig, 1987; Finster et al., 1998; Janssen et al., 1996; Pikuta et al., 2003; Sorokin et al., 2008, 2011) as well as for two thermophiles, Dissulfuribacter thermophilus and Dissulfurimicrobium hydrothermalis (Slobodkin et al., 2013, 2016). Beyond the class Deltaproteobacteria, this ability is known for only one species, Thermosulfurimonas disarmata, belonging to the phylum Thermodesulfovibrio (Slobodkin et al., 2012). Here we report the isolation and characterization of a novel chemolithotrophic, thermophilic, sulfite-reducing and disproportionalizing bacterium from a shallow-sea hydrothermal vent.

Strain SH388T was isolated from a mixed sample of sand, hydrothermal fluid (thermal water and dissolved gases) and seawater collected at a shallow, submarine hydrothermal vent located at a depth of 12 m and at 200–250 m offshore (Kunashir Island, Kurils, Russia). Temperature and pH at the sampling site (44°29.469′N 146°06.247′E) varied within the range 60–84 °C and pH 6.0–6.5, respectively. Samples were taken anaerobically in tightly stoppered 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, 18.0 g NaCl, 4.33 g MgCl₂·6H₂O, 2.0 g NaHCO₃, 1 ml trace element solution (Slobodkin et al., 2012) and 1 ml vitamin solution (Wolin et al., 1963). 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 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 a final concentration 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 50 °C for 15 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 cells was observed in the highest growth-positive dilution (10⁻⁸). Attempts to obtain separate colonies were unsuccessful either with 1% Gelrite gellan gum or with 1% agar as solidifying agent in the medium with or without ferrihydrite. A pure culture of strain SH388T 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 cellular fatty acids (CFA) composition were performed as described previously (Slobodkina et al., 2016). Genomic DNA of strain SH388T was extracted using the method of Marmur (1961) and purified using Wizard MaxiPreps DNA Purification Resin (Promega). 16S rRNA gene amplification and sequencing were done as described previously (Slobodkina et al., 2012). Pairwise similarity values were calculated by means of the EzTaxon server (http://www.ezbiocloud.net/eztaxon; Kim et al., 2012). Alignment with a representative set of related 16S rRNA gene sequences, evolutionary analysis and phylogenetic tree reconstruction were performed as described previously (Slobodkina et al., 2016).

Cells of strain SH388T were straight rods with rounded ends, 0.2–0.4 μm in diameter and 1.0–2.5 μm in length, growing singly or in pairs. Cells were motile due to a single polar flagellum (Fig. 1a). Formation of endospores was not observed. Ultrathin sections of strain SH388T revealed a Gram-negative cell wall type with an outer membrane (Fig. 1b). The temperature range for growth of strain SH388T was 25–58 °C with an optimum at 50 °C. No growth was detected at 26 or 60 °C after incubation for 3 weeks. The pH range for growth was 6.5–7.0, with optimum growth at pH 6.6–6.5. No growth was detected at pH 5.0 or 7.5. Growth of strain SH388T was observed at NaCl concentrations ranging from 0.5 to 4.0% (w/v) with an optimum at 2.0–2.5% (w/v), but no growth was evident below 0.4 or above 4.5% (w/v) NaCl.

Strain SH388T grew lithoautotrophically with molecular hydrogen as an electron donor, sodium sulfite as an electron acceptor and bicarbonate/CO₂ as a carbon source. Sulfite could be replaced by SO₂ gas [SO₂/CO₂ (15:85, v/v) in the gas phase] that resulted in growth with the same specific growth rate and final cell yield. Sulfite or SO₂ gas reduction was accompanied by hydrogen consumption and formation of hydrogen sulfide; the presence of ferrihydrite was not essential. In the absence of molecular hydrogen, strain SH388T grew by disproportionation of sulfite (5 mM), SO₂ gas [SO₂/CO₂ (15:85, v/v) in the gas phase] and elemental...
sulfur (5 g l⁻¹), but not thiosulfate (15 mM). The growth was accompanied by accumulation of sulfate and conversion of ferrihydrite to black, non-magnetic precipitate, indicating the production of sulfide. For disproportionation of elemental sulfur, the presence of ferrihydrite was required; disproportionation of sulfate and SO₂ gas could proceed without ferrihydrite. Addition of pyruvate, malate (10 mM each), glucose, fructose and sucrose (2 g l⁻¹ each) to medium with sodium sulfite slightly increased the growth rate but did not increase the final cell number. Acetate, propionate, butyrate, formate, methanol, ethanol, n-propanol, i-propanol, lactate, fumarate, succinate, glycerol (10 mM each), peptone and yeast extract (2 g l⁻¹ each) did not stimulate growth of strain SH388ᵀ with sodium sulfite. The new isolate did not grow and did not reduce sulfate (15 mM), nitrate (10 mM) or ferrihydrite \([90 \text{ mmol Fe(III) l}^{-1}]\) with hydrogen \([\text{H}_2/\text{CO}_2 (80:20, \text{v/v})]\), acetate, lactate, pyruvate, succinate, ethanol (10 mM each) or peptone (2 g l⁻¹ each), and did not ferment these substances in anaerobic conditions. CFAs of strain SH388ᵀ grown in medium with hydrogen and sulfite and harvested in the late exponential phase of growth comprised a mixture of saturated and monounsaturated straight-chain and branched fatty acids (Table S1, available in the online Supplementary Material). The major cellular fatty acids were i-C₁₅ : 0 and C₁₆ : 0 (32.4 and 23.2 % of the total, respectively) with lesser amounts of C₁₈ : 1ω₇ (7.8 %), i-C₁₇ : 1ω₈ (6.8 %), C₁₇ : 1ω₆ and C₁₈ : 1ω₉ (3.7 %) each. It also did not grow and did not reduce elemental sulfur (5 g l⁻¹), thiosulfate (15 mM), fumarate, 9,10-anthraquinone 2,6-disulfonate (10 mM each) or oxygen (2.0 or 20 %, v/v, in the gas phase) with hydrogen as electron donor. The isolate did not grow by oxidizing elemental sulfur (5 g l⁻¹) or thiosulfate (15 mM) with nitrate (10 mM) or oxygen (2.0 or 20 %, v/v, in the gas phase). Strain SH388ᵀ did not grow aerobically with acetate, pyruvate, succinate (10 mM each), glucose, fructose, maltose, sucrose, arabinose or peptone (2 g l⁻¹ each), and did not ferment these substances in anaerobic conditions. CFAs of strain SH388ᵀ grown in medium with hydrogen and sulfate and harvested in the late exponential phase of growth comprised a mixture of saturated and monounsaturated straight-chain and branched fatty acids (Table S1, available in the online Supplementary Material). The major cellular fatty acids were i-C₁₅ : 0 and C₁₆ : 0 (32.4 and 23.2 % of the total, respectively) with lesser amounts of C₁₈ : 1ω₇ (7.8 %), i-C₁₇ : 1ω₈ (6.8 %), C₁₇ : 1ω₆ and C₁₈ : 1ω₉ (3.7 %) each.

### Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain SH388ᵀ among the members of the class Deltaproteobacteria. The tree was reconstructed using the maximum-likelihood method. Trees reconstructed by 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.

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(6.0 %) and C14:0 (5.7 %). Other fatty acids were present in low or trace amounts (<5 %). The G+C content of the genomic DNA of strain SH388T was 64.6 mol% (Tm).

A comparison of 1521 nt of 16S rRNA gene sequence of strain SH388T with those available in the GenBank and EzTaxon server databases showed that the strain belonged to the class Deltaproteobacteria (Fig. 2), displaying highest sequence similarity to Dissulfuribacter thermophilus S69T (91.6 %) and Dissulfurimicrobium hydrothermale Sh68T (90.4 %) and being distantly related (less than 88.6 % 16S rRNA gene sequence similarity) to the species of the orders Syntrophobacterales, Desulfofabaclerales and Desulfuromonadales.

Strain SH388T was isolated from a shallow-sea hydrothermal vent where it participates in biogeochemical cycling of sulfur, most probably as a primary producer. It is the first thermophilic disproportionator of sulfur compounds isolated from a shallow-sea environment to our knowledge. SH388T shares the distinctive physiological features of the most closely related species — the ability to disproportionate sulfur compounds at elevated temperatures, combined with the inability to accomplish dissimilatory sulfate reduction. In addition to significant phylogenetic distance, strain SH388T differs from related strains in genomic DNA G+C content, CFA profile and in some physiological characteristics, including Fe(III) reduction and thiosulfate utilization (Table 1). On the basis of phylogenetic position and phenotypic properties, we propose to classify strain SH388T as the type strain of a novel species of a new genus in the class Deltaproteobacteria, Dissulfurirhabdus thermomarina gen. nov., sp. nov.

Table 1. Characteristics that distinguish strain SH388T from representatives of the most closely related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>Source of isolation</td>
<td>Shallow-sea hydrothermal vent</td>
<td>Deep-sea hydrothermal vent</td>
<td>Terrestrial hydrothermal pond</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>25–58</td>
<td>28–70</td>
<td>30–65</td>
</tr>
<tr>
<td>Temperature optimum (°C)</td>
<td>50</td>
<td>61</td>
<td>50–52</td>
</tr>
<tr>
<td>pH range</td>
<td>5.0–7.0</td>
<td>5.6–7.9</td>
<td>5.2–7.5</td>
</tr>
<tr>
<td>pH optimum</td>
<td>6.0–6.5</td>
<td>6.8</td>
<td>6.0–6.2</td>
</tr>
<tr>
<td>NaCl (% w/v) range</td>
<td>0.5–4.0</td>
<td>0.9–5.0</td>
<td>0–2.3</td>
</tr>
<tr>
<td>Fe(III) reduction</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>S2O32− disproportionation</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth on H2S2O32−</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth stimulation by:</td>
<td>Pyruvate, malate, glucose, fructose, sucrose</td>
<td>Fumarate, maleinate</td>
<td>Succinate</td>
</tr>
<tr>
<td>Major CFAs</td>
<td>i-C15:0 and C16:0</td>
<td>C18:1ω7, C19:0 and cyc-C19:0</td>
<td>C16:0, C17:0, C18:0, C19:0 and C20:0</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>64.6</td>
<td>40.5</td>
<td>49.0</td>
</tr>
</tbody>
</table>

Cells are rod-shaped. Cell wall of Gram-negative type. Thermophilic. Anaerobic. Neutrophilic. Do not form endospores. Chemolithoautotrophic growth by reduction or disproportionation of sulfur compounds. Member of the class Deltaproteobacteria. The type species is Dissulfurirhabdus thermomarina.

Description of Dissulfurirhabdus thermomarina sp. nov.

Dissulfurirhabdus thermomarina (ther. mo. ma.ri’na. Gr. adj. thermos warm; L. fem. adj. marina of the sea, marine; N.L. fem. adj. thermomarina warm and marine, referring to the site of isolation).

Has the following properties in addition to those given in the description of the genus. Cells are straight rods, 0.2–0.4 µm in diameter and 1.0–2.5 µm in length, growing singly or in pairs. Cells are motile with a single polar flagellum. Gram-stain-negative. Thermophilic. Growth occurs at 25–58 °C (optimum at 50 °C, pH 5.0–7.0 (optimum pH 6.0–6.5) and NaCl concentrations of 0.5–4.0 % (w/v) (optimum 2.0–2.5 %, w/v NaCl). Grows chemolithoautotrophically using hydrogen as an electron donor, sulfite or SO2 gas as an electron acceptor and bicarbonate/CO2 as a carbon source. Able to grow by disproportionation of sulfite, SO2 and elemental sulfur with sulfide and sulfate formation. Does not reduce sulfate, nitrate or ferrihydrite with hydrogen, acetate, lactate, pyruvate, succinate, ethanol or peptone. Does not oxidize elemental sulfur or thiosulfate with nitrate or oxygen. Does not grow aerobically with acetate, pyruvate, succinate, glucose, fructose, maltose, sucrose, arabinose and peptone and does not ferment them.

The type strain, SH388T (=DSM 100025T=VKM B-2960T), was isolated from a shallow, submarine hydrothermal vent.
(Kuril Islands, Russia). The DNA G+C content of the type strain is 64.6 mol% (Tm).

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References


