Sulfurihydrogenibium kristjanssonii sp. nov., a hydrogen- and sulfur-oxidizing thermophile isolated from a terrestrial Icelandic hot spring

Gilberto E. Flores,1 Yitai Liu,1 Isabel Ferrera,1 Terry J. Beveridge2 and Anna-Louise Reysenbach1

1Department of Biology, Portland State University, PO Box 751, Portland, OR 97207-0751, USA
2Department of Molecular and Cellular Biology, College of Biological Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Three thermophilic, aerobic, hydrogen- and sulfur-oxidizing bacteria were isolated from an Icelandic hot spring near the town of Hveragerdi and share ≈99 % 16S rRNA gene sequence similarity. One of these isolates, designated strain I66287, was selected for further characterization. Strain I66287 is a motile rod, 1.5–2.5 μm long and about 0.5 μm wide. Growth occurred between 40 and 73 °C (optimally at 68 °C), at pH 5.3–7.8 (optimally at pH 6.6) and at NaCl concentrations between 0 and 0.5 % (w/v). Strain I66287 grew with H2, S0 or S2O32− as an electron donor with O2 (up to 25 %, v/v; optimally at 4–9 %) as the sole electron acceptor. CO2 and succinate were utilized as carbon sources but no organic compounds, including succinate, could be used as an energy source. The G+C content of the genomic DNA was determined to be 28.1 mol%. Phylogenetic analysis of the 16S rRNA gene sequence indicated that strain I66287 is a member of the genus Sulfurihydrogenibium, the closest cultivated relative being the recently described strain Sulfurihydrogenibium rodmani UZ3-5T (98.2 % sequence similarity). On the basis of the physiology and phylogeny of this organism, strain I66287 represents a novel species of the genus Sulfurihydrogenibium, for which the name Sulfurihydrogenibium kristjanssonii sp. nov. is proposed. The type strain is I66287 (=DSM 19534T = OCM 901T = ATCC BAA-1535T).

The order Aquificales, which comprises thermophiles belonging to the families Aquificaceae, Desulfurobacteriaceae and Hydrogenothermaceae, is considered to be one of the deepest branching lineages of the domain Bacteria (Burggraf et al., 1992; Pitulle et al., 1994; Di Giulio, 2003a, b, c; Barion et al., 2007). Members of two of these families, the Aquificaceae and the Hydrogenothermaceae, are widespread in terrestrial hydrothermal systems and include those belonging to the genera Hydrogenobacter, Sulfurihydrogenibium and Thermocrinis (Reysenbach et al., 2000; Skirnisdottir et al., 2003; Takacs et al., 2001; Spear et al., 2005; Purcell et al., 2007; Hetzer et al., 2007; Ferrera et al., 2007). Most representatives of these genera appear as filamentous biomass in hot-spring streams and are often associated with sulfur or iron deposits (Reysenbach et al., 2005). Recently, several members of the genus Sulfurihydrogenibium have been isolated from hydrothermal systems in geographically diverse locations, including Japan (Sulfurihydrogenibium subterraneum HGMK-1T; Takai et al., 2003), the Azores (Sulfurihydrogenibium azorense Az-Fu1T; Aguiar et al., 2004), Yellowstone National Park (USA) (Sulfurihydrogenibium yellowstonense SS-5T; Nakagawa et al., 2005), New Zealand (strain CP.B2; Hetzer et al., 2007) and Kamchatka, Russia (Sulfurihydrogenibium rodmani UZ3-5T; O’Neill et al., 2008). These studies have identified physiological properties common among members of this genus; several differences have also been revealed. For example, all described isolates are able to oxidize S0 and S2O32− with O2 acting as an electron acceptor, but only S. azorense Az-Fu1T, S. subterraneum HGMK-1T and strain CP.B2 can also oxidize H2 (Takai et al., 2003; Aguiar et al., 2004; Nakagawa et al., 2005; Hetzer et al., 2007; O’Neill et al., 2008). Clone libraries of 16S rRNA gene sequences from Icelandic hot-spring filamentous identified relatives of Sulfurihydrogenibium (Skirnisdottir et al., 2000), and here we report the isolation of a novel member of the genus Sulfurihydrogenibium obtained from Iceland. The isolation of this novel species serves to expand the known geographical distribution and metabolic diversity of the genus Sulfurihydrogenibium.
Thick, grey filaments were collected along the outflow channel of an Icelandic hot spring near the town of Hveragerdi (64° 0.1901′ N 021° 11.606′ W) and transferred into sterile 150 ml serum bottles. The temperature and pH at the sampling site were 68 °C and approximately 6.0. Subsamples were later used to enrich for microaerophilic sulfur-oxidizers in 5 ml modified MS medium, described below (Boone et al., 1989). Enrichment cultures were incubated at 70 °C and monitored for changes in turbidity. Within 24 h, cultures appeared turbid and were examined for growth by using phase-contrast microscopy. Several of the cultures, i.e. those comprising strains I6628T, I66735 and I6517, exhibited growth in the form of motile rods. Cultures were subsequently purified by several series of dilution-to-extinction transfers and their purity was verified by 16S rRNA gene sequencing. The three isolates were >99 % identical on the basis of partial 16S rRNA gene sequences, and strain I6628T was selected for further characterization.

The medium used for the isolation and characterization of strain I6628T was modified MS medium (Boone et al., 1989) and contained the following (l⁻¹): 5 g elemental sulfur, 0.8 g NaOH, 0.48 g KCl, 1 g MgCl₂·6H₂O, 7 g MgSO₄·7H₂O, 2 g Na₂S₂O₃·5H₂O, 0.48 g CaCl₂·2H₂O, 0.2 g NH₄Cl, 0.4 g K₂HPO₄·3H₂O and 10 ml trace element stock solution (Ferguson & Mah, 1983). The medium was prepared with distilled water under constant bubbling with CO₂. The pH was adjusted to 6.0 with sulfuric acid prior to autoclaving at 105 °C for 60 min to prevent melting of the elemental sulfur. After autoclaving, oxygen was added to a final concentration of 4 % (v/v).

Routine observations of the three isolates were made using an Olympus BX60 phase-contrast microscope. Further detailed examination of strain I6628T was done using electron microscopy as described previously (Nakagawa et al., 2005). In brief, for thin sectioning, cells were fixed in 2 % (v/v) glutaraldehyde followed by 2 % (w/v) osmium tetroxide and staining en bloc with 2 % (w/v) uranyl acetate, as outlined by Beveridge et al. (2007). Cells were dehydrated through an ethanol series and embedded in LR White resin. Once sectioned, cell samples were mounted on carbon- and Formvar-coated 200-mesh grids and stained with uranyl acetate and lead citrate. For negative stains, the grids were coated with a thin suspension of cells, blotted dry and stained with 2 % uranyl acetate. All transmission electron microscopy was done with a Philips CM10 microscope operating at 80 kV under standard operating conditions.

As shown by the thin sections, cells of strain I6628T were Gram-negative, motile, straight to slightly curved rods with a mean length of 1.5–2.5 μm and a width of approximately 0.5 μm (Fig. 1a). Cells sometimes possessed humps along their length, indicating some pleomorphism (Fig. 1b). Cells occurred singly or in filaments, each consisting of a few cells (not shown). Flagella were apparent on negatively stained cells, especially at the poles (Fig. 1a). Thin sections

**Fig. 1.** Electron micrographs of a negatively stained cell (a) and a thin section (b) of strain I6628T. Internal membranes or fibres are indicated by arrows in (b). Bars, 1 μm (a) and 500 nm (b).
Strain I6628T was tested for the ability to oxidize a variety of inorganic electron donors with O2 (4 %, v/v) or nitrate (as NaN3; 0.1 %, w/v) serving as the terminal electron acceptors. Donors tested included H2 (145 kPa), S0 (3 %, w/v), thiosulfate (as Na2S2O3; 0.1 %, w/v), sulfite (as Na2SO3; 0.1 %, w/v), arsenite (as Na2AsO3; 5 mM), selenite (as Na2SeO3; 5 mM) and Fe2+ (as FeCl2; 4H2O; 5 mM). Growth was observed only with H2, S0 and S2O32− in the presence of O2. A variety of inorganic electron acceptors were also tested with H2 (145 kPa) as the electron donor, and included S0 (3 %, w/v), O2 (1−25 %, v/v), thiosulfate (0.1 %, w/v), sulfate (0.1 %, w/v), nitrate (as NaNO3; 0.1 %, w/v), nitrite (as NaNO2; 0.01 and 0.1 %, w/v), Fe3+ (as ferric citrate; 5 mM), arsenate (as NaAsO3; 7H2O; 5 mM) and selenate (as Na2SeO4; 5 mM). Strain I6628T was able to utilize only O2 (up to 25 %) as a terminal electron acceptor, showing optimal growth between 4 and 9 % (data not shown).

To test the novel isolate for heterotrophic and fermentative growth, a variety of organic carbon sources were added to modified MS medium in the absence of CO2, S0 and S2O32−. Growth was monitored in the presence and absence of both O2 as an electron acceptor and H2 as an electron donor. Potential energy substrates and carbon sources were added at 0.1 and 0.01 % (w/v; v/v for liquids) and included yeast extract, Bacto peptone, trypticase peptone, sucrose, glucose, starch, sodium formate, Casamino acids, formaldehyde, formamide, sodium citrate, sodium propionate, sodium acetate, 2-propanol, mannose, succinate and oxalate (Aguiar et al., 2004). Cultures that exhibited growth were transferred at least twice to ensure that growth was not the result of carry-over from the initial transfer. Strain I6628T was only able to utilize succinate as a carbon source with H2 as an electron donor and O2 as an acceptor. The results of all of the growth experiments and comparisons with other Sulfurihydrogenibium isolates are summarized in Table 1.

Genomic DNA used for sequencing and G+C content determination was extracted from a 1 l culture by using the Qiaogen Genomic-tip 100/G DNA extraction kit according to the manufacturer’s protocol for bacterial culture preparations. The thermal denaturation method (Marmur & Doty, 1962) was used to determine the genomic G+C content of strain I6628T. The DNA G+C content of strain I6628T was found to be 28.1 mol%, which is the lowest value reported for any described Sulfurihydrogenibium species (Table 1).

Amplification of the 16S rRNA gene and subsequent purification and sequencing were conducted as described previously (Ferrera et al., 2007). An almost-complete 16S rRNA gene sequence (1486 nt) was assembled using the software AUTOASSEMBLER and was compared, using BLAST, against the NCBI non-redundant database. The ARB program (http://www.arb-home.de; Ludwig et al., 2004) was used to align the 16S rRNA gene sequences according to secondary structure constraints. A similarity matrix using 1430 nt was constructed using a subset of closely related Sulfurihydrogenibium species. Strain I6628T is most closely related to the 16S rRNA gene sequence of an environmental isolate from Iceland, designated strain SRI-240 (99.5 % sequence similarity; Skirnisdottir et al., 2000). Only unambiguous nucleotide positions were used in the phylogenetic analysis (~1300 bp). Neighbour-joining (1000 bootstrap replications) and maximum-likelihood (100 bootstrap replications) analyses (PAUP* 4.0 beta 10; Swofford, 2003) were performed as described previously (Ferrera et al., 2007) (Fig. 2).

On the basis of the 16S rRNA gene sequence analysis, strain I6628T forms a distinct clade within the Sulfurihydrogenibium group (99 % maximum-likelihood bootstrap value) with the sequence of an environmental clone (SRI-240; Skirnisdottir et al., 2000) from an Icelandic hot spring, and represents a novel cultivated species within this genus. Of the species of Sulfurihydrogenibium isolated to date, the strain closest to I6628T is S. rodmanii UZ3-5T (98.2 % 16S rRNA gene sequence similarity) (Fig. 2), which was isolated from Kamchatka, Russia. Strain I6628T shows 16S rRNA gene sequence similarities of 98, 97.5 and 96 % with respect to S. azorense Az-Fu1T, S. subterraneum HGMK-1T and S. yellowstonense SS-5T (Fig. 2), respectively.

The members of the Aquificales appear to be the predominant primary producers in circumneutral terrestrial hot-spring streams at temperatures above the limits for photosynthesis (Reysenbach et al., 2005; Spear et al., 2005) and in conditions where hydrogen, sulfur/sulfide and oxygen are readily available. Micro-organisms belonging to the genus Sulfurihydrogenibium tend to be the predominant members of the Aquificales present in circumneutral terrestrial springs with elevated sulfide concentrations. This situation contrasts with that found in low-sulfide springs, in which other members of the Aquificales that preferentially oxidize hydrogen, e.g. Thermocrinis and Hydrogenobacter, predominate (Skirnisdottir et al., 2000); both Thermocrinis and Hydrogenobacter can also oxidize reduced sulfur compounds (Huber et al., 1998; Stöhr et al.,
Table 1. Comparison of physiological traits of strain I6628T and described members of the genus \textit{Sulfurhydrogenibium}

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain I6628T</th>
<th>\textit{S. rodmanii} UZ3-5T</th>
<th>\textit{S. yellowstonense} SS-5T</th>
<th>\textit{S. azorense} Az-Fu1T</th>
<th>\textit{S. subterraneum} HGMK-1T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
<td>Terrestrial hot spring, Iceland</td>
<td>Terrestrial hot spring, Kamchatka, Russia</td>
<td>Terrestrial hot spring, Yellowstone National Park, USA</td>
<td>Terrestrial hot spring, the Azores, Portugal</td>
<td>Subsurface gold mine, Hishikari, Japan</td>
</tr>
<tr>
<td>Cell size (µm) (length × width)</td>
<td>1.5–2.5 × 0.4–0.5</td>
<td>1.19–1.42 × 0.72–0.79</td>
<td>1.2–2.8 × 0.6–0.8</td>
<td>0.9–2.0 × 0.4–0.5</td>
<td>1.5–2.5 × 0.3–0.5</td>
</tr>
<tr>
<td>Temperature range (optimum) (°C)</td>
<td>40–73 (68)</td>
<td>55–80 (74–75)</td>
<td>55–78 (70)</td>
<td>50–73 (68)</td>
<td>40–70 (65)</td>
</tr>
<tr>
<td>pH range (optimum)</td>
<td>5.3–7.8 (6.6)</td>
<td>5.0–7.0 (3.5–6.47)</td>
<td>6.0–8.0 (7.5)</td>
<td>5.5–7.0 (6.0)</td>
<td>6.4–8.8 (7.5)</td>
</tr>
<tr>
<td>NaCl range (optimum) (%) v/w</td>
<td>0–0.5 (0)</td>
<td>0–0.9 (0)</td>
<td>0–0.6 (0)</td>
<td>0–0.25 (0.1)</td>
<td>0–4.8 (0.5)</td>
</tr>
<tr>
<td>Electron donors</td>
<td>H₂, S⁰, S₂O₃⁻⁻</td>
<td>S⁰, S₂O₃⁻⁻</td>
<td>S⁰, S₂O₃⁻⁻</td>
<td>H₂, S⁰, S₂O₃⁻⁻, SO₃⁻⁻, Fe²⁺⁺⁺, AsO₃⁻⁻⁻⁺⁺</td>
<td>H₂, S⁰, S₂O₃⁻⁻</td>
</tr>
<tr>
<td>Electron acceptor(s)</td>
<td>O₂ (up to 25%, v/v)</td>
<td>O₂ (up to 14%, v/v)</td>
<td>O₂ (up to 18%, v/v)</td>
<td>S⁰, Fe²⁺⁺⁺, O₂ (up to 9%, v/v), SO₃⁻⁻⁻, AsO₃⁻⁻⁻⁺⁺</td>
<td>Fe²⁺⁺⁺, O₂, NO⁻⁻⁻⁻⁻, HAsO₃⁻⁻⁻⁻⁻, SeO₃⁻⁻⁻⁻⁻⁻</td>
</tr>
<tr>
<td>Organic carbon source(s)</td>
<td>Succinate (with H₂ as electron donor)</td>
<td>None</td>
<td>Yeast extract, Bacto peptone, trypticase peptone, sucrose, glucose, starch, formate, Casamino acids, citrate, propionate, acetate</td>
<td>Yeast extract, Bacto peptone, trypticase peptone, Casamino acids</td>
<td>Acetate</td>
</tr>
<tr>
<td>Internal structures</td>
<td>Stacked membranes/fibres</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Stacked membranes/fibres</td>
<td>No internal structures reported</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>28.1</td>
<td>35</td>
<td>32</td>
<td>33.6</td>
<td>31.3</td>
</tr>
</tbody>
</table>

*We have recently had difficulty growing this organism under these conditions.*
2001; Eder & Huber 2002). However, it appears that *Sulfurihydrogenibium* species might be more adapted to high-sulfide environments and are able to outcompete other members of the *Aquificales* (Skirnisdottir et al., 2000). Furthermore, some isolates, e.g. *S. yellowstonense* SS-5T, lack the suite of hydrogenases present in ‘*Aquifex aeolicus*’ VF5 and *S. azorense* Az-Fu1T (A.-L. Reysenbach, unpublished results). The isolation of strain I6628T under sulfur-oxidizing conditions serves to illustrate the importance of sulfide to the physiological ecology of the genus *Sulfurihydrogenibium*.

Strain I6628T shares several of the physiological properties associated with recognized species of the genus *Sulfurihydrogenibium*, including the preference for low concentrations of NaCl and O₂ and the ability to oxidize S⁰ and S₂O³⁻ (Table 1). In contrast, only three of the described isolates, strain I6628T, *S. azorense* Az-Fu1T (Aguiar et al., 2004) and *S. subterraneum* HGMK-1T (Takai et al., 2003), can oxidize H₂. Additionally, only I6628T, *S. rodmanii* UZ3-5T (O’Neill et al., 2008) and *S. yellowstonense* SS-5T (Nakagawa et al., 2005) use only O₂ as an electron acceptor. Strain I6628T also possesses heterotrophic capabilities similar to those of *S. azorense* Az-Fu1T, *S. subterraneum* HGMK-1T and *S. yellowstonense* SS-5T, but is much more restricted, using only succinate as an organic carbon source. Therefore, it appears that strain I6628T possesses a combination of the physiological capabilities of all *Sulfurihydrogenibium* species described to date. On the basis of these phylogenetic and physiological characteristics, strain I6628T represents a novel species of the genus *Sulfurihydrogenibium*, for which the name *Sulfurihydrogenibium kristjanssonii* sp. nov. is proposed.

**Description of *Sulfurihydrogenibium kristjanssonii* sp. nov.**

*Sulfurihydrogenibium kristjanssonii* (krist.jans’son.i.i. N.L. masc. gen. n. *kristjanssonii* of Kristjansson, in honour of Jakob Kristjansson for his long-term commitment to the description and exploration of thermophile biodiversity in Iceland, which includes some of the first work done on the *Aquificales*).

Cells are motile, Gram-negative, straight to slightly curved rods with mean lengths of 1.5–2.5 μm and widths of approximately 0.5 μm. Occur singly or in filaments consisting of a few cells. Grows between 40 and 73 °C (optimally at 68 °C), between pH 5.3 and 7.8 (optimally at pH 6.6) and at NaCl concentrations between 0 and 0.5 % (w/v). Grows with H₂, S⁰ and S₂O³⁻ as electron donors and O₂ as sole electron acceptor (up to 25 %, v/v). Facultatively heterotrophic, being capable of using succinate and CO₂ as carbon sources. The G+C content of the genomic DNA of the type strain is 28.1 mol%.

The type strain, I6628T (=DSM 19534T =OCM 901T =ATCC BAA-1535T), was isolated from the outflow channel of a hot spring near the town of Hveragerdi, Iceland.

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