Thermodesulfobacterium hydrogeniphilum sp. nov., a thermophilic, chemolithoautotrophic, sulfate-reducing bacterium isolated from a deep-sea hydrothermal vent at Guaymas Basin, and emendation of the genus Thermodesulfobacterium

Christian Jeanthon, Stéphane L’Haridon, Valérie Cueff, Amy Banta, Anna-Louise Reysenbach and Daniel Prieur

A thermophilic, non-spore-forming, marine, sulfate-reducing bacterium, strain SL6T, was isolated from deep-sea hydrothermal sulfides collected at Guaymas Basin. The Gram-negative-staining cells occurred singly or in pairs as small, highly motile rods. The temperature range for growth was 50–80 °C with an optimum at 75 °C. The pH range for growth at 70 °C was 6.3–6.8, with an optimum at 6.5. The NaCl concentration range for growth was 5–55 g l⁻¹ with an optimum at 30 g l⁻¹. H₂ and CO₂ were the only substrates for growth and sulfate reduction. However, growth was stimulated by several organic compounds. Sulfur, thiosulfate, sulfite, cystine, nitrate and fumarate were not used as electron acceptors. Pyruvate, lactate and malate did not support fermentative growth. Desulfoviridin was not detected. The G+C content of the genomic DNA was 28 mol%. On the basis of 16S rRNA sequence analysis, strain SL6T is related to members of the genus Thermodesulfobacterium. However, the novel organism possesses phenotypic and phylogenetic traits that differ from those of its closest relatives. Therefore, it is proposed that this isolate, which constitutes the first marine representative of this genus, should be described as the type strain of a novel species, Thermodesulfobacterium hydrogeniphilum sp. nov. The type strain is SL6T (= DSM 14290T = JCM 11239T). Because of the phenotypic characteristics of the novel species, it is also proposed that the description of the genus Thermodesulfobacterium requires emendation.

Keywords: deep-sea hydrothermal vent, thermophile, Bacteria, Thermodesulfobacterium, sulfate reduction

INTRODUCTION

Phylogenetically diverse thermophilic sulfate-reducers have been isolated from both marine high-temperature environments, i.e. hydrothermal systems and deep offshore petroleum reservoirs. Marine sulfate-reducing representatives of the domain Archaea are restricted to hyperthermophilic members of the genus Archaeoglobus (Stetter, 1992). This genus contains three validly described species, Archaeoglobus fulgidus (Stetter et al., 1987; Stetter, 1988), Archaeoglobus profundus (Burggraf et al., 1990) and Archaeoglobus veneficus (Huber et al., 1997). The sulfate-reducers A. fulgidus and A. profundus have been isolated from diverse deep petroleum reservoirs (Stetter et al., 1993; Beeder et al., 1994; L’Haridon et al., 1995) and deep-sea hydro-
thermal vents at Guaymas Basin (Burggraf et al., 1990), whereas the sulfite-reducer A. veneficus has been found to occur in several chimney samples collected at the Mid-Atlantic Ridge (23° N) and East Pacific Rise (9° N). With the exception of Desulfotomaculum thermocisternum, a Gram-positive spore-former isolated from North Sea oil reservoir formation water (Nilsen et al., 1996), all the thermophilic, marine sulfate-reducers of the domain Bacteria described to date belong to the δ-Proteobacteria. These are Thermodesulfobacterium norvegicum (Beeder et al., 1995) and Desulfacinum infernum (Rees et al., 1995), both of which were isolated from North Sea petroleum reservoirs, the hydrocarbon-degrading strain TD3, isolated from a deep-sea vent site in the Guaymas Basin (Rueter et al., 1994), Desulfacinum hydrothermal and a novel species belonging to the genus Thermodesulfobacterium, isolated from a marine, shallow-water hydrothermal vent site in the Aegean Sea (Sievert & Kuever, 2000).

Members of the genus Thermodesulfobacterium are non-spore-forming, thermophilic, sulfate-reducing bacteria (Widdel, 1992) that branch deeply within the domain Bacteria (Olsen et al., 1994b; Castro et al., 2000). To date, no representatives of this genus have been isolated from marine, high-temperature habitats. The genus Thermodesulfobacterium includes three described species: Thermodesulfobacterium commune and Thermodesulfobacterium hveragerdense, respectively isolated from microbial mats in hot springs at Yellowstone National Park and in Iceland (Zeikus et al., 1983; Sonne-Hansen & Ahring, 1999), and Thermodesulfobacterium thermophilum (Tao et al., 1996; De Vos & Trüper, 2000) [previously named Desulfuvibrio thermophilus by Rozanova & Khudyakova (1974) and later reclassified illegitimately as Thermodesulfobacterium mobile by Rozanova & Pivovarova (1988)], isolated from hot stratral water from a petroleum reservoir. These species have a relatively restricted type of nutrition and are incomplete oxidizers, using H₂, lactate and pyruvate as electron donors for sulfate reduction.

In this paper, we describe the isolation and characterization of a novel thermophilic, sulfate-reducing isolate, strain SL6, which was obtained from a deep-sea hydrothermal vent site at Guaymas Basin.

**METHODS**

**Sample collection.** Hydrothermal chimneys and sediments were collected in 1991 from the Guaymas Basin hydrothermal vent field (27° 01' N, 111° 24' 30" W) at a depth of 2000 m, during the Guaynaut cruise. Using the port manipulator of the submersible Nautile, the samples were placed in the submersible insulated basket for the trip to the surface. Once they had been transferred on board, they were put into 50 ml glass vials and flooded with a sterile solution of 3% (w/v) sea salts (Sigma). The vials were then closed tightly with butyl rubber stoppers (Bellco), pressurized with N₂ (100 kPa), reduced with sodium sulfide when required and stored at 4 °C until they were processed (recently).

**Enrichment cultures and purification.** Enrichments were performed anaerobically in 50 or 100 ml vials respectively containing 10 or 20 ml medium (Miller & Wolin, 1974). The enrichment medium consisted of (1⁴-distilled water): 30 g sea salts (Sigma), 1 g NH₄Cl, 0.35 g K₂HPO₄, 3.46 g PIPES, 1 g NaHCO₃, 2 g Difco yeast extract, 2 g Difco peptone, 1 g sodium acetate, 0.5 g cysteine hydrochloride, 1 ml trace-element mixture (Widdel & Bak, 1992), 30 mg sodium tungstate, 0.5 mg sodium selenate, 1 ml vitamin mixture (Widdel & Bak, 1992), 1 ml thiamin solution (Widdel & Bak, 1992), 0.05 mg vitamin B₆, 1 ml growth-stimulating factors (Pfenning et al., 1981) and 1 mg resazurin. The pH of the medium was adjusted to 6.5 using 5 N HCl before autoclaving. H₂/CO₂ (80:20; 200 kPa) was used as the gas phase. Cultures were incubated at 65 °C and the pH of the medium was readjusted after 1 h incubation. Enrichments that produced sulfide were subcultured into the same medium that had been solidified with 0.7% (w/v) Phytage by using shake dilution series (Widdel & Bak, 1992). Tubes were incubated at 65 °C for 7 days under a H₂/CO₂ atmosphere (80:20; 200 kPa) and colonies were then removed and subcultured until a pure culture was obtained. Purity was checked at 65 °C aerobically in Marine broth (Difco) and anaerobically in Marine broth supplemented with 5 mM glucose. Stock cultures of isolate SL₆ were stored in culture medium at 4 °C. For long-term storage, pure cultures were stored at −80 °C in the same medium containing 5% (w/v) DMSO.

**Culture medium and conditions.** Isolate SL₆ was routinely grown in the following medium containing (1⁴-distilled water): 30 g NaCl, 3 g MgCl₂, 2H₂O, 4 g Na₂SO₄, 0.5 g KCl, 0.25 g NH₄Cl, 0.2 g K₂HPO₄, 3.46 g PIPES, 0.15 g CaCl₂, 2H₂O, 0.5 g Difco yeast extract, 1 mM sodium acetate, 1 ml trace-element mixture (Widdel & Bak, 1992), 0.25 mg sodium tungstate, 50 µg sodium selenate, 1 ml vitamin mixture (Widdel & Bak, 1992), 1 ml thiamin solution (Widdel & Bak, 1992), 0.05 mg vitamin B₆, 1 ml growth-stimulating factors (Pfenning et al., 1981) and 1 mg resazurin. The pH of the medium was adjusted to 6.5 using 5 M HCl before autoclaving. After autoclaving, the pH of the medium was readjusted to 6.5 at room temperature. H₂/CO₂ (80:20; 200 kPa) was used as the gas phase. Cultures were incubated at 70 °C and the pH of the medium was readjusted after 1 h incubation.

**Determination of growth.** Growth was monitored by measuring changes in turbidity at 600 nm by inserting anoxic culture tubes directly into a Spectronic 20D spectrophotometer (Bioblock). Direct cell counts were determined using cells stained with acridine orange and were counted by epifluorescence microscopy using an ocular grid (Hobbie et al., 1977). All growth experiments were performed in duplicate.

**Determination of growth parameters.** These experiments were performed without agitation. The influence of pH on growth was determined in the culture medium. The medium was adjusted to the desired pH, measured at ambient temperature by injecting 10% (w/v) sterile, anaerobic, stock solutions of 10% (w/v) NaHCO₃ and 8% (w/v) Na₂CO₃. In order to determine the NaCl range for growth, the NaCl concentration was varied while the concentrations of the other inorganic components were maintained.

**Determination of growth requirements.** The following organic substrates were tested as electron donors [with N₂/CO₂ (20:20; 200 kPa) as the headspace] or carbon sources [with H₂ (100%; 100 kPa) as the headspace] in the presence of...
20 mM sulfate (added substrate concentrations, in mM or w/v, are given in parentheses): acetate (15), formate (15), methanol (0.5%), monomethylamine (0.2%), malate (10), propionate (10), glutamate (5), fumarate (10), trypthone (0.1%), peptone (0.1%), valerate (5), isovalerate (5), butyrtye (10), isobutyrate (5), 2-methylbutyrate (5), 3-methylbutyrate (5), glucose (5), fructose (5), ethanol (10), propanol (5), pyruvate (10), lactate (20), succinate (10), caprylate (2.5), caproate (5), caprate (2.5) and heptanoate (5). These substrates were tested at the same concentrations as stimulating agents for growth, with H₂/CO₂ (80:20; 200 kPa) as the headspace. Ammonium chloride (5 mM), nitrate (5 mM), peptone (0.1%) and trypthone (0.1%) were tested for suitability as nitrogen sources. Elemental sulfur (1%), thiosulfate (10 mM), sulfite (2 mM), cystine (1%), nitrate (5 mM) and fumarate (10 mM) were tested as potential electron acceptors in sulfate-free medium.

Desulfoviridin. The presence of desulfoviridin was determined by adding NaN₃ to a resuspended pellet of cells and viewing the preparation under UV light, as described by Postgate (1959). Desulfovibrio giganteus (DSM 4123T) was used as a positive control.

Antibiotic susceptibility. The sensitivity of strain SL6T to ampicillin, chloramphenicol, kanamycin, penicillin G, streptomycin, tetracycline and rifampicin (Sigma) as standards. Antibiotic susceptibility was routinely to observe the bacteria and to obtain photomicrographs. Gram staining was carried out as described by Conn et al. (1957). For negative staining, 20 µl of a bacterial suspension fixed with 2% (w/v) glutaraldehyde was dropped on Formvar- and carbon-coated grids (400 mesh) and stained with 4% (w/v) uranyl acetate. Electron micrographs were taken using a Philips model EM201 transmission electron microscope with an acceleration voltage of 80 kV.

Light and electron microscopy. An Olympus BH-2 microscope equipped with an Olympus OM-2 camera was used routinely to observe the bacteria and to obtain photomicrographs. Gram staining was carried out as described by Conn et al. (1957). For negative staining, 20 µl of a bacterial suspension fixed with 2% (w/v) glutaraldehyde was dropped on Formvar- and carbon-coated grids (400 mesh) and stained with 4% (w/v) uranyl acetate. Electron micrographs were taken using a Philips model EM201 transmission electron microscope with an acceleration voltage of 80 kV.

H₂S production. H₂S production was evaluated by adding 500 µl of a solution of CuSO₄ (5 mM) and HCl (50 mM) to a 250 µl culture grown at 70 °C. The dark-brown precipitate, demonstrating the presence of sulfide, was compared with uninoculated medium incubated under the same conditions.

DNA base composition. Genomic DNA of strain SL6T was isolated by using the procedure described by Charbonnier & Forterre (1994). The DNA was purified on a caesium chloride gradient (Sambrook et al., 1989) and purity was checked spectrophotometrically. The G+C content of the DNA was determined from the melting point, according to the method of Marmur & Doty (1962), using Escherichia coli DNA (G+C content 52 mol%), Clostridium perfringens DNA (24 mol%) and Micrococcus luteus DNA (73 mol%) (all from Sigma) as standards.

PCRamplification and sequencing of the 16S rDNA. The 16S rDNA genes from strain SL6T genomic DNA were amplified, with a PCR, in reactions containing 50 mM KCl, 30 mM Tris/HCl (pH 8.3), 1.5 mM MgCl₂, 200 µM each of dATP, dCTP, dGTP and dTTP, 0.05% Igepal (Sigma), 1 U Taq polymerase and 20 pmol of each primer (5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-GGTTACCTTGTTACGACTT-3'), corresponding to positions 8–27 and 1492–1510 in the E. coli rDNA sequence (Brosius et al., 1978). The sample was denatured by heating the reaction mixture at 94 °C for 2 min and the thermal profile then consisted of 30 cycles of annealing at 50 °C for 30 s, extension at 72 °C for 90 s and denaturation at 94 °C for 30 s. A final extension step was carried out at 72 °C for 10 min. PCR products (approx. 1500 bp) were purified by using the PCRpure Spin kit (ISC) and cloned into the vector pCR2.1 by using the TOPO-TA cloning kit (Invitrogen). Plasmid DNA was prepared using an alkaline-lysis method (Inc. Perfect Prep kit). Sequencing reactions were performed using the ABI PRISM BigDye Terminator Cycle Sequencing kit and an ABI 310 Genetic Analyzer (PE Biosystems) according to the manufacturer’s protocol. The complete sequence on both strands was obtained by using vector-specific primers and a suite of 16S rDNA-specific primers to generate an overlapping set of sequences that were assembled into one contiguous sequence. The PCR product was also sequenced directly and no polymorphisms were observed.

Phylogenetic analysis of the rDNA genes. The 16S rDNA sequences were aligned manually with the sequences of representatives of related genera obtained from the Ribosomal Database Project (Maidak et al., 1999) or from recent GenBank releases. The secondary structure was used as a guide to ensure that only homologous regions were compared. In total, 1451 nucleotides were sequenced and 1241 sequences that were assembled into one contiguous sequence. The PCR product was also sequenced directly and no polymorphisms were observed.

RESULTS AND DISCUSSION

Enrichment and isolation

The enrichment medium used in this study was originally designed to enable investigation of the presence of novel thermophilic methanogens in Guaymas Basin hydrothermal samples. Chimney and sediment suspensions (1 ml) were inoculated into 10 ml enrichment medium and incubated at 65 and 80 °C in 50 ml vials with H₂/CO₂ as the gas phase (80:20; 200 kPa) without shaking. At 80 °C within 2–3 days, five of the samples showed growth of coccoid cells that fluoresced intense green at 420 nm. The hyperthermophilic strains isolated from these cultures were further identified as methanogens (Jeannot et al., 1999). An intense H₂S odour developed after 8 days in one of the cultures incubated at 65 °C. The prevailing organism in this enrichment culture, a motile, rod-shaped bacterium designated strain SL6T, was isolated and studied in detail.

Phenotypic characteristics

Cells of strain SL6T appeared as small rods, approximately 0.5–0.8 µm long and 0.4–0.5 µm wide (Fig. 1a, b), and stained Gram-negative. They occurred singly or in pairs. Each cell appeared to be highly motile by means of a single polar flagellum (Fig. 1b). In the stationary growth phase, some rods became longer and chains containing up to five to six cells were formed. Moreover, round, poorly refractile bodies (1–1.5 µm in diameter) were observed occasionally in stationary-phase cultures. Spores were not produced.

Strain SL6T grew at temperatures between 50 and 80 °C, with an optimum around 75 °C, whereas no
growth was detected at 45 or 81 °C (Fig. 2a). Growth was observed between pH 6.5 and 6.8, the optimum being around pH 6.5 (data not shown). No growth was detected at pH 6.2 or 6.9 after 96 h incubation at 70 °C.

Growth could be observed at NaCl concentrations ranging from 5 to 55 g l⁻¹ (Fig. 2b), the optimum being approximately 30 g l⁻¹. No growth was observed at NaCl concentrations of 0 or 60 g l⁻¹ after 96 h incubation at 70 °C. Under optimal growth conditions (i.e. optimal temperature, pH and NaCl concentration) without agitation, the doubling time of strain SL6ᵀ was around 3.1 h.

The novel isolate was a strictly anaerobic organism, growing chemolithoautotrophically by sulfate reduction using molecular hydrogen as the electron donor. Hydrogen was essential for growth. Growth was accompanied by exponential H₂S production that followed the growth curve (data not shown). Growth was prevented in the presence of low levels of oxygen. Of the alternative possible electron acceptors tested in combination with H₂ as the electron donor, sulfur, thiosulfate, cystine, sulfate, nitrate and fumarate could not be used by strain SL6ᵀ. In the presence of H₂, CO₂ and sulfate, the lag phase was reduced when acetate (even at 1 mM), fumarate, 3-methylbutyrate, glutamate, yeast extract, peptone or tryptone was added to the culture medium. Under the same conditions, glucose, fructose and valerate had no effect on growth, whereas malate, ethanol, formate, isovalerate, propionate, butyrate, isobutyrate, 2-methylbutyrate, propanol and succinate were slightly inhibitory. Growth was inhibited completely in the presence of pyruvate, lactate, caprylate, caproate, caprate or heptanoate. No growth was observed when the following compounds were used as electron donors [with N₂/CO₂ (80:20; 200 kPa) as the headspace] or as carbon sources [with H₂ (100%; 100 kPa) as the headspace] in the presence of 20 mM sulfate: acetate, formate, methanol, monomethylamine, malate, propionate, glutamate, fumarate, tryptone, peptone, valerate, isovalerate, butyrate, isobutyrate, 2-methylbutyrate, 3-methylbutyrate, glucose, fructose, ethanol, propanol, pyruvate, lactate, succinate, caprylate, caproate, caprate and heptanoate. No fermentative growth was obtained on pyruvate, lactate or malate. Ammonium was used preferentially as the nitrogen source, but growth also occurred in the presence of nitrate, peptone or tryptone. Desulfoviridin was absent in strain SL6ᵀ.

The growth of strain SL6ᵀ was inhibited by ampicillin, chloramphenicol and rifampicin at the lowest concentration tested (25 μg ml⁻¹). The organism did not grow in the presence of tetracycline (50 μg ml⁻¹). Growth occurred in the presence of penicillin G (100 μg ml⁻¹), kanamycin and streptomycin (each at 200 μg ml⁻¹).

**DNA base composition**

The G+C content of the DNA of strain SL6ᵀ, determined using the thermal denaturation method, was 28 mol%.

**16S rDNA sequence analysis**

The 16S rRNA sequence analysis showed that strain SL6ᵀ always clustered together with all other cultivated members of the genus *Thermodesulfobacterium*. 

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**Fig. 1.** (a) Phase-contrast micrograph of strain SL6ᵀ; bar, 5 μm. (b) Electron micrograph of a negatively stained cell of strain SL6ᵀ showing a single polar flagellum; bar, 500 nm.

**Fig. 2.** Effect of (a) temperature (in the presence of 30 g NaCl l⁻¹ at pH 6.5) and (b) NaCl concentration (at 70 °C and at pH 6.5) on growth of strain SL6ᵀ.
Thermodesulfobacterium hydrogeniphilum sp. nov.

Fig. 3. Phylogenetic tree showing the position of Thermodesulfobacterium hydrogeniphilum SL6T. The tree was generated using maximum-likelihood analysis. The scale bar represents the number of fixed mutations per nucleotide position. The numbers at the branch nodes are bootstrap values based on 100 bootstrap trees (bootstrap values below 50 are represented by asterisks).

Strain SL6T was more closely related to OPB45 (97.2% similarity), an environmental clone sequence obtained from a hot spring at Yellowstone National Park (Hugenholtz et al., 1998) (Fig. 3). It also placed strain SL6T as a close relative of Thermodesulfobacterium commune YSRA-1T (96.8% similarity) and Thermodesulfobacterium hveragerdense JSPT (96.2% similarity). Lower similarity values were obtained with environmental clone sequences SRI-93 (96.0%) and SRI-27 (95.1%), retrieved from hot-spring microbial mats (Skirnisdottir et al., 2000).

Strains SL6T and OPB45 clustered together in 38% of the bootstrap trees. However, because of the similarity of all the 16S rRNA sequences in the Thermodesulfobacterium group, it was difficult to resolve this association, and other phylogenetic methods did not result in higher bootstrap values. Our results confirmed that the overall position of the Thermodesulfobacterium group and Thermotogales is not clearly resolved. The Thermotogales most often branched more deeply than the Thermodesulfobacterium group (39 of 100 bootstrap resamplings), or the two orders grouped together (36 of 100 bootstrap trees); occasionally, the Thermodesulfobacterium group branched more deeply than the Thermotogales (11 of the 100 bootstrap trees).

Ecological relevance

Prior to this report, Thermodesulfobacterium species had been isolated only from terrestrial hot springs (Zeikus et al., 1983; Sonne-Hansen & Ahring, 1999) and oil reservoirs (Rozanova & Khudyakova, 1974; Christensen et al., 1992; L’Haridon et al., 1995). Here, we provide the description of the first organism belonging to this genus to be isolated from a marine environment and, more specifically, from a deep-sea hydrothermal vent site at Guaymas Basin. Its substrates (hydrogen, CO₂, sulfate) are commonly found in hydrothermal fluids and the surrounding sea water (Jannasch & Mottl, 1985). Acetate, which stimulates the growth of strain SL6T in the presence of these substrates, is also present at Guaymas Basin hydrothermal vent sites (Martens, 1990). Since the optimal range of temperatures for growth of strain SL6T (60–75 °C) covers the temperature range for which sulfate reduction has been measured in sediment slurries collected at Guaymas Basin (Jørgensen et al., 1990), it appears that strain SL6T may contribute to primary production at these sites.

Taxonomic position

When a number of different taxonomic parameters were compared, strain SL6T differed from the three described Thermodesulfobacterium species (Table 1). The novel isolate differs from them in its salinity range and optimum for growth, in its chemolithoautotrophic mode of life and in its inability to reduce thiosulfate. Its physiological properties, in combination with the results of the 16S rRNA gene sequence comparison, indicate that strain SL6T represents a novel species within the genus Thermodesulfobacterium. We propose to name this novel species Thermodesulfobacterium hydrogeniphilum sp. nov., with strain SL6T as the type strain.

According to the description of the genus Thermodesulfobacterium by Zeikus et al. (1983), its members are chemo-organotrophic, fermentative sulfate-reducers that use lactate, pyruvate and hydrogen as electron donors and sulfate and thiosulfate as electron acceptors for growth. However, Thermodesulfobacterium hydrogeniphilum is a chemolithoautotrophic organism unable to support a fermentative metabolism and unable to use thiosulfate as an electron acceptor. Since the most recent description of the genus (Hatchikian & Ollivier, 2001) is also not in agreement with the phenotypic characteristics of Thermodesulfobacterium hveragerdense and Thermodesulfobacterium hydrogeniphilum, we propose that the description of the genus Thermodesulfobacterium should be emended as described below.

Emended description of the genus Thermodesulfobacterium

Straight, rod-shaped cells, approximately 0.3–2.8 µm in size, occurring singly, in pairs or in chains. Possess an outer wall membrane layer. Gram-negative. Cells may form ‘blebs’ at their ends. Contain non-phytanyl ether-linked lipids, cytochrome c₅ and desulfofuscidin but no desulfoviridin. Thermophilic. Strict anaerobes
**Table 1. Main characteristics of cultivated members of the genus Thermodesulfobacterium**

Data were obtained from Rozanova & Pivovarova (1988), Zeikus et al. (1983) and Sonne-Hansen & Ahring (1999). Electron donors were tested with CO$_2$ as the carbon source. All taxa used H$_2$ + acetate (2 mM) as an electron donor. (+), Slight growth; ND, not determined.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain SL6$^T$</th>
<th>T. commune</th>
<th>T. thermophilum</th>
<th>T. hveragerdense</th>
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<td>Cell size ($\mu$m)</td>
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<td>34</td>
<td>38 (31)*</td>
<td>40</td>
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<td>0</td>
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<td>70–74</td>
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<td>H$_2$</td>
<td>+†</td>
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</table>

*The value in parentheses was determined by Henry et al. (1994).
†Autotrophic growth.

that use sulfate as electron acceptor; the sulfate is reduced to sulfide. Nutritionally versatile (chemoorganotrophic or chemolithoautotrophic). Acetate (1–2 mM) may be required for growth with H$_2$ and CO$_2$ in the presence of sulfate. Occurs in thermal environments including hot springs, hot oil reservoirs and deep-sea hydrothermal vents. The G + C content of the DNA is between 28 and 40 mol%. The type species is *Thermodesulfobacterium commune*.

Description of *Thermodesulfobacterium hydrogeniphilum* sp. nov.

*Thermodesulfobacterium hydrogeniphilum* (hy.dro.ge.ni.phi.lum. N.L. n. *hydrogenum* hydrogen; Gr. n. *philos* friend; N.L. adj. *hydrogeniliking*, since growth depends upon the presence of hydrogen).

Each cell is highly motile by means of a single polar flagellum. Cells occur singly and in pairs and are 0.5–0.8 µm long by 0.4–0.5 µm wide. Growth occurs between 50 and 80 °C, the optimum being approximately 75 °C (doubling time 186 min). Growth occurs between pH 6.3 and 6.8, the optimum being approximately pH 6.5, and at NaCl concentrations ranging from 5 to 55 g l$^{-1}$, the optimum being approximately 30 g l$^{-1}$. Strictly chemolithoautotrophic. Sulfate serves as an electron acceptor in the presence of H$_2$. In the presence of H$_2$, CO$_2$ and sulfate, acetate, fumarate, 3-methylbutyrate, glutamate, yeast extract, peptone and tryptone stimulate growth. Sulfur, thiosulfate, sulfite, cystine, fumarate and nitrate are not used as electron acceptors. Growth is inhibited by ampicillin, chloramphenicol and rifampicin at 25 µg ml$^{-1}$. Growth occurs in the presence of tetracycline at 25 µg ml$^{-1}$, penicillin G at 100 µg ml$^{-1}$, streptomycin at 200 µg ml$^{-1}$ and kanamycin at 200 µg ml$^{-1}$. The G + C content of the DNA of the type strain is 28 mol% (thermal denaturation method).

The type strain is SL6$^T$ (= DSM 14290$^T$ = JCM 11239$^T$), which was obtained from deep-sea hydrothermal vent sulfides at Guaymas Basin.

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