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

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A thermophilic, non-spore-forming, marine, sulfate-reducing bacterium, strain SL6[T], 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}. H$_2$ and CO$_2$ 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 SL6[T] 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 SL6[T] (= DSM 14290[T] = JCM 11239[T]). Because of the phenotypic characteristics of the novel species, it is also proposed that the description of the genus *Thermodesulfobacterium* requires emendation.

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 *Thermodesulforhabdus norvegicus* (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 hydrothermale* and a novel species belonging to the genus *Thermodesulforhabdus*, 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 *Desulfovibrio thermophilus* by Rozanova & Khudyakova (1974) and later reclassified illegitimately as *Thermodesulfobacterium mobile* by Rozanova & Pivovarova (1988)], isolated from hot stratal water from a petroleum reservoir. These species have a relatively restricted type of nutrition and are incomplete oxidizers, using H_2, 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[T], 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 N_2 (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 (l^{-1} distilled water): 30 g sea salts (Sigma), 1 g NH_4Cl, 0·35 g KH_2PO_4, 3·46 g PIPES, 1 g $NaHCO_3$, 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 B_{12}, 1 ml growth-stimulating factors (Pfennig *et al.*, 1981) and 1 mg resazurin. The pH of the medium was adjusted to 6·5 using 5 M HCl before autoclaving. H_2/CO_2 (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) Phytagel by using shake dilution series (Widdel & Bak, 1992). Tubes were incubated at 65 °C for 7 days under a H_2/CO_2 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[T] 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[T] was routinely grown in the following medium containing (l^{-1} distilled water): 30 g NaCl, 3 g $MgCl_2$. $2H_2O$, 4 g Na_2SO_4, 0·5 g KCl, 0·25 g NH_4Cl, 0·2 g KH_2PO_4, 3·46 g PIPES, 0·15 g $CaCl_2$. $2H_2O$, 0·5 g Difco yeast extract, 1 mM sodium acetate, 1 ml trace-element mixture (Widdel & Bak, 1992), 0·2 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 B_{12}, 1 ml growth-stimulating factors (Pfennig *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. H_2/CO_2 (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) $NaHCO_3$ and 8% (w/v) Na_2CO_3. 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 N_2/CO_2 (80:20; 200 kPa) as the headspace] or carbon sources [with H_2 (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), tryptone (0·1 %), peptone (0·1 %), valerate (5), isovalerate (5), butyrate (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 H_2/CO_2 (80:20; 200 kPa) as the headspace. Ammonium chloride (5 mM), nitrate (5 mM), peptone (0·1 %) and tryptone (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 NaOH to a resuspended pellet of cells and viewing the preparation under UV light, as described by Postgate (1959). *Desulfovibrio giganteus* (DSM 4123[T]) was used as a positive control.

Antibiotic susceptibility. The sensitivity of strain SL6[T] to ampicillin, chloramphenicol, kanamycin, penicillin G, streptomycin, tetracycline and rifampicin (Sigma) was tested at concentrations of 25, 50, 100 and 200 μg ml^{-1}.

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-/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.

H_2S production. H_2S production was evaluated by adding 500 μl of a solution of $CuSO_4$ (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 SL6[T] 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.

PCR amplification and sequencing of the 16S rDNA. The 16S rRNA genes from strain SL6[T] genomic DNA were amplified, with a PCR, in reactions containing 50 mM KCl, 30 mM Tris/HCl (pH 8·3), 1·5 mM $MgCl_2$, 200 μM each of dATP, dCTP, dGTP and dTTP, 0·05 % Igepal (Sigma), 1 U *Taq* polymerase and 20 pmol of each primer (5′-AGAGTTTG-ATCCTGGCTCAG-3′ and 5′-GGTTACCTTGTTACGA-CTT-3′, corresponding to positions 8–27 and 1492–1510 in the *E. coli* rRNA 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 were used in the phylogenetic analysis. Distance trees were constructed by using the least-squares algorithm of De Soete (1983). Maximum-likelihood trees and corresponding bootstrap proportions were constructed by using the program fastDNAmL (Olsen *et al.*, 1994a). Bootstrap values were obtained for 100 replicate trees.

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 H_2/CO_2 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 (Jeanthon *et al.*, 1999). An intense H_2S 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 SL6[T], was isolated and studied in detail.

Phenotypic characteristics

Cells of strain SL6[T] 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 SL6[T] grew at temperatures between 50 and 80 °C, with an optimum around 75 °C, whereas no
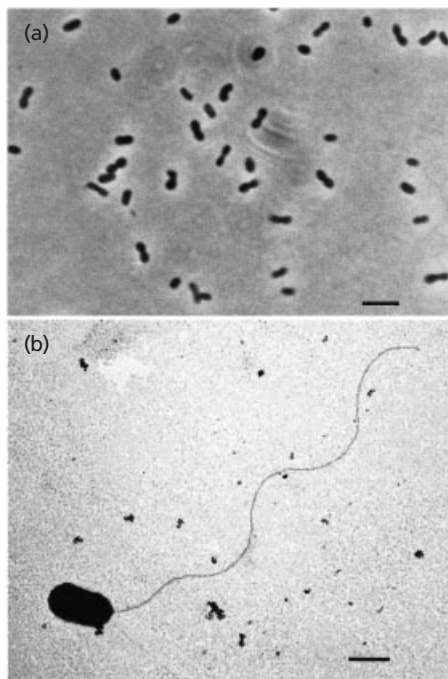
Fig. 1. (a) Phase-contrast micrograph of strain SL6^T; bar, 5 μm. (b) Electron micrograph of a negatively stained cell of strain SL6^T showing a single polar flagellum; bar, 500 nm.
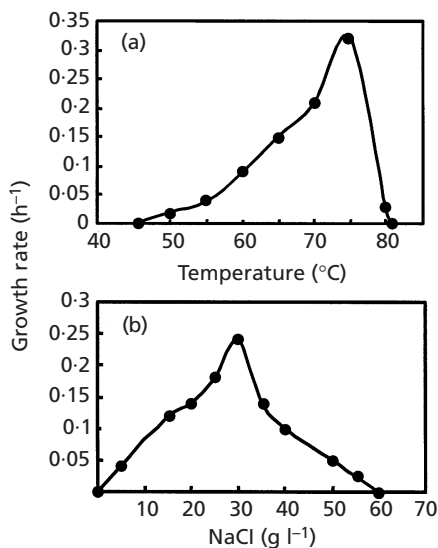
Fig. 2. Effect of (a) temperature (in the presence of 30 g NaCl l^{-1} at pH 6·5) and (b) NaCl concentration (at 70 °C and at pH 6·5) on growth of strain SL6^T.

growth was detected at 45 or 81 °C (Fig. 2a). Growth was observed between pH 6·3 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 SL6^T 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 H$_2$S 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 H$_2$ as the electron donor, sulfur, thiosulfate, cystine, sulfite, nitrate and fumarate could not be used by strain SL6^T. In the presence of H$_2$, CO$_2$ 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 N$_2$/CO$_2$ (80:20; 200 kPa) as the headspace] or as carbon sources [with H$_2$ (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 SL6^T.

The growth of strain SL6^T 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 SL6^T, determined using the thermal denaturation method, was 28 mol%.

16S rDNA sequence analysis

The 16S rRNA sequence analysis showed that strain SL6^T always clustered together with all other cultivated members of the genus *Thermodesulfobacterium*.
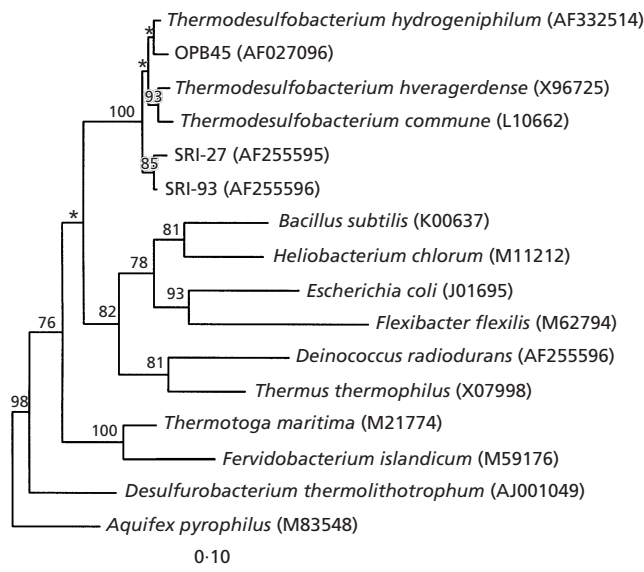
Fig. 3. Phylogenetic tree showing the position of *Thermodesulfobacterium hydrogeniphilum* SL6^T. The tree was generated using maximum-likelihood analysis. The scale bar represents the number of fixed mutations per nucleotide position. The numbers at the branch nodes are bootstrap values based on 100 bootstrap trees (bootstrap values below 50 are represented by asterisks).

Strain SL6^T 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 SL6^T as a close relative of *Thermodesulfobacterium commune* YSRA-1^T (96·8 % similarity) and *Thermodesulfobacterium hveragerdense* JSP^T (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 SL6^T 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, CO_2, sulfate) are commonly found in hydrothermal fluids and the surrounding sea water (Jannasch & Mottl, 1985). Acetate, which stimulates the growth of strain SL6^T 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 SL6^T (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 SL6^T may contribute to primary production at these sites.

Taxonomic position

When a number of different taxonomic parameters were compared, strain SL6^T 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 SL6^T represents a novel species within the genus *Thermodesulfobacterium*. We propose to name this novel species *Thermodesulfobacterium hydrogeniphilum* sp. nov., with strain SL6^T 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 c_3 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.

Characteristic	Strain SL6^T	*T. commune*	*T. thermophilum*	*T. hveragerdense*
Cell size (µm)	$0.4–0.5 \times 0.5–0.8$	0.3×0.9	0.6×2.0	0.5×2.8
Motility/flagellation	+/Single polar	−	+/Single polar	−
G+C content (mol %)	28	34	38 (31)*	40
Optimal salinity (g l^{-1})	30	0	1	0
Optimal temperature (°C)	75	70	65	70–74
Electron donors:				
H$_2$	+†	−	−	−
Formate	−	ND	+	−
Lactate	−	+	+	+
Pyruvate	−	+	+	+
Fermentation of:				
Pyruvate	−	+	+	+
Lactate	−	−	(+)	−
Electron acceptors:				
Thiosulfate	−	+	+	+
Sulfite	−	−	+	+

* 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 (chemo-organotrophic 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. *hydrogeniphilum* hydrogen-liking, 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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