Deferribacter autotrophicus sp. nov., an iron(III)-reducing bacterium from a deep-sea hydrothermal vent

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A thermophilic, anaerobic, chemolithoautotrophic bacterium (designated strain SL50^T) was isolated from a hydrothermal sample collected at the Mid-Atlantic Ridge from the deepest of the known World ocean hydrothermal fields, Ashadze field (12° 58' 21" N 44° 51' 47" W) at a depth of 4100 m. Cells of strain SL50^T were motile, straight to bent rods with one polar flagellum, 0.5–0.6 µm in width and 3.0–3.5 µm in length. The temperature range for growth was 25–75 °C, with an optimum at 60 °C. The pH range for growth was 5.0–7.5, with an optimum at pH 6.5. Growth of strain SL50^T was observed at NaCl concentrations ranging from 1.0 to 6.0% (w/v) with an optimum at 2.5% (w/v). The generation time under optimal growth conditions for strain SL50^T was 60 min. Strain SL50^T used molecular hydrogen, acetate, lactate, succinate, pyruvate and complex proteinaceous compounds as electron donors, and Fe(III), Mn(IV), nitrate or elemental sulfur as electron acceptors. The G + C content of the DNA of strain SL50^T was 28.7 mol%. 16S rRNA gene sequence analysis revealed that the closest relative of strain SL50^T was *Deferribacter abyssi* JR^T (95.5% similarity). On the basis of its physiological properties and phylogenetic analyses, the isolate is considered to represent a novel species, for which the name *Deferribacter autotrophicus* sp. nov. is proposed. The type strain is SL50^T (=DSM 21529^T=VKPM B-10097^T). *Deferribacter autotrophicus* sp. nov. is the first described deep-sea bacterium capable of chemolithoautotrophic growth using molecular hydrogen as an electron donor and ferric iron as electron acceptor and CO₂ as the carbon source.

Fe(III)-reducing micro-organisms play an important role in the cycling of carbon and metals in various anaerobic ecosystems including thermal environments. Thermophilic iron reducers have been found in continental hot springs, deep terrestrial subsurface and submarine petroleum reservoirs (Slobodkin, 2005). However, the diversity of this group of prokaryotes in deep-sea hydrothermal ecosystems has been much less studied (Miroshnichenko & Bonch-Osmolovskaya, 2006). At present, thermophilic and hyperthermophilic iron-reducing micro-organisms recovered from deep-sea habitats include three representatives of *Archaea* (Slobodkin *et al.*, 2001; Kashefi *et al.*, 2002; Reysenbach *et al.*, 2006) and two species of *Bacteria*,

Geothermobacter ehrlichii (Kashefi *et al.*, 2003) and *Deferribacter abyssi* (Miroshnichenko *et al.*, 2003).

The order *Deferribacterales* represents a deep lineage in the domain *Bacteria* (Cole *et al.*, 2007). At the time of writing, the family *Deferribacteraceae* (Huber & Stetter, 2002) comprised three genera, *Deferribacter*, *Flexistipes* and *Geovibrio*. Currently, the genus *Deferribacter* consists of three species, *D. thermophilus* (Greene *et al.*, 1997), *D. desulfuricans* (Takai *et al.*, 2003) and *D. abyssi* (Miroshnichenko *et al.*, 2003). The type species of the genus, *D. thermophilus*, was isolated from a high-temperature, seawater-flooded oil reservoir located in the North Sea. The other two micro-organisms were isolated from deep-sea hydrothermal vents. *D. desulfuricans* was obtained from the Suiyo Seamount hydrothermal chimney and *D. abyssi* was isolated from the Rainbow hydrothermal vent field of the Mid-Atlantic Ridge. All recognized species

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SL50^T is EU407777.

of the genus *Deferribacter* are strictly anaerobic, thermophilic organisms capable of the oxidation of a variety of complex organic compounds and organic acids in the presence of diverse electron acceptors. In this paper, we report the isolation and characterization of a novel species of the genus *Deferribacter* from the deepest of the known World ocean hydrothermal fields.

Strain SL50^T was isolated from a sample of a fragment of the hydrothermal structure. The sample was collected in March 2007 during the Serpentine cruise at the Ashadze hydrothermal field (12° 58' 21" N 44° 51' 47" W) on the Mid-Atlantic Ridge at a depth of 4100 m. For the sample collection, sterilized microbiological boxes filled with sterile freshwater were prepared onboard. Active chimney samples were collected by the ROV Victor. On site, after opening the box lid, the freshwater was replaced by *in situ* seawater, the chimney fragment was introduced and the lid was closed. All following operations were done onboard under sterile conditions. Boxes with samples were stored at 4 °C. An enrichment culture was initiated by inoculation of 10 % (w/v) of the sample into anaerobically prepared, bicarbonate-buffered, sterile (135 °C, 1 h) liquid medium with lactate (1.5 g l⁻¹) as an electron donor and poorly crystalline Fe(III) oxide (90 mM) as an electron acceptor. Medium composition and preparation techniques have been described previously (Slobodkin *et al.*, 1999). Isolate SL50^T was purified from the enrichment by serial dilution in the same medium. A pure culture was obtained with sodium acetate (18 mM) as electron donor and potassium nitrate (10 mM) as electron acceptor by using the agar-shake dilution technique with an agar block in the tube (1.5 % agar in growth medium) in medium of the following composition [per litre distilled water: 0.34 g KCl, 4.00 g MgCl₂·6H₂O, 0.25 g NH₄Cl, 0.14 g CaCl₂·2H₂O, 0.14 g K₂HPO₄, 18.00 g NaCl, 5.00 g NaHCO₃, 0.20 g yeast extract (Difco), 0.002 g Fe(NH₄)₂(SO₄)₂·7H₂O, 1 ml trace-element solution (Slobodkin *et al.*, 1997), 10 ml vitamin solution (Wolin *et al.*, 1963), 0.001 g resazurin, 0.50 g Na₂S·9H₂O, gas phase CO₂ (100 %)]. Physiological studies on substrate and electron acceptor utilization, temperature, pH and salinity ranges for growth, light and electron microscopy, analytical techniques, DNA extraction and determination of the G+C content were performed as described previously (Slobodkin *et al.*, 1999). Growth of strain SL50^T with poorly crystalline Fe(III) was determined by direct cell counts using light microscopy after dissolving the iron precipitate in a solution of ammonium oxalate (28 g l⁻¹)/oxalic acid (15 g l⁻¹). pH measurements and pH-metre calibration were carried out at 60 °C. NO, N₂O and N₂ were detected by GC (Molsiv 5A column at 40 °C; Ar). Ammonium and nitrite were determined by HPLC with a conductivity detector (Aquilon C1P column, 4 mM HNO₃ for ammonium and Aquilon A1.2 column, 3.5 mM carbonate buffer for nitrite). 16S rRNA gene amplification, sequencing and sequence analysis were done as described previously (Zavarzina *et al.*, 2002).

In agar-shake cultures, brown lens-shaped colonies (0.2–0.5 mm in diameter) of strain SL50^T appeared after 7–10 days of incubation at 50 °C. Vegetative cells of strain SL50^T were straight to bent rods, 0.5–0.6 µm in width and 3.0–3.5 µm in length. The cells occurred singly, in pairs or in short chains, and had one polar flagellum. Spores were not observed. Ultrathin sectioning of cells of strain SL50^T revealed that the cell wall had a typical Gram-negative structure.

The temperature range for growth of strain SL50^T was 25–75 °C, with an optimum at 60 °C. No growth was detected at 80 °C or at temperatures up to 22 °C after incubation for 3 weeks. The pH range for growth was 5.0–7.5, with an optimum at pH 6.5. No growth was observed at pH 4.5 or 8.0. Growth of strain SL50^T was observed at NaCl concentrations ranging from 1.0 to 6.0 % (w/v) with an optimum at 2.5 % (w/v), but no growth was evident in 0 or 7.0 % NaCl (w/v). Yeast extract was not necessary for growth of strain SL50^T but strongly stimulated it. Potential electron acceptors were tested with sodium acetate (18 mM) or sodium lactate (1.5 g l⁻¹) as an energy source in the presence of 0.20 g yeast extract l⁻¹. Nitrate (potassium salt, 10 mM), elemental sulfur (10 g l⁻¹), ferric citrate (5 mM), poorly crystalline Fe(III) oxide (90 mM), Mn(IV) supplied as 25 mM of MnO₂ and 9,10-anthraquinone 2,6-disulfonate (AQDS; 20 mM) were used as an electron acceptor for growth of strain SL50^T. Sulfate (14 mM), thiosulfate and fumarate (20 mM each) were not reduced and did not support growth. Poorly crystalline Fe(III) oxide was reduced to black magnetic precipitate with high Fe(II) content. During Mn(IV) reduction, black insoluble MnO₂ turned to a light-brown precipitate that almost disappeared after prolonged incubation. No changes in colour or precipitate amount were observed in uninoculated controls with 0.20 g yeast extract l⁻¹ in growth media containing poorly crystalline Fe(III) oxide, Mn(IV) or AQDS during the incubation period at 60 °C. Elemental sulfur was reduced to hydrogen sulfide (Cord-Ruwisch, 1985). Nitrate was reduced to ammonium; NO, N₂O or nitrite were not produced in measurable amounts.

Strain SL50^T was able to grow on peptone and yeast extract (10 g l⁻¹ each), formate, acetate, lactate, pyruvate, fumarate, malate, propionate, succinate, maleinate and maltose (25 mM each) as electron donors and potassium nitrate (10 mM) as electron acceptor in the presence of 0.20 g yeast extract l⁻¹. Maltose was completely oxidized to CO₂ without formation of soluble fermentation products. Strain SL50^T could grow chemolithoautotrophically in the absence of yeast extract, using molecular hydrogen as an electron donor and poorly crystalline Fe(III) oxide (90 mM) as electron acceptor and CO₂ as the carbon source. When Mn(IV) was used as an electron acceptor, H₂/CO₂ (80/20, v/v) was utilized and supported growth in the presence of yeast extract (0.20 g l⁻¹). With nitrate or sulfur as an electron acceptor molecular hydrogen did not support chemolithoautotrophic growth or growth in the presence of 0.20 g yeast extract l⁻¹. Strain SL50^T was not

able to utilize casein, tryptone, starch (10 g l⁻¹ each), methanol, ethanol, n-propanol, iso-propanol, n-butanol, (20 mM each), fructose, xylose, cellobiose, sucrose, L-arabinose (25 mM each), glycerol, butyrate or benzoate (20 mM each) with potassium nitrate (10 mM) as electron acceptor.

The G + C content of the genomic DNA of strain SL50^T was 28.7 mol% (*T*_m). A comparison of 1543 nucleotides of the 16S rRNA gene sequence of strain SL50^T with those available in the GenBank database showed that strain SL50^T belonged to the genus *Deferribacter* (Fig. 1). Only 16S rRNA gene sequences of type strains of species with validly published names were included in the analyses. The 16S rRNA gene sequence of the novel isolate had the highest similarity with that of *D. abyssi* JR^T (95.5%). The levels of 16S rRNA gene sequence similarity with other members of the genus *Deferribacter* were 94.3–94.6%. The trees constructed by using maximum-likelihood and maximum-parsimony algorithms displayed the same topology (data not shown). Transversion analysis (Woese *et al.*, 1991) did not affect the phylogenetic position of the novel strain.

The novel isolate described in this report represents a micro-organism capable of reduction of Fe(III) and Mn(IV) as well as nitrate and sulfur, which are also common substances in deep-sea hydrothermal environments. Strain SL50^T shared many phenotypic features with recognized representatives of the genus *Deferribacter* (Table 1). Species of the genus *Deferribacter*, with the exception of *D. thermophilus*, were isolated from deep-sea hydrothermal vents. According to the electron microscopic analysis all of them have a Gram-negative type of cell-wall structure. Cells of all *Deferribacter* species are rod-shaped with a polar flagellum and grow under anaerobic conditions by the oxidation of a variety of complex organic compounds and organic acids in the presence of diverse electron acceptors, but not by fermentation. However, strain SL50^T differed from all representatives of the genus *Deferribacter* by its ability to grow chemolithoautotrophically, utilizing hydrogen as an electron donor, CO₂ as a carbon source and poorly

crystalline Fe(III) oxide as an electron acceptor. Strain SL50^T had the widest range of utilized electron acceptors of the recognized species of this genus. Unlike the type species of the genus, *D. thermophilus*, strain SL50^T was able to reduce sulfur. The ability of strain SL50^T to reduce both Fe(III) and Mn(IV) differentiated it from *D. abyssi*, which cannot utilize Mn(IV) and *D. desulfuricans*, which is unable to use either of these metals as electron acceptors. In contrast to the type species of the genus and the phylogenetically closest species, *D. abyssi*, strain SL50^T could grow on formate and propionate as substrates. Other significant characteristics that differentiate strain SL50^T from all recognized representatives of the genus are nitrate reduction to ammonium, but not to nitrite, and the ability to use disaccharide (maltose) as an electron donor with nitrate as electron acceptor. On the basis of the phylogenetic, phenotypic and physiological properties that clearly differentiate strain SL50^T from recognized species of the genus *Deferribacter*, we propose that strain SL50^T represents the type strain of a novel species, with the name *Deferribacter autotrophicus* sp. nov.

Description of *Deferribacter autotrophicus* sp. nov.

Deferribacter autotrophicus (au.to.tro'phi.cus. N.L. masc. adj. *autotrophicus* autotrophic).

Cells are motile by means of one polar flagellum, straight to bent rods, 0.5–0.6 µm in width and 3.0–3.5 µm in length; spores were not observed. Cells form brown lens-shaped colonies (0.2–0.5 mm in diameter) in agar-shake cultures. Temperature range for growth is 25–75 °C, with an optimum at 60 °C. pH range for growth is 5.0–7.5, with an optimum at pH 6.5. Growth occurs at NaCl concentrations ranging from 1.0 to 6.0% (w/v), with an optimum at 2.5% (w/v). Anaerobic. Capable of chemolithoautotrophic growth using molecular hydrogen as an electron donor and ferric iron as electron acceptor and CO₂ as the carbon source. Anaerobically oxidizes peptone, yeast extract (10 g l⁻¹ each), formate, acetate, lactate, pyruvate, fumarate, malate, propionate, succinate, maleinate and maltose with

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences indicating the position of strain SL50^T within representative members of the order *Deferribacterales*. The 16S rRNA gene sequence of *Clostridium butyricum* was included as an outgroup. GenBank accession numbers are given in parentheses. Bootstrap percentages (based on 500 replications) greater than 70% are indicated at nodes. Bar, 5 substitutions per 100 nt.

Table 1. Characteristics that differentiate strain SL50^T from other *Deferribacter* species

Strains: 1, strain SL50^T (*D. autotrophicus* sp. nov.); 2, *D. abyssi* JR^T (data from Miroshnichenko *et al.*, 2003); 3, *D. thermophilus* BMA^T (Greene *et al.*, 1997); 4, *D. desulfuricans* SSM1^T (Takai *et al.*, 2003). +, Positive; –, negative.

Characteristic	1	2	3	4
Temperature range (°C)	25–75	45–65	50–65	40–70
Optimal temperature (°C)	60	60	60	60–65
Electron acceptor				
Elemental sulfur	+	+	–	+
Fe(III)	+	+	+	–
Mn(IV)	+	–	+	–
Electron donor				
Ethanol	–	–	–	+
Formate	+	–	–	+
Propionate	+	–	–	+
Malate	+	–	–	+
Maltose	+	–	–	–
Chemolithoautotrophic growth with electron acceptor				
Fe(III)	+	–	–	–
Elemental sulfur	–	+	–	–
Potassium nitrate	–	+	–	–
DNA G + C content (mol%)	28.7	30.8	34.0	38.6

sulfur, nitrate, Mn(IV) or Fe(III) as electron acceptor in the presence of 0.20 g yeast extract l^{–1}. Casein, tryptone, starch, methanol, ethanol, n-propanol, iso-propanol, n-butanol, fructose, xylose, cellobiose, sucrose, L-arabinose, glycerol, butyrate and benzoate are not utilized with nitrate as electron acceptor. Does not reduce fumarate, sulfate, thiosulfate or oxygen (20 %, v/v in the gas phase). The G + C content of the DNA of the type strain is 28.7 mol% (*T_m*).

The type strain, SL50^T (=DSM 21529^T=VKPM B-10097^T), was isolated from a hydrothermal vent field of the Mid-Atlantic Ridge.

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