Deferribacter abyssi sp. nov., an anaerobic thermophile from deep-sea hydrothermal vents of the Mid-Atlantic Ridge

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Two strains of thermophilic, anaerobic, chemolithoautotrophic bacteria, designated JR^T and DR, were isolated from hydrothermal samples collected on the Mid-Atlantic Ridge from the Rainbow (36° 16' N, 33° 54' W) and Menez Gwen (37° 50' N, 31° 50' W) vent fields, respectively. Cells of both isolates were short, straight- to vibrio-shaped, motile rods with one polar flagellum, and were Gram-negative and non-sporulating. Strain JR^T was characterized in detail. It was found to grow optimally at pH 6.5–6.7, at 60 °C and in the presence of 30 g NaCl l⁻¹. Strain JR^T could use molecular hydrogen, acetate, succinate, pyruvate and proteinaceous compounds as electron donors, and elemental sulfur, nitrate or Fe(III) as electron acceptors. No fermentation of organic substrates occurred. The G + C content of the DNA of strain JR^T was 30.8 mol%. Strain DR (= DSM 14927) possessed the same morphology and pH, temperature and salinity optima and ranges, and used the same electron acceptors as strain JR^T. On the basis of their 16S rDNA sequences (1517 nucleotides), strains JR^T and DR were identical and distantly related to *Deferribacter thermophilus* and *Deferribacter desulfuricans* (95.3 and 95.2 % sequence similarity, respectively). Based on their phenotypic and phylogenetic characteristics, it is proposed that both strains are members of a new species of the genus *Deferribacter*, for which the name *Deferribacter abyssi* (type strain JR^T = DSM 14873^T = JCM 11955^T) is proposed.

Members of the phylum *Deferribacteres* are organized into a single class, order and family (Garrity & Holt, 2001). The family *Deferribacteraceae* (Huber & Stetter, 2002) is composed of three genera, *Deferribacter*, *Flexistipes* and *Geovibrio*, which form one separate lineage on the 16S-rRNA-based phylogenetic tree with sequence similarity values of around 89 %. Currently, the only two species within the genus *Deferribacter* are *Deferribacter thermophilus* (Greene *et al.*, 1997) and *Deferribacter desulfuricans* (Takai *et al.*, 2003). The type species of the genus, *D. thermophilus*, was isolated from the production water of Beatrice oil field, a high-temperature, sea-water-flooded oil reservoir located in the North Sea, UK. The recently described second species of the genus, *D. desulfuricans*, was obtained from a deep-sea

hydrothermal vent chimney at the Suiyo Seamount in the Izu-Bonin Arc, Japan. Both described species 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 report, we describe a novel species of this genus, two representatives of which were obtained from two different deep-sea hydrothermal vent fields of the Mid-Atlantic Ridge.

Strains JR^T and DR were isolated from hydrothermal samples collected in May 2001 at the Rainbow (36° 16' N, 33° 54' W) and Menez Gwen (37° 50' N, 31° 50' W) vent fields of the Mid-Atlantic Ridge. At Rainbow (at a depth of 2400 m), an *in situ* growth chamber or vent cap (Reysenbach *et al.*, 2000) designed to concentrate the micro-organisms discharged by hydrothermal emissions was deployed using the hydraulic arm of the remotely operated vehicle (ROV) *Victor*. After *in situ* incubation for

The GenBank accession numbers for the 16S rDNA sequences of *Deferribacter abyssi* strains JR^T and DR are AJ515882 and AJ515881, respectively.

2 days, the vent cap was closed by the hydraulic arm of the ROV, before transportation to the surface. The fluid from the cap was used in further work. At Menez Gwen (at a depth of 850 m), a chimney fragment was collected and placed in an insulated container for the trip to the surface. No temperature measurements were done at either site. Once on board the ship, samples were immediately transferred 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 and stored at 4 °C until further processing at the laboratory.

For the enrichment of organisms, the following basal medium was used ($g\ l^{-1}$, unless indicated otherwise): NH_4Cl , 0.33; KCl , 0.33; KH_2PO_4 , 0.33; $CaCl_2 \cdot 2H_2O$, 0.33; $MgCl_2 \cdot 6H_2O$, 0.33; $NaCl$, 25.0; yeast extract, 0.1; $NaHCO_3$, 0.3; sodium acetate, 2; trace elements (Balch *et al.*, 1979), 10 ml l^{-1} ; vitamins (Wolin *et al.*, 1963), 10 ml l^{-1} . Elemental sulfur (10 g l^{-1}) or nitrate (2 g l^{-1}) was added as the electron acceptor. The media were prepared anaerobically and dispensed into 17 ml Hungate tubes; the headspace (7 ml) was filled with N_2 . The pH of the media before sterilization was 6.5.

After 3–5 days incubation at 60 °C, abundant growth of motile, straight- to vibrio-shaped organisms was detected in both types of enrichment medium. From the enrichment on sulfur-containing medium, strain JR^T was obtained by the agar-shake dilution technique in the same basal medium supplemented with polysulfides (Widdel & Pfennig, 1992) and solidified with 1.5% agar (Difco). Strain DR was obtained by the same technique, but on the solid medium supplemented with sodium nitrate (1 g l^{-1}) from the corresponding enrichment.

Strain JR^T was studied in detail. Cells of this strain were small rods of about 1.5–2 μm in length and 0.4–0.5 μm in width, which occurred singly, in pairs or in small chains. They were motile by means of one polar flagellum (Fig. 1a). In the stationary phase of growth, some rods took a spherical shape. Spores were never observed. Thin sections (Bonch-Osmolovskaya *et al.*, 1990) revealed that the cell wall of strain JR^T had a typical Gram-negative structure (Fig. 1b). Strain JR^T grew over a temperature range of 45–65 °C, with optimal growth observed at 60 °C. At 60 °C, the strain grew between pH 6.0 and 7.2, with an optimum around pH 6.5. Strain JR^T required NaCl for growth and grew at NaCl concentrations ranging from 10 to 50 g l^{-1} , with an optimum at 30 g NaCl l^{-1} . When air was added to the headspace of the tubes filled with basal medium (5 ml), supplemented with 2 g yeast extract l^{-1} and elemental sulfur, up to final oxygen concentrations of 0.1–1%, no growth of strain JR^T was observed.

Strain JR^T was found to grow using acetate, pyruvate, succinate, yeast extract, tryptone, peptone, Bio-trypticase, Bacto-tryptone (2 g l^{-1} , organic acids as their sodium salts) and molecular hydrogen [H_2/CO_2 (8:2, v/v, atmospheric

Fig. 1. Negatively stained whole cell (a) and thin-sectioned cell (b) of strain JR^T. Bars, 0.5 μm .

pressure)] as electron donors, and sulfur or nitrate as electron acceptors. It was not able to utilize glucose, sucrose, xylose, lactose, fructose, starch, methanol, ethanol, formate, propionate, butyrate, malate with or without sulfur (10 g l^{-1}) or nitrate (sodium salt, 1 g l^{-1}). Elemental sulfur was reduced to hydrogen sulfide (Trüper & Schlegel, 1964) in the course of sulfur reduction. Nitrate was reduced to nitrite; NO, N_2O or ammonium were not detected by methods described elsewhere (Miroshnichenko *et al.*, 2003c). The doubling time under the optimal growth conditions, on the medium with yeast extract and elemental sulfur, was around 55 min, with the final cell concentration exceeding 10^8 cells ml^{-1} . No growth on fermentable substrates occurred in the absence of electron acceptors. Strain JR^T could also grow chemolithoautotrophically on the basal medium in the absence of yeast extract, using molecular hydrogen as the electron donor, sulfur or nitrate as electron acceptors and CO_2 as the carbon source. The strain was also shown to be able to grow with amorphous Fe(III) oxide (90 mM) (Slobodkin *et al.*, 1999) or Fe(III) citrate (20 mM) as electron acceptors and acetate, succinate or molecular hydrogen as electron donors with approximately the same doubling time and cell yield as with other electron acceptors. Growth of strain JR^T with molecular hydrogen and amorphous Fe(III) oxide was, however, possible only in the presence of yeast extract (50 mg l^{-1} minimal concentration). Amorphous Fe(III) oxide was reduced to a black magnetic precipitate with a high Fe(II) content. Sulfate, thiosulfate, nitrite, malate (sodium salts, 1 g l^{-1}) and Mn(IV) supplied as 20 mM of MnO_2 were not used as electron acceptors and did not support the growth on any of the substrates. Growth of strain JR^T was inhibited by chloramphenicol, penicillin, rifampicin, streptomycin but not by tetracycline (all tested at a concentration of

100 µg ml⁻¹ at 60 °C). Because of its unstable growth, strain DR was characterized only tentatively. Nevertheless, it was found to have the same morphology, optima and ranges of pH, temperature and salinity and used the same electron acceptors as strain JR^T.

The G + C content of strain JR^T DNA, determined according to Miroshnichenko *et al.* (1994), was 30.8 mol%. Analysis of the almost-complete 16S rRNA gene sequences of strains JR^T and DR (1517 nucleotides), done as described previously (Rainey *et al.*, 1995; Maidak *et al.*, 2001; Jukes & Cantor 1969), indicated that their 16S sequences were identical and 95.3 and 95.2% similar to those of *D. thermophilus* BMA^T and *D. desulfuricans* DSM 14783^T, respectively. The binary similarity value for the latter two strains was 97.9%. A phylogenetic dendrogram (Fig. 2), displaying the position of strain JR^T in relation to the two described species of the genus *Deferribacter*, was reconstructed from a distance matrix using the treeing algorithm of De Soete (1983). Most branching points are supported by high bootstrap values. The three *Deferribacter* species were distantly related to *Flexistipes sinuarabici* DSM 4947^T (87.1–87.6% sequence similarity), the two species of *Geovibrio* (84.6–85.9% sequence similarity) and *Denitrovibrio acetiphilus* N2460^T (83.3–84.4% sequence similarity).

Enrichment and isolation experiments performed with samples collected from deep-sea hydrothermal fluids and chimneys initially revealed the predominance of sulfur-reducing thermophiles. Recent culture and isolation studies

Fig. 2. Phylogenetic position of strain JR^T within the family *Deferribacteraceae*. The dendrogram is derived from a neighbour-joining analysis of almost-complete 16S rRNA gene sequences. Numbers at branching points are bootstrap values (expressed as percentages of 500 resamplings). The 16S rRNA gene sequence of *Aquifex pyrophilus* was used as an outgroup. Bar, estimated sequence divergence.

have, however, reported that nitrate and Fe(III) may also represent alternative electron acceptors widely used by phylogenetically diverse organisms in these environments. Many of the newly discovered thermophilic prokaryotes inhabiting deep-sea hydrothermal vents have been shown to be capable of denitrification. Representatives of the family *Thermaceae*, *Oceanithermus profundus* and *Vulcanithermus mediatlanticus* (Miroshnichenko *et al.*, 2003a, b), are able to reduce nitrate to nitrite, members of the genus *Persephonella* (Götz *et al.*, 2002) are able to produce nitrogen in the course of nitrate reduction, whereas *Caldithrix abyssi* (Miroshnichenko *et al.*, 2003c), *Caminibacter hydrogeniphilus* (Alain *et al.*, 2002) and *Thermovibrio ruber* (Huber *et al.*, 2002) are able to reduce nitrate to ammonium as the end product of denitrification. The presence in deep-sea hydrothermal vents of thermophilic dissimilatory Fe(III)-reducing micro-organisms, including representatives of the genus *Deferribacter*, was first demonstrated by denaturing-gradient gel electrophoresis (DGGE) experiments performed on enrichment cultures (Slobodkin *et al.*, 2001). However, to date, the archaeon *Geoglobus ahangari* (Kashefi *et al.*, 2002) is the only example of a deep-sea hydrothermal vent micro-organism that is capable of dissimilatory Fe(III) reduction in growing cultures. Strain JR^T, one of the new isolates described in this report, represents another group of micro-organisms inhabiting deep-sea hydrothermal vents – moderately thermophilic bacteria able to reduce Fe(III) compounds as well as nitrate and sulfur. Strain JR^T shares many common phenotypic features with the type species of the genus *Deferribacter*, *D. thermophilus* (Table 1). However, strain JR^T differs from *D. thermophilus* and *D. desulfuricans* by its ability to grow lithoautotrophically, utilizing hydrogen as an electron donor, CO₂ as a carbon source and elemental sulfur or nitrate as electron acceptors.

Table 1. Characteristics useful for differentiating strain JR^T from other *Deferribacter* spp.

Strain/species: 1, strain JR^T; 2, *D. thermophilus* (Greene *et al.*, 1997); 3, *D. desulfuricans* (Takai *et al.*, 2003). +, Positive; –, negative.

Characteristic	1	2	3
Temperature range (°C)	45–65	50–65	40–70
Optimal temperature (°C)	60	60	60–65
Electron acceptor:			
Elemental sulfur	+	–	+
Fe(III)	+	+	–
Mn(IV)	–	+	–
Electron donor:			
Ethanol	–	–	+
Formate	–	–	+
Propionate	–	–	+
Pyruvate	+	–	+
Lithoautotrophic growth	+	–	–
G + C content (mol%)	30.8	34	38.6

Other significant characteristics that differentiate strain JR^T from *D. thermophilus* are its ability to reduce sulfur and inability to use Mn(IV) as an electron acceptor. In contrast to *D. desulfuricans*, strain JR^T is able to reduce Fe(III). On the basis of the results of phylogenetic, genosystematic and physiological studies that clearly differentiate strain JR^T from known species of the genus *Deferribacter*, we propose to describe it as the type strain of a new species of the genus, namely, *Deferribacter abyssi*. Strain DR (=DSM 14927) represents the second strain of the same species.

Description of *Deferribacter abyssi* sp. nov.

Deferribacter abyssi (a.bys'si. L. fem. gen. n. *abyssi* of immense depths, living in the depths of the ocean).

Small rods that are straight- to vibrio-shaped. Cells are 1.5–2.0 µm long and 0.4–0.5 µm wide, with the Gram-negative type of cell wall. Motile by means of one polar flagellum. Anaerobic. Moderately thermophilic, growing between 45 and 65 °C, with optimum growth at 60 °C. Neutrophilic, growing between pH 6.0 and 7.2, with optimum growth at pH 6.5–6.7. Grows at NaCl concentrations ranging from 10 to 50 g l⁻¹, with optimum growth at 30 g NaCl l⁻¹. Capable of chemolithoautotrophic growth with hydrogen as an electron donor, elemental sulfur or nitrate as electron acceptors and CO₂ as a carbon source. Anaerobic oxidation of acetate, pyruvate, succinate and proteinaceous substrates by using sulfur, nitrate or Fe(III) as electron acceptors. Not capable of fermentation. Sensitive to rifampicin, chloramphenicol, vancomycin, penicillin and streptomycin but resistant to tetracycline. Isolated from hydrothermal vent fields of the Mid-Atlantic Ridge.

The type strain is JR^T (=DSM 14873^T =JCM 11955^T). The G+C content of its DNA is 30.8 mol%. Isolated from the Rainbow (36° 16' N, 33° 54' W) hydrothermal vent field of the Mid-Atlantic Ridge.

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