***Thermoanaerobacter siderophilus* sp. nov., a novel dissimilatory Fe(III)-reducing, anaerobic, thermophilic bacterium**

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A thermophilic, anaerobic, spore-forming, dissimilatory Fe(III)-reducing bacterium, designated strain SR4^T, was isolated from sediment of newly formed hydrothermal vents in the area of the eruption of Karymsky volcano on the Kamchatka peninsula. Cells of strain SR4^T were straight-to-curved, peritrichous rods, 0.4–0.6 µm in diameter and 3.5–9.0 µm in length, and exhibited a slight tumbling motility. Strain SR4^T formed round, refractile, heat-resistant endospores in terminally swollen sporangia. The temperature range for growth was 39–78 °C, with an optimum at 69–71 °C. The pH range for growth was 4.8–8.2, with an optimum at 6.3–6.5. Strain SR4^T grew anaerobically with peptone as carbon source. Amorphous iron(III) oxide present in the medium stimulated the growth of strain SR4^T; cell numbers increased with the concomitant accumulation of Fe(II). In the presence of Fe(III), strain SR4^T grew on H₂/CO₂ and utilized molecular hydrogen. Strain SR4^T reduced 9,10-anthraquinone-2,6-disulfonic acid, sulfite, thiosulfate, elemental sulfur and MnO₂. Strain SR4^T did not reduce nitrate or sulfate and was not capable of growth with O₂. The fermentation products from glucose were ethanol, lactate, H₂ and CO₂. The G+C content of DNA was 32 mol%. 16S rDNA sequence analysis placed the organism in the genus *Thermoanaerobacter*. On the basis of physiological properties and phylogenetic analysis, it is proposed that strain SR4^T (= DSM 12299^T) should be assigned to a new species, *Thermoanaerobacter siderophilus* sp. nov.

Keywords: *Thermoanaerobacter*, Fe(III) reduction, Mn(IV) reduction, thermophiles, magnetite

INTRODUCTION

Reduction of Fe(III) by micro-organisms has important implications in the cycling of iron and organic matter and has been intensively studied in marine and freshwater anoxic sediments and submerged soils (Lovley, 1991, 1995; Nealson & Saffarini, 1994). Dissimilatory Fe(III)-reducing micro-organisms have also been found in a variety of thermobiotic environments, including sediments of hydrothermal vents and hot springs (Slobodkin *et al.*, 1995; Slobodkin & Wiegel, 1997), sedimentary basins in the deep ter-

restrial subsurface (Boone *et al.*, 1995; Liu *et al.*, 1997) and submarine petroleum reservoirs (Greene *et al.*, 1997).

Dissimilatory Fe(III)-reducing micro-organisms can be subdivided into several physiological groups according to the electron donor used for Fe(III) reduction: (i) fermentative, which use Fe(III) reduction as a minor pathway for electron flow while fermenting sugars or amino acids; (ii) hydrogen-oxidizing; (iii) sulfur-oxidizing; and (iv) organisms that oxidize poorly fermentable substrates, such as organic acids, alcohols and aromatic compounds.

Iron-reducing thermophiles available in pure culture are represented by several phylogenetically diverse micro-organisms. The aerobic archaeon *Sulfolobus*

Abbreviation: AQDS, 9,10-anthraquinone-2,6-disulfonic acid.

The GenBank accession number for the 16S rDNA sequence of strain SR4^T is AF120479.

acidocaldarius reduces Fe(III) with elemental sulfur (Brock & Gustafson, 1976). The anaerobic *Bacillus infernus* reduces Fe(III) with formate or lactate as the electron donor (Boone *et al.*, 1995). The anaerobic (eu)bacteria *Deferribacter thermophilus* (Greene *et al.*, 1997) and *Thermoterrabacterium ferrireducens* (Slobodkin *et al.*, 1997) couple Fe(III) reduction with the oxidation of complex organic substrates, organic acids, alcohols and molecular hydrogen. Recently, the ability to reduce Fe(III) with glycerol under oxygen limitation was shown for the moderately thermophilic acidophiles *Sulfobacillus thermosulfidooxidans*, *Sulfobacillus acidophilus* and *Acidimicrobium ferrooxidans* (Bridge & Johnson, 1998).

In this paper, we describe an anaerobic, dissimilatory Fe(III)-reducing, thermophilic bacterium, *Thermoanaerobacter siderophilus* sp. nov., isolated from hydrothermal vents on the Kamchatka peninsula.

METHODS

Environmental samples. Samples of sediments and water were collected in March 1997 from newly formed hydrothermal vents in the area of the eruption of Karymsky volcano on the Kamchatka peninsula. The temperatures at the sampling sites ranged from 70 to 94 °C, pH from 6.1 to 7.1 and E_h from -215 to -305 mV.

Media and cultivation. A basal medium used for enrichment, isolation and cultivation of Fe(III)-reducing bacteria was prepared anaerobically by boiling and cooling it under CO₂ (100%) gas phase. The basal medium contained (l⁻¹ distilled water): 0.33 g KH₂PO₄, 0.33 g NH₄Cl, 0.33 g KCl, 0.33 g MgCl₂·2H₂O, 0.33 g CaCl₂·2H₂O, 2.0 g NaHCO₃, 10 g peptone, 0.20 g yeast extract (Difco), 10 ml vitamin solution (Wolin *et al.*, 1963) and 1 ml trace-element solution (Slobodkin *et al.*, 1997). The pH was adjusted to 6.5–6.8 (at 25 °C) with 10% (w/v) NaOH. No reducing agent was added to the medium. Fe(III) was provided in the form of amorphous iron(III) oxide at about 90 mmol Fe(III) l⁻¹ medium. The amorphous iron(III) oxide was synthesized by titrating a solution of FeCl₃ with 10% (w/v) NaOH to pH 9.0. The pH of the autoclaved medium measured at 70 °C was 6.8–6.9.

Unless otherwise noted, enrichments and pure cultures were grown in 10 ml medium in Hungate tubes under an atmosphere of CO₂ (100%). All transfers and sampling of cultures were performed with syringes and needles. The medium was heat-sterilized at 135 °C for 30 min. All incubations were at 70 °C unless otherwise noted.

Physiological studies. Growth of bacteria in medium containing amorphous iron(III) oxide or other insoluble compounds was determined by direct counting with a phase-contrast microscope and a counting chamber. In media with soluble components, growth was determined by counting and by measuring the increase in optical density at 600 nm (Spekol 10; Carl Zeiss Jena).

The ability of the organism to grow on different substrates was determined in basal medium, in which peptone was replaced by autoclaved or filter-sterilized substrates, both in the presence and in the absence of amorphous iron(III) oxide. When Fe(III) was omitted, the medium was pre-reduced with Na₂S·9H₂O (0.5 g l⁻¹). The potential for

molecular hydrogen to serve as an electron donor was studied in 60-ml flasks containing 10 ml medium and H₂ (100%) or H₂/CO₂ (5:95 v/v) as the gas phase. The cultures were incubated for 2 weeks and the ability to utilize a particulate substrate was judged from culture growth and Fe(II) accumulation. A medium in which the organic carbon source had been omitted was used as a control.

The ability to use various electron acceptors was studied in the basal medium containing peptone (10 g l⁻¹) as a sole electron donor, in which amorphous iron(III) oxide was omitted. The electron acceptors were added from autoclaved stock solutions. MnO₂ was prepared by the method of Lovley & Phillips (1988). The medium was pre-reduced with Na₂S·9H₂O (0.5 g l⁻¹) in the experiments with sulfate, sulfite, thiosulfate and elemental sulfur. No reducing agent was present in media containing O₂, MnO₂ or 9,10-anthraquinone-2,6-disulfonic acid (AQDS). Both reduced and reducing-agent-free media were used in nitrate-amended experiments. Cultures grown in pre-reduced basal medium without any electron acceptor were used as inocula (5% v/v). The use of the electron acceptors was judged from culture growth (for all acceptors), sulfide production (for sulfate, sulfite, thiosulfate and elemental sulfur), change of visible colour of the medium or precipitate (for MnO₂ and AQDS) and from the accumulation of Mn(II) (for MnO₂).

Temperature, pH and NaCl concentration ranges for growth and susceptibility to antibiotics were determined in basal medium in which amorphous iron(III) oxide was omitted and peptone was replaced by glucose (5 g l⁻¹). The medium was pre-reduced with Na₂S·9H₂O (0.5 g l⁻¹). The pH range for growth was determined at 70 °C. The pH was adjusted with sterile stock solutions of HCl or NaOH and measured at 70 °C with a model PHM 82 pH meter (Radiometer) equipped with a temperature probe and calibrated at 70 °C.

Microscopy. Routine examinations and cell counting were performed under a phase-contrast Amplival microscope (Carl Zeiss Jena). Transmission electron microscopy was performed with a model JEM-100 electron microscope (JEOL) as described previously (Bonch-Osmolovskaya *et al.*, 1990). Gram staining was performed according to Norris & Swain (1971).

Analytical techniques. Fe(III) reduction was monitored by measuring the accumulation of Fe(II) over time. Fe(II) was measured by adding a 0.5 ml sample from the culture to 5 ml 0.6 M HCl. After 24 h extraction, HCl-soluble Fe(II) was determined with 2,2'-dipyridyl (Balashova & Zavarzin, 1980). Iron-containing precipitate was analysed by X-ray diffraction analysis (Slobodkin *et al.*, 1995). Mn(II) was analysed by atomic absorption spectrophotometry after HCl extraction (Lovley & Phillips, 1988). Determination of short-chain organic acids, alcohols and gaseous products of metabolism was performed by GC; sulfide was determined by a colorimetric method as described previously (Slobodkin & Bonch-Osmolovskaya, 1994).

Cultivation of reference strains. *Thermoanaerobacter sulfurophilus* strain L-64^T (= DSM 11584^T) was from the culture collection of the Laboratory of Hyperthermophilic Microbial Communities, Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia. *Thermoanaerobacter wiggelii* strain Rt8.B1^T (= DSM 10319^T) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. The strains were routinely cultured in the mineral medium described above with glucose (5 g l⁻¹) as the main energy source. Tests for

Fe(III) reduction were performed in the basal medium with amorphous iron(III) oxide as an electron acceptor; the electron donors were 20 mM lactate (for *Thermoanaerobacter sulfurophilus*), 20 mM glycerol (for *Thermoanaerobacter wiegelsii*) or 1% w/v peptone (for both strains). The cultivation temperature was 60 °C for *Thermoanaerobacter sulfurophilus* and 65 °C for *Thermoanaerobacter wiegelsii*.

DNA characteristics. The DNA was extracted and purified by the method of Marmur (1961). Its base composition was determined from the melting point according to Marmur & Doty (1962). The molecular mass of genome DNA was determined based on optical renaturation-rate measurements (Gillis *et al.*, 1970). DNA–DNA hybridization studies were performed by the optical reassociation method as described previously (Krivenko *et al.*, 1990).

16S rRNA sequence studies. The 16S rRNA gene was selectively amplified from genomic DNA by PCR using 5'-AGAGTTTGATCCTGGCTCAG-3' as the forward primer and 5'-TACGGTTACCTTGTACGACTT-3' as the reverse primer (Lane, 1991).

The PCR was carried out in 100 µl reaction mixture containing 1 µg DNA template, 200 µM (each) primers, 200 µM (each) dNTPs and 3 U Tet-z polymerase (Bio-Master) in reaction buffer (100 mM Tris/HCl pH 8.3, 500 mM KCl, 20 mM MgCl₂). The temperature cycling was done by using 30 amplification cycles of 1 min at 94 °C, 1 min at 42 °C and 1 min at 72 °C. The final extension was carried out at 72 °C for 6 min. The PCR products were purified using the PCR-prep kit (Promega) as recommended by the manufacturer. The 16S rRNA gene was sequenced in both directions with the use of forward and reverse primers. DNA sequencing was performed by using Sequenase version 2.0 (USB).

The 16S rDNA sequence was aligned with a representative set of 16S rRNA sequences obtained from the Ribosomal Database Project or from recent GenBank releases by using MULTALIN software (Corpet, 1988). Positions that had not been sequenced in one or more reference organisms were omitted and a total of 1593 nucleotides were used in the analysis. Pairwise evolutionary distances were computed by using the correction of Jukes & Cantor (1969). The rooted phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) with bootstrap analysis of 100 trees using the programs of the TREECON package (Van de Peer & De Wachter, 1994).

Nucleotide sequence accession numbers. The accession numbers of the sequences used as references were as follows: *Thermoanaerobacter thermohydrosulfuricus* DSM 567^T, L09161; *Thermoanaerobacter sulfurophilus* L-64^T, Y16940; *Thermoanaerobacter wiegelsii* Rt8.B1^T, X92513; *Thermoanaerobacter acetoethylicus* ATCC 33265^T, L09163; *Thermoanaerobacter kivui* DSM 2030^T, L09160; *Thermoanaerobacter brockii* DSM 1457^T, L09165; *Thermoanaerobacter mathranii* A3^T, Y11279; *Thermoanaerobacter thermocopriae* JT-3^T, L09167; *Thermoanaerobacter ethanolicus* JW-200^T, L09162; *Desulfotomaculum thermobenzoicum* TSB^T, L15628; *Thermoanaerobacterium thermosulfurigenes* E100-69^T, L09161; *Dictyoglomus thermophilum* H-6-12^T, X69194; *Anaerobranca horikoshii* JW/YL-138^T; *Bacillus infernus* TH-22^T, U20384; *Moorella thermoautotrophica* JW 701/5^T, X58354; *Deferribacter thermophilus* BMA1^T, U75602; *Syntrophospora bryantii* DSM 3014B^T, M26491; and *Thermoterrabacterium ferrireducens* JW/AS-Y7^T, U76363.

RESULTS

Enrichment and isolation

Four sediment/water samples obtained from newly formed hydrothermal vents in the area of the eruption of Karymsky volcano on the Kamchatka peninsula were used for enrichment of thermophilic, dissimilatory Fe(III)-reducing micro-organisms. Basal anaerobic medium, in which peptone was an electron donor and amorphous iron(III) oxide was provided as an electron acceptor, was inoculated with 10% (w/v) of the sample and incubated at 85 °C in the dark. After 2 weeks incubation, no accumulation of Fe(II) was observed. After that, the same flasks were incubated at 70 °C. After 72–96 h cultivation, in two enrichments, non-magnetic, brown amorphous iron(III) oxide was converted to a black, solid material of less volume, which was strongly attracted to a magnet and contained a significant amount of Fe(II). For the isolation of a pure culture, the enrichment with the fastest rate of Fe(III) reduction (sampling point: T = 94 °C, pH = 6.1, E_h = -215 mV) was chosen. After three successive 5% (w/v) transfers, the enrichment was repeatedly serially diluted to extinction in the basal medium in which iron(III) oxide was replaced by AQDS (20 mM). The highest dilution that was positive for AQDS reduction (10⁻⁶) was serially diluted to extinction in agar shake tubes (1.5% Bacto Agar) in the basal medium with AQDS. Single colonies were removed and subcultured in liquid basal medium with amorphous iron(III) oxide. Light microscopic observation revealed that the organism in these cultures was a spore-forming rod. The culture was then transferred (5% v/v) to fresh basal medium with AQDS, autoclaved at 121 °C for 90 min to kill vegetative cells and incubated at 70 °C. After 48 h incubation, this culture was serially diluted to extinction in agar shake tubes (1.5% Bacto Agar) in basal medium with AQDS and the procedure of isolation and subculturing of single colonies was repeated. After that, the culture was considered to be pure and was designated as strain SR4^T.

Colony and cell morphology

In agar-shake cultures, the colonies appeared after 18–24 h. The colonies were uniformly round, 0.5–1.0 mm in diameter and white. When grown in the basal medium with peptone and external electron acceptor [amorphous iron(III) oxide, AQDS, sulfite or thiosulfate], the vegetative cells of strain SR4^T were straight-to-curved rods, 0.4–0.6 µm in diameter and 3.5–9.0 µm in length (Fig. 1a, b). The cells occurred singly or in short chains, were peritrichous and exhibited a slight tumbling motility. The cells stained Gram-positive in both the exponential and stationary growth phases. Strain SR4^T formed round, refractile endospores in terminally swollen sporangia. Maximal sporulation was observed in liquid medium with AQDS: up to 10% of the cells sporulated during the late exponential phase. The cultures survived 90 min

Fig. 1. Electron micrographs of strain SR4^T cells grown in basal medium with peptone as an electron donor and sulfite as an electron acceptor. (a) Negatively stained whole-cell specimen. (b) Cell with peritrichous flagella (negative staining). (c) Ultrathin section showing cell-wall layers. (d) Ultrathin section showing cell division. Bars, 1 µm.

exposure to 121 °C, thus confirming that the spores were heat resistant.

Ultrathin sectioning of strain SR4^T revealed a distinct peptidoglycan layer in the cell wall (Fig. 1c). The cells appeared to divide via a septation mechanism, with the formation of V-shaped membrane invaginations (Fig. 1d).

Growth and Fe(III) reduction

Strain SR4^T grew anaerobically with peptone as the main organic carbon source (Fig. 2). Amorphous iron(III) oxide present in the medium stimulated the growth of strain SR4^T. The number of cells increased with the concomitant accumulation of Fe(II). Without an external electron acceptor, the growth yield of SR4^T on peptone was reduced; addition of Na₂S·9H₂O (0.5 g l⁻¹) to decrease the redox potential of the medium did not stimulate growth significantly. During the growth of strain SR4^T in medium with Fe(III), the production of molecular hydrogen was lower than during growth

Fig. 2. Growth of and Fe(II) and H₂ production by strain SR4^T with peptone as a substrate with or without amorphous iron(III) oxide as an electron acceptor. (a) Growth in the presence (●) and absence (○) of Fe(III), and Fe(II) production (▲). (b) H₂ production in the presence (●) and absence (○) of Fe(III).

Fig. 3. Effect of temperature on the growth of strain SR4^T. One hundred per cent was equivalent to a specific growth rate of 0.24 h⁻¹.

without Fe(III). Reduction of Fe(III) was not observed in non-inoculated medium with peptone (1% w/v), incubated at 70 °C.

In the presence of amorphous iron(III) oxide, strain SR4^T grew on H₂/CO₂ (80:20 or 5:95 v/v) and utilized molecular hydrogen. The consumption of hydrogen did not exceed 5 mmol H₂ l⁻¹ culture. For each mole of H₂ consumed, 2.15 ± 0.32 mol (mean ± SD for five cultures) Fe(II) was produced. Cell number increased

Fig. 4. Effect of pH on the growth of strain SR4^T. One hundred per cent was equivalent to a specific growth rate of 0.24 h⁻¹.

from $0.9 \pm 0.3 \times 10^6$ cells ml⁻¹ in inoculated cultures before growth to $2.5 \pm 0.5 \times 10^7$ cells ml⁻¹ in outgrown cultures (mean $\pm$ SD for five cultures). Without external electron acceptors, growth on H₂/CO₂ and consumption of H₂ were not observed.

The products of amorphous iron(III) oxide reduction with peptone and molecular hydrogen as electron donors were magnetite and siderite.

Physiological characteristics

The temperature range for growth of strain SR4^T was 39–78 °C, with an optimum at 69–71 °C (Fig. 3). No growth was detected at 79 °C or at temperatures of 36 °C or lower after 3 weeks incubation. The strain grew in a pH range from 4.8 to 8.2, with an optimum at 6.3–6.5 (Fig. 4). No growth was detected at pH 4.6 or 8.4. Growth of SR4^T was observed at NaCl concentrations ranging from 0 to 3.5% (w/v), with no growth evident at 4.0% (w/v).

The substrates utilized by strain SR4^T in the presence, as well as in the absence, of Fe(III) as an electron acceptor included peptone (10 g l⁻¹), yeast extract (10 g l⁻¹), beef extract (10 g l⁻¹), casein (10 g l⁻¹), starch (10 g l⁻¹), glycerol (20 mM), pyruvate (20 mM), glucose (25 mM), sucrose (25 mM), fructose (25 mM), maltose (25 mM), xylose (25 mM), cellobiose (25 mM) and sorbitol (25 mM). Strain SR4^T used H₂/CO₂ (80:20 v/v) in the presence of Fe(III). Fe(III) was stimulatory for growth of strain SR4^T with all substrates utilized; however, Fe(III) was chemically reduced in sterile controls in the experiments with carbohydrates. Thus, the results of the test on stimulation of growth by Fe(III) on carbohydrates should be considered equivocal. Strain SR4^T did not use formate (20 mM), acetate (20 mM), lactate (20 mM), methanol (20 mM), ethanol (20 mM), propan-1-ol (20 mM), propan-2-ol (20 mM), butan-1-ol (20 mM), propionate (20 mM), *n*-butyrate (20 mM), succinate (20 mM), malate (20 mM), maleate (20 mM), glycine (20 mM), alanine (20 mM), arginine (20 mM), L-arabinose (25 mM), olive oil (10 ml l⁻¹), xylan

Fig. 5. Phylogenetic tree showing the position of *Thermoanaerobacter siderophilus* SR4^T. The scale bar represents the expected number of changes per sequence position.

(10 g l⁻¹), carboxymethylcellulose (10 g l⁻¹) or filter paper (10 g l⁻¹), with or without Fe(III) as an electron acceptor. The fermentation products from glucose were ethanol, lactate, H₂ and CO₂.

Strain SR4^T reduced amorphous iron(III) oxide (90 mM), AQDS (20 mM), sulfite (5 mM), thiosulfate (20 mM), elemental sulfur (150 mM) and MnO₂ (20 mM). Sulfite, thiosulfate and elemental sulfur were reduced to hydrogen sulfide. Reduction of MnO₂ resulted in the formation of a whitish precipitate composed of the Mn(II) state. Strain SR4^T did not reduce nitrate (20 mM) or sulfate (20 mM) and was not capable of growth with O₂ (20% v/v in the gas phase).

Chloramphenicol, neomycin, polymyxin B and kanamycin completely inhibited growth at concentrations of 100 µg ml⁻¹ medium. Penicillin, ampicillin, streptomycin and novobiocin at 100 µg ml⁻¹ did not inhibit growth.

DNA characteristics and phylogenetic analysis

The G + C content of the genomic DNA of strain SR4^T was 32 mol% (*T_m*). The molecular mass of genomic DNA of strain SR4^T was 3.14×10^9 Da.

We determined an almost complete 16S rDNA sequence for strain SR4^T, corresponding to positions 11–1506 of *Escherichia coli* numbering. Phylogenetic analysis of 16S rDNA sequences placed strain SR4^T in the cluster comprising members of the genus *Thermoanaerobacter*, with a mean sequence identity of 95.9% (Fig. 5). The closest relatives of strain SR4^T were

Table 1. Specific elements of the secondary structure of 16S rRNA in different *Thermoanaerobacter* species

Helix types present in each region of the 16S rRNA are shown for each taxon.

Species	Region of 16S rRNA sequence (<i>E. coli</i> numbering)			
	80	1040	1140	1440
<i>T. siderophilus</i> strain SR4 ^T	1	1	2	2
<i>T. wiegelii</i>	1	2	2	2
<i>T. sulfurophilus</i>	1	2	2	2
<i>T. acetoehtylicus</i>	1	3	1	1
<i>T. ethanolicus</i>	1	3	1	1
<i>T. kivui</i>	2	2	2	1
<i>T. thermohydrosulfuricus</i>	2	1	1	1
<i>T. mathranii</i>	3	2	2	2
<i>T. brockii</i>	3	2	2	2
<i>T. thermocopriae</i>	3	2	2	2

Thermoanaerobacter wiegelii and *Thermoanaerobacter sulfurophilus* (levels of identity 98.1 and 97.9%, respectively). However, bootstrap values for the branching point of strain SR4^T (less than 50%) indicate that its position is not yet conclusively resolved.

The affiliation of strain SR4^T to the genus *Thermoanaerobacter* is also supported by additional secondary structure analysis. Comparison of the 16S rRNA sequences of *Thermoanaerobacter* species with small-subunit rRNA of prokaryotes revealed the presence of unique long versions of certain helices around positions 80, 1040, 1140 and 1440 (*E. coli* numbering) (Rainey *et al.*, 1993). The number and type of long versions of these helices vary in different *Thermoanaerobacter* species (Table 1). Strain SR4^T differed from all validly described *Thermoanaerobacter* species by the combination of specific versions of these helices.

Considering the high levels of identity (more than 97%) between the 16S rRNA sequences of strain SR4^T and *Thermoanaerobacter wiegelii* and *Thermoanaerobacter sulfurophilus*, quantitative DNA–DNA hybridization experiments were performed. The levels of DNA reassociation were: between strain SR4^T and *Thermoanaerobacter wiegelii*, 50%, and between strain SR4^T and *Thermoanaerobacter sulfurophilus*, 48%. These data confirm that strain SR4^T is a new species of the genus *Thermoanaerobacter*.

DISCUSSION

Iron-containing compounds are abundant in some terrestrial, geothermally heated sediments in Kamchatka (Karpov, 1976). The fact that strain SR4^T

was isolated from a sample that was collected within 5 months of the formation of hydrothermal vents in the area of the eruption of Karymsky volcano indicates early colonization of high-temperature environments by micro-organisms capable of Fe(III) reduction.

Strain SR4^T is a facultative Fe(III) reducer, capable of oxidizing organic substrates in the presence and absence of Fe(III). The mechanism of microbial Fe(III) reduction with complex organic substances, such as peptone, is unknown and may include fermentative as well as respiratory types of dissimilatory Fe(III) reduction. Greater cell numbers were obtained in media containing Fe(III), suggesting that Fe(III) reduction may play a role in energy conservation. Strain SR4^T is also capable of molecular-hydrogen oxidation in the presence of Fe(III). Although the net consumption of H₂ was relatively low, it was enough for sustainable growth and formation of a magnetic precipitate. The finding that strain SR4^T can reduce Mn(IV) extends the known upper temperature limit for biological manganese reduction to 76 °C.

The anaerobic, thermophilic, endospore-forming bacteria that are unable to reduce sulfate are currently placed in the genera *Clostridium*, *Thermoanaerobacter*, *Thermoanaerobacterium*, *Caloramator* and *Moorella* (Collins *et al.*, 1994; Lee *et al.*, 1993; Wiegel, 1986). The results of 16S rDNA sequence analysis placed strain SR4^T in the cluster composed of *Thermoanaerobacter* species.

The capacity for dissimilatory Fe(III) reduction has not been tested among the members of the genus *Thermoanaerobacter*. The involvement of *Thermoanaerobacter* species in Fe(III) reduction in the deep subsurface was suggested by Liu *et al.* (1997) on the basis of molecular analysis; however, pure cultures of the micro-organisms were not obtained. We tested two species of *Thermoanaerobacter* most closely related to strain SR4^T on the basis of 16S rDNA identity, and found both *Thermoanaerobacter wiegelii* (Cook *et al.*, 1996) and *Thermoanaerobacter sulfurophilus* (Bonch-Osmolovskaya *et al.*, 1997) to be capable of dissimilatory Fe(III) reduction during growth with peptone as the electron donor and amorphous iron(III) oxide as the electron acceptor. However, the rate of Fe(III) reduction was considerably lower than that observed with strain SR4^T. *Thermoanaerobacter wiegelii* and *Thermoanaerobacter sulfurophilus* did not reduce amorphous iron(III) oxide significantly with glycerol or lactate, respectively, as electron donors. These findings indicate that the capacity for dissimilatory Fe(III) reduction with complex organic substrates as electron donors may be widespread among the members of the genus *Thermoanaerobacter*. The stimulation of growth of strain SR4^T by Fe(III) resembles the positive effect of thiosulfate or elemental sulfur on the growth of some *Thermoanaerobacter* species (Fardeau *et al.*, 1994; Faudon *et al.*, 1995; Bonch-Osmolovskaya *et al.*, 1997) and probably has a similar mechanism.

Table 2. Characteristics that differentiate *T. siderophilus* from *T. wiedelii* and *T. sulfurophilus*

Characteristic	<i>T. siderophilus</i>	<i>T. wiedelii</i>	<i>T. sulfurophilus</i>
Reduction of elemental sulfur	+	—	+
Temperature optimum (°C)	69–71	65–68	55–60
pH optimum	6.3–6.5	6.8	6.8–7.2
G + C content of DNA (mol %)	32	35	30
Mass of genome DNA (Da)*	3.14×10^9	3.77×10^9	3.94×10^9

* Masses of genomes of *T. wiedelii* and *T. sulfurophilus* were determined in this study.

Strain SR4^T differs from *Thermoanaerobacter wiedelii* and *Thermoanaerobacter sulfurophilus* in the temperature and pH optima, capacity for reduction of elemental sulfur, G+C content of DNA and size of genome (Table 2). The fact that strain SR4^T does not belong to any of the described *Thermoanaerobacter* species is also supported by the presence of a specific combination of long versions of helices in four regions of 16S rRNA. On the basis of physiological properties and phylogenetic analysis, we propose that strain SR4^T should be assigned to a new species of the genus *Thermoanaerobacter*.

Description of *Thermoanaerobacter siderophilus* sp. nov.

Thermoanaerobacter siderophilus (si.de.ro'phil.us. Gr. n. *sideros* iron; Gr. adj. *philos* loving; M.L. adj. *siderophilus* iron-loving).

Cells are straight-to-curved rods, 0.4–0.6 µm in diameter and 3.5–9.0 µm in length, forming round, refractile, heat-resistant endospores in terminally swollen sporangia. Cells occur singly and exhibit slight tumbling motility due to peritrichous flagellation. Anaerobic. The temperature range for growth is 39–78 °C, with an optimum at 69–71 °C. Neutrophilic: pH range for growth is from 4.8 to 8.2, with an optimum at 6.3–6.5. Growth occurs in an NaCl concentration range of 0–3.5 % (w/v). The substrates utilized in the presence, as well in the absence, of Fe(III) as an electron acceptor include peptone, yeast extract, beef extract, starch, glycerol, pyruvate, glucose, sucrose, fructose, maltose, xylose, cellobiose and sorbitol. Utilizes molecular hydrogen in the presence of Fe(III). No growth occurs with formate, acetate, lactate, methanol, ethanol, propan-1-ol, propan-2-ol, butan-1-ol, propionate, *n*-butyrate, succinate, malate, maleate, glycine, alanine, arginine, L-arabinose, olive oil, xylan or cellulose, either with or without Fe(III) as an electron acceptor. The fermentation products from glucose are ethanol, lactate, H₂ and CO₂. Reduces amorphous iron(III) oxide, AQDS, sulfite, thiosulfate, elemental sulfur and MnO₂. The products of amorphous iron(III) oxide reduction are magnetite and siderite. Sulfite, thiosulfate and elemental sulfur are reduced to hydrogen sulfide. Does not reduce nitrate

or sulfate and is incapable of growth with O₂. Growth is inhibited by chloramphenicol, neomycin, polymyxin B and kanamycin but not by penicillin, ampicillin, streptomycin or novobiocin. G + C content of DNA of the type strain is 32 mol %. The habitat is hydrothermal vents in the area of Karymsky volcano on the Kamchatka peninsula, Russia. The type strain is SR4^T, which has been deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen under the accession number DSM 12299^T.

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