Thermovenabulum ferriorganovorum gen. nov., sp. nov., a novel thermophilic, anaerobic, endospore-forming bacterium

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A thermophilic, anaerobic, spore-forming bacterium (strain Z-9801[T]) was isolated from a terrestrial hydrothermal source in the Uzon caldera on the Kamchatka peninsula. Cells of strain Z-9801[T] were straight, sometimes branched rods, 0·5–0·6 μm in diameter and 1·5–7·0 μm in length, with peritrichous flagella. The temperature range for growth was 45–76 °C, with an optimum at 63–65 °C. The pH range for growth was 4·8–8·2, with an optimum at 6·7–6·9. The substrates utilized by strain Z-9801[T] included peptone, yeast extract, beef extract, Casamino acids, starch, pyruvate, melibiose, sucrose, fructose, maltose, xylose and ribose. The fermentation products from melibiose were ethanol, acetate, H_2 and CO_2. Strain Z-9801[T] used H_2 in the presence of Fe(III) and an organic electron donor. Strain Z-9801[T] reduced Fe(III), Mn(IV), nitrate, fumarate, sulfite, thiosulfate, elemental sulfur and 9,10-anthraquinone 2,6-disulfonate. The G+C content of strain Z-9801[T] DNA was 36 mol%. 16S rDNA sequence analysis revealed that the isolated organism forms a separate branch within the *Bacillus/Clostridium* group. On the basis of physiological properties and phylogenetic analysis, it is proposed that strain Z-9801[T] (= DSM 14006[T] = UNIQEM 210[T]) should be assigned to a novel species of a new genus, *Thermovenabulum ferriorganovorum* gen. nov., sp. nov.

Keywords: Fe(III) reduction, thermophiles, endospores

INTRODUCTION

Thermophilic bacteria attract considerable attention from researchers because of their biotechnological potential and the evolutionary traits of these micro-organisms. A large number of species belonging to the domain *Bacteria* are able to reduce various inorganic compounds including ferric iron, one of the most abundant elements on Earth (Lovley, 1991, 1995; Nealson & Saffarini, 1994; Slobodkin *et al.*, 1999a; Straub *et al.*, 2001). The capacity for Fe(III) reduction is known among thermophilic bacteria with different taxonomic affiliations (Boone *et al.*, 1995; Greene *et al.*, 1997; Slobodkin *et al.*, 1997, 1999b, c; Vargas *et al.*, 1998; Kieft *et al.*, 1999).

Thermophilic endospore-forming anaerobes have so far been found only within the *Bacillus/Clostridium* division of the *Bacteria*, where they are grouped into several genera (Collins *et al.*, 1994). Spore-forming thermophiles have been isolated from a wide range of natural and man-made ecosystems (Lowe *et al.*, 1993) and are probably the earliest colonizers of newly formed thermal environments by virtue of the high heat resistance of their endospores (Slobodkin *et al.*, 1999b).

In this paper, we report the isolation of a thermophilic, anaerobic, endospore-forming bacterium, strain Z-9801[T]. This organism was isolated during a search for Fe(III)-reducing micro-organisms able to utilize molecular hydrogen as an electron donor for iron reduction. Our isolation attempts, conducted using a medium with a low concentration of yeast extract,

have led to the isolation of a bacterium with a fermentative type of metabolism. While growth of strain Z-9801[T] was obligately dependent on the presence of H_2 and Fe(III) at low concentrations of yeast extract, H_2 was not utilized for Fe(III) reduction as a sole electron donor. Strain Z-9801[T] reduced Fe(III) with organic substances as electron donors; however, Fe(III) did not stimulate its growth. Based on its phenotypic and phylogenetic characteristics, strain Z-9801[T] is described as the type species of a new genus as *Thermovenabulum ferriorganovorum* gen. nov., sp. nov.

METHODS

Environmental samples. Samples of sediments and water were collected in September 1997 from the 'Zhelezistyi' (Ferrous) hydrothermal source in the Uzon caldera on the Kamchatka peninsula. This source is an opening, 5–7 cm in diameter, that emits hot water and gas bubbles, mostly composed of CO_2. It is located on the bottom of shallow hot spring and surrounded by ochre deposits, rich in Fe(III), 30–40 cm in diameter and 5–10 cm deep. The temperature at the sampling site was 63 °C and the pH was 6·8–7·0.

Media and cultivation. A basal medium used for enrichment and cultivation of Fe(III)-reducing bacteria was prepared anaerobically by boiling and cooling it under a H_2/CO_2 (80:20, v/v) gas phase. The basal medium contained (l^{-1} distilled water): 0·33 g KH_2PO_4, 0·33 g NH_4Cl, 0·33 g KCl, 0·33 g $MgCl_2$. $6H_2O$, 0·33 g $CaCl_2$. $2H_2O$, 0·70 g $NaHCO_3$, 0·20 g yeast extract (Difco), 10 ml vitamin solution (Wolin *et al.*, 1963) and 1 ml trace element solution (Slobodkin *et al.*, 1997). No reducing agent was added to the medium. Fe(III) was provided in the form of amorphous Fe(III) oxide at 90 mmol Fe(III) l^{-1} medium. The amorphous Fe(III) oxide was synthesized by titrating a solution of $FeCl_3$ with 10 % (w/v) NaOH to pH 9·0. The pH of the autoclaved medium measured at 60 °C was 6·8–7·0. The medium used for isolation of a pure culture had the same composition as a basal medium except that amorphous Fe(III) oxide was omitted and 9,10-anthraquinone 2,6-disulfonate (AQDS) (20 mM) was added to the medium as an electron acceptor.

Enrichments and pure cultures were grown in 60-ml serum bottles with rubber stoppers and metal caps containing 10 ml medium under an atmosphere of H_2/CO_2 (80:20, v/v). 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 65 °C unless otherwise noted.

Physiological studies. Growth of bacteria in medium containing amorphous Fe(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 the gas phase of H_2/CO_2 was replaced by a gas phase of N_2/CO_2 (80:20, v/v) and autoclaved or filter-sterilized substrates were added to the medium, both in the presence and absence of amorphous Fe(III) oxide. When Fe(III) was omitted, the medium was pre-reduced with Na_2S. $9H_2O$ (0·5 g l^{-1}). The cultures were incubated for 2 weeks and the ability to utilize a particular substrate was judged from culture growth and Fe(II)

accumulation. Medium in which the energy source had been omitted was used as a control.

The ability of the isolated strain to use various electron acceptors was studied in the basal medium, without amorphous Fe(III) oxide, in two variants: with H_2 and yeast extract [atmosphere of H_2/CO_2 (80:20, v/v), 0·2 g yeast extract l^{-1}] and with beef extract [3·0 g l^{-1}, atmosphere of N_2/CO_2 (80:20, v/v)] as electron donors. The soluble electron acceptors were added from autoclaved stock solutions; MnO_2 and elemental sulfur were added to the medium before sterilization and these media were then autoclaved at 109 °C for 1 h. MnO_2 was prepared by the method of Lovley & Phillips (1988). The medium was pre-reduced with Na_2S. $9H_2O$ (0·5 g l^{-1}) in the experiments with sulfate, sulfite, thiosulfate, elemental sulfur and fumarate. No reducing agent was present in media containing O_2, MnO_2 or AQDS. In nitrate-amended experiments, NH_4Cl was omitted from the basal medium and sodium nitrate (20 mM) was provided as a potential electron acceptor; both reduced and reducing-agent-free media were used. Cultures grown in pre-reduced basal medium with beef extract without any electron acceptor were used as inocula (5 % v/v). The use of 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 (for AQDS), accumulation of Mn(II) (for MnO_2) and from the production of ammonium from nitrate.

Temperature, pH and NaCl concentration ranges for growth and susceptibility to antibiotics were determined under an atmosphere of N_2/CO_2 (80:20, v/v) in modified basal medium in which amorphous Fe(III) oxide was omitted and beef extract (3 g l^{-1}) and fumarate (20 mM) were added. The medium was pre-reduced with Na_2S. $9H_2O$ (0·5 g l^{-1}). The pH range for growth was determined at 60 °C. The pH was adjusted with sterile stock solutions of HCl (6 M) or NaOH (10 %) and measured at 60 °C with a model PHM 82 pH meter (Radiometer) equipped with a temperature probe and calibrated at 60 °C.

Microscopy. Routine examinations and cell counting were performed under a phase-contrast ZETOPAN Reichert optical anoptral microscope. Phase-contrast micrographs of bacteria were taken using agar-coated slides (Pfennig & Wagner, 1986). Transmission electron microscopy was performed with a model JEM-100 electron microscope (JEOL) as described previously (Bonch-Osmolovskaya *et al.*, 1990).

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 of extraction, HCl-soluble Fe(II) was determined with 2,2′-dipyridyl (Balashova & Zavarzin, 1980). Mn(II) was analysed by atomic absorption spectrophotometry after HCl extraction (Lovley & Phillips, 1988). Ammonium was analysed with Nessler's reagent. Determination of short-chain organic acids, alcohols and gaseous products of metabolism was performed by GC (Slobodkin & Bonch-Osmolovskaya, 1994). Sulfide was determined by a colorimetric method, as described previously (Slobodkin & Bonch-Osmolovskaya, 1994).

DNA characteristics. 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).
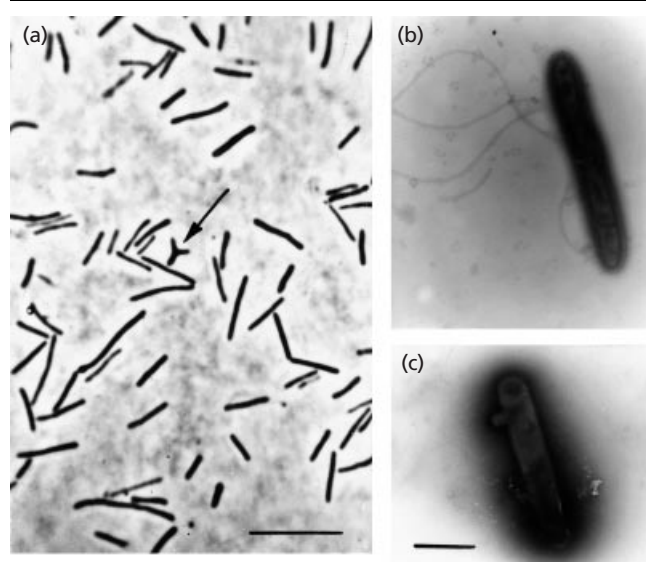
16S rRNA sequence studies. The 16S rRNA gene was

selectively amplified from genomic DNA by PCR and sequenced as described previously (Slobodkin *et al.*, 1999b).

The 16S rDNA sequence was aligned with a representative set of 16S rRNA sequences obtained from the Ribosomal Database Project (Maidak *et al.*, 2000) or from recent GenBank releases by using CLUSTAL software (Higgins & Sharp, 1988). Positions that had not been sequenced in one or more reference organisms were omitted from the analysis. Pairwise evolutionary distances were computed by using the correction of Jukes & Cantor (1969). The unrooted phylogenetic trees were constructed by the neighbour-joining method using the programs of the TREECON package (Van de Peer & De Wachter, 1994), by the maximum-likelihood method using the PUZZLE program (Strimmer & von Haeseler, 1996) and by the maximum-parsimony method using the program DNAPARS of the PHYLIP package (Felsenstein, 1989) with bootstrap analysis of 100 trees.
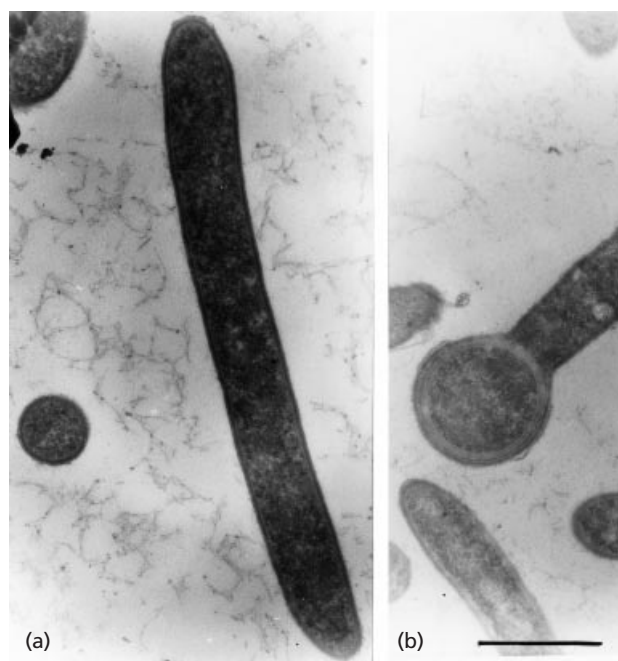
RESULTS AND DISCUSSION

Enrichment and isolation

A sample of sediment/water collected from a hydrothermal source surrounded with ochre deposits in the Uzon caldera on the Kamchatka peninsula was used for enrichment of thermophilic, dissimilatory Fe(III)-reducing micro-organisms. Basal anaerobic medium, in which molecular hydrogen and 0·2 g yeast extract l^{-1} were potential electron donors and amorphous Fe(III) oxide was provided as an electron acceptor, was inoculated with 10% (w/v) of the sample and incubated at 60 °C in the dark. After 72–96 h of cultivation, non-magnetic, brown, amorphous Fe(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). After three

Fig. 2. Ultrathin sections of strain Z-9801^T showing cell-wall layers of a vegetative cell (a) and a sporulating cell (b). Bar, 1 μm.

successive 5% (w/v) transfers, the enrichment was serially diluted repeatedly to extinction in the basal medium in which Fe(III) oxide was replaced by AQDS. The highest dilution that was positive for AQDS reduction (10^{-6}) was serially diluted to extinction in roll tubes (Hungate, 1969) with 1·5% Bacto agar in the basal medium with AQDS. Single colonies were removed and subcultured in liquid basal medium with amorphous Fe(III) oxide. After repeating the procedure of isolation and subculturing of single colonies, the culture was considered to be pure and was designated strain Z-9801^T.

Colony and cell morphology

In agar roll-tube cultures, colonies appeared after 24–48 h. The colonies were lens-shaped, 0·1–0·2 mm in diameter and white. Vegetative cells of strain Z-9801^T were straight, sometimes branched rods, 0·5–0·6 μm in diameter and 1·5–7·0 μm in length (Fig. 1a, b). Protrusions of cell envelope and cytoplasm, 0·1–0·3 μm in length, were observed on some cells (Fig. 1c). Cells occurred singly or in short chains, were peritrichously flagellated and exhibited a slight tumbling motility. Strain Z-9801^T formed round, refractile endospores in terminally swollen sporangia. Maximum sporulation was observed in liquid medium with beef extract and fumarate: up to 15% of the cells sporulated during the late exponential phase. The cultures survived a 90 min exposure to 121 °C, confirming that the spores were heat resistant. Ultrathin sectioning of strain Z-9801^T revealed a distinct peptidoglycan layer in the cell wall (Fig. 2).

Fig. 1. Cell morphology of strain Z-9801^T grown in basal medium with molecular hydrogen, yeast extract (0·2 g l^{-1}) and AQDS. (a) Phase-contrast micrograph. Arrow indicates branched cell. Bar, 10 μm. (b) Electron micrograph of negatively stained cell with peritrichous flagella. (c) Electron micrograph of cell with protrusion (negative staining). Bar, 1 μm.
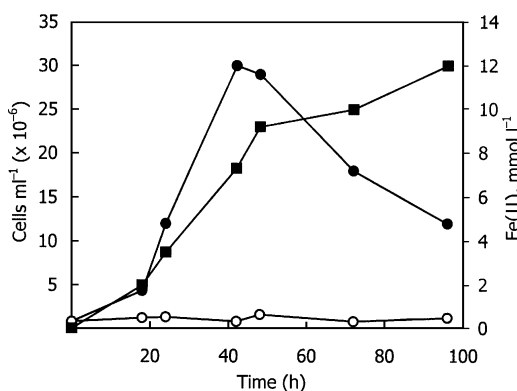
Fig. 3. Growth of and Fe(II) production by strain Z-9801^T with molecular hydrogen and yeast extract (0·2 g l^{-1}) as potential electron donors with or without amorphous Fe(III) oxide as an electron acceptor. Growth in the presence (●) and absence (○) of Fe(III) and Fe(II) production (■) are shown.

Physiological characteristics

The temperature range for growth of strain Z-9801^T was 45–76 °C, with an optimum at 63–65 °C. No growth was detected at 78 or 43 °C after 3 weeks of incubation. The strain grew at pH 4·8–8·2, with an optimum at 6·7–6·9. No growth was detected at pH 4·6 or 8·4. Growth of Z-9801^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 Z-9801^T in the presence, as well as in the absence, of Fe(III) included peptone (10 g l^{-1}), yeast extract (10 g l^{-1}), beef extract (10 g l^{-1}), Casamino acids (10 g l^{-1}), starch (10 g l^{-1}) and pyruvate (20 mM). Melibiose (25 mM), sucrose (25 mM), fructose (25 mM), maltose (25 mM), xylose (25 mM) and ribose (25 mM) were utilized in the absence of Fe(III). Fe(III) was chemically reduced by carbohydrates in sterile controls; the test for carbohydrate utilization in the presence of Fe(III) is therefore equivocal. Strain Z-9801^T grew and reduced Fe(III) in medium with molecular hydrogen and yeast extract (0·2 g l^{-1}) (Fig. 3). The consumption of H$_2$ (100, 10 or 5 %, v/v, initial concentration) was not observed. However, strain Z-9801^T did not grow when Fe(III) was omitted or H$_2$ was replaced by N$_2$, indicating that both Fe(III) and H$_2$ were essential for growth under these conditions. When the cultivation medium was additionally supplemented with beef extract (3 g l^{-1}), strain Z-9801^T grew relatively better (up to 4·5–5·0 × 10^7 cells ml^{-1}) and utilized molecular hydrogen [2·05 ± 0·18 mmol H$_2$ l^{-1} culture (mean ± standard deviation for five cultures)]. Without Fe(III), consumption of H$_2$ was not observed. Strain Z-9801^T did not grow without yeast extract; at least 0·1 g yeast extract l^{-1} was required for sustainable growth. Strain Z-9801^T did not use formate (20 mM), acetate (20 mM), lactate (20 mM), methanol (20 mM), ethanol (20 mM), glycerol (20 mM), propionate (20 mM), fumarate (20 mM), glucose (25 mM), mannose (25 mM), galactose (25 mM), cellobiose (25 mM), L-arabinose (25 mM) or glycogen (10 g l^{-1}), with or without Fe(III). The fermentation products from melibiose were ethanol, acetate, H$_2$ and CO$_2$.

Strain Z-9801^T reduced amorphous Fe(III) oxide (90 mM), Fe(III) citrate (20 mM), AQDS (20 mM), fumarate (20 mM), sulfite (2 mM), thiosulfate (20 mM), elemental sulfur (150 mM), nitrate (20 mM) and MnO$_2$ (20 mM). The product of amorphous Fe(III) oxide reduction was a black magnetic precipitate. Sulfite, thiosulfate and elemental sulfur were reduced to hydrogen sulfide. Nitrate was reduced to ammonium. Reduction of MnO$_2$ resulted in the formation of a whitish precipitate composed of the Mn(II) state. Strain Z-9801^T did not reduce sulfate (20 mM), Fe(III) EDTA (15 mM) or Fe(III) nitrilotriacetate (20 mM) and was not capable of growth with O$_2$ (2 %, v/v, in the gas phase). None of the electron acceptors utilized, except fumarate, stimulated growth of strain Z-9801^T. Fe(III) stimulated growth of strain Z-9801^T only in medium with molecular hydrogen and 0·2 g yeast extract l^{-1}.

Chloramphenicol, neomycin, polymyxin B, kanamycin and streptomycin inhibited growth completely at concentrations of 100 µg ml^{-1} medium. Penicillin at 100 µg ml^{-1} did not inhibit growth.

DNA characteristics and phylogenetic analysis

The G+C content of the genomic DNA of strain Z-9801^T was 36 mol% (T_m). We determined an almost complete 16S rDNA sequence (1464 nucleotides) for strain Z-9801^T, corresponding to positions 8–1489 of the *Escherichia coli* numbering. According to initial phylogenetic analysis by BLAST, the highest level of sequence similarity was found to species of the genus *Thermoanaerobacter* of the low-G+C Gram-positive subdivision of the *Bacteria*; however, the sequence identity did not exceed 89 %. Several phylogenetic trees were constructed by changing the selection of reference organisms and regions of alignment uncertainty resulting from the presence of long inserts in 16S rDNA of some members of the *Bacillus/Clostridium* group (Rainey *et al.*, 1993; Slobodkin *et al.*, 1999b) were omitted from the sequence analysis. A final comparison of 1165 nucleotides of the 16S rDNA sequence of strain Z-9801^T was made with the 39 closest reference bacterial strains, mostly of the *Syntrophomonas/Thermoanaerobacter* group, and used for the reconstruction of a phylogenetic tree and calculation of sequence similarity. The levels of sequence similarity of strain Z-9801^T were almost equal to all reference strains used in the analysis (84·4–88·5 %). Strain Z-9801^T was not clustered with any genus of the *Bacillus/Clostridium* group in the phylogenetic tree constructing by the neighbour-joining method (Fig. 4). The trees constructed from the maximum-likelihood and maximum-parsimony algorithms had the same topology (data not shown). The G+C content of the 16S rDNA of strain Z-9801^T
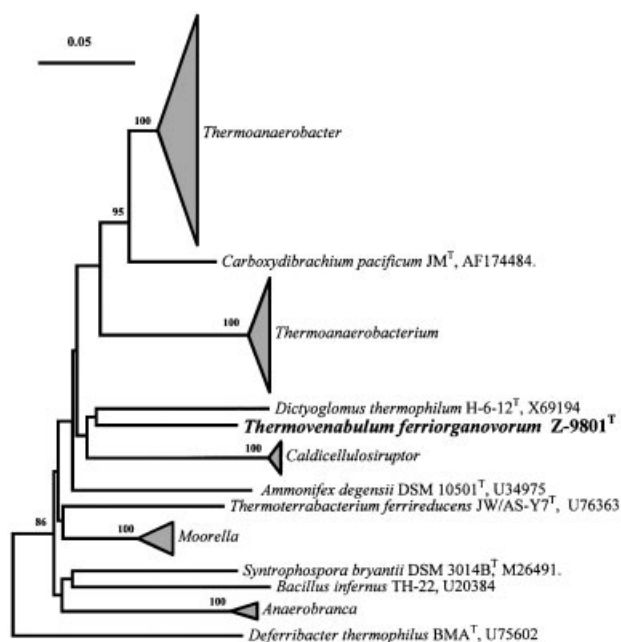
Fig. 4. Phylogenetic tree showing the position of *Thermovenabulum ferriorganovorum* Z-9801[T]. Bar, 0·05 expected changes per sequence position. Bootstrap values (expressed as percentages of 100 replications) are shown at branch points; values greater than 80 % were considered significant. Triangles represent multifurcations containing the following sequences: *Thermoanaerobacter thermohydrosulfuricus* DSM 567[T] (L09161); *Thermoanaerobacter sulfurophilus* L-64[T] (Y16940); *Thermoanaerobacter wiegelii* Rt8.B1[T] (X92513); *Thermoanaerobacter acetoethylicus* ATCC 33265[T] (L09163); *Thermoanaerobacter kivui* DSM 2030[T] (L09160); *Thermoanaerobacter brockii* subsp. *brockii* DSM 1457[T] (L09165); *Thermoanaerobacter mathranii* A3[T] (Y11279); *Thermoanaerobacter thermocopriae* JT3-3[T] (L09167); *Thermoanaerobacter ethanolicus* JW 200[T] (L09162); *Thermoanaerobacter lactiethylicus* SEBR 5268[T] (U14330); *Thermoanaerobacter siderophilus* SR4[T] (AF120479); *Thermoanaerobacterium thermosulfurigenes* E100-69[T] (L09161); *Thermoanaerobacterium aotearoense* JW/SL-NZ613[T] (X93359); *Thermoanaerobacterium saccharolyticum* DSM 7060[T] (L09169); *Thermoanaerobacterium xylanolyticum* DSM 7097[T] (L09172); *Thermoanaerobacterium thermosaccharolyticum* ATCC 7956[T] (M59119); *Caldicellulosiruptor owensensis* OL[T] (U80596); *Caldicellulosiruptor kristjanssonii* DSM 12137[T] (AJ004811); *Caldicellulosiruptor lactoaceticus* DSM 9545[T] (X82842); *Caldicellulosiruptor saccharolyticus* DSM 8903[T] (AF130258); *Moorella thermoautotrophica* JW 701/5 (X58354); *Moorella glycerini* DSM 11254[T] (U82327); *Moorella thermoacetica* DSM 521[T] (X58352); *Anaerobranca horikoshii* JW/YL-138[T] (U21809); *Anaerobranca gottschalkii* DSM 13577[T] (AF203703).

(59·1 mol %) fell into the range typical for other thermophilic prokaryotes. Transversion analysis (Woese *et al.*, 1991) did not affect the position of the strain in the phylogenetic tree.

The thermophilic, anaerobic, endospore-forming bacteria are currently placed into the genera *Clostridium*, *Desulfotomaculum*, *Thermoanaerobacter*, *Thermoanaerobacterium*, *Caloramator*, *Moorella*, *Thermacetogenium* and *Anoxybacillus* (Wiegel, 1986; Lee *et al.*,

1993; Collins *et al.*, 1994; Hattori *et al.*, 2000; Pikuta *et al.*, 2000). The results of 16S rDNA sequence analysis did not allow to us to assign strain Z-9801[T] to any of these genera. Besides that, the unique features of strain Z-9801[T] are the formation of protrusions of the cell envelope and branched cells, properties that have not been reported so far for other thermophilic, spore-forming bacteria. On the basis of these findings, we propose that strain Z-9801[T] should be placed in a new genus as *Thermovenabulum ferriorganovorum* gen. nov., sp. nov.

Description of *Thermovenabulum* gen. nov.

Thermovenabulum (Ther.mo.ve.na′bul.um. Gr. adj. *thermos* hot; L. neut. n. *venabulum* a hunting spear; N.L. neut. n. *Thermovenabulum* hot, hunting spear-shaped cell, referring to the branched cell morphology).

Rod-shaped, Gram-type positive, endospore-forming (eu)bacteria. Form branched cells and protrusions of cell envelope. Anaerobic and thermophilic. Neutrophilic. Grow organotrophically on a number of fermentable substrates. Reduce Fe(III), Mn(IV), nitrate, sulfite, thiosulfate, elemental sulfur and fumarate. The type species is *Thermovenabulum ferriorganovorum*.

Description of *Thermovenabulum ferriorganovorum* sp. nov.

Thermovenabulum ferriorganovorum (fer.ri.or.ga.no. vo′rum. L. n. *ferrum* iron; N.L. n. *organum* organic compound; L. v. *voro* to eat, consume; N.L. adj. *ferriorganovorum* using iron and organic compounds).

Cells are straight, sometimes branched rods, 0·5–0·6 μm in diameter and 1·5–7·0 μm in length, forming round, refractile, heat-resistant endospores in terminally swollen sporangia. Cells occur singly or in short chains and exhibit slight tumbling motility due to peritrichous flagellation. The temperature range for growth is 45–76 °C, with an optimum at 63–65 °C. The pH range for growth is 4·8–8·2, with an optimum at 6·7–6·9. Growth occurs in NaCl concentrations of 0–3·5 % (w/v). Anaerobic. Substrates utilized include peptone, yeast extract, beef extract, Casamino acids, starch, pyruvate, melibiose, sucrose, fructose, maltose, xylose and ribose. Utilizes molecular hydrogen in the presence of Fe(III) and an organic electron donor. No growth occurs with formate, acetate, propionate, lactate, methanol, ethanol, glycerol, glucose, mannose, galactose, arabinose, cellobiose or glycogen. The fermentation products from melibiose are ethanol, acetate, H_2 and CO_2. Reduces amorphous Fe(III) oxide, Fe(III) citrate, AQDS, fumarate, nitrate, sulfite, thiosulfate, elemental sulfur and MnO_2. Nitrate is reduced to ammonium. Sulfite, thiosulfate and elemental sulfur are reduced to hydrogen sulfide. None of the electron acceptors utilized, except fumarate, stimulates growth. Does not reduce sulfate and is

incapable of growth with O_2. Growth is inhibited by chloramphenicol, neomycin, polymyxin B, kanamycin and streptomycin but not by penicillin. The $G+C$ content of DNA is 36 mol%. The habitat is a freshwater hydrothermal source in the Uzon caldera on the Kamchatka peninsula, Russia.

The type strain is Z-9801[T] ($=$ DSM 14006[T]), which has also been deposited in the Unique Micro-organism Classification and Storage Laboratory of the Institute of Microbiology, Russian Academy of Sciences, under the accession number UNIQEM 210[T].

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