Fervidicola ferrireducens gen. nov., sp. nov., a thermophilic anaerobic bacterium from geothermal waters of the Great Artesian Basin, Australia

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A strictly anaerobic, thermophilic bacterium, designated strain Y170^T, was isolated from a microbial mat colonizing thermal waters of a run-off channel created by the free-flowing waters of a Great Artesian Basin (GAB) bore well (New Lorne bore; registered number 17263). Cells of strain Y170^T were slightly curved rods (1.2–12×0.8–1.1 µm) and stained Gram-negative. The strain grew optimally in tryptone-yeast extract-glucose medium at 70 °C (temperature range for growth was 55–80 °C) and pH 7 (pH range for growth was 5–9). Strain Y170^T grew poorly on yeast extract as a sole carbon source, but not on tryptone (0.2 %). Yeast extract could not be replaced by tryptone and was obligately required for growth on tryptone, peptone, glucose, fructose, galactose, cellobiose, mannose, sucrose, xylose, mannitol, formate, pyruvate, Casamino acids and threonine. No growth was observed on arabinose, lactose, maltose, raffinose, chitin, xylan, pectin, starch, acetate, benzoate, lactate, propionate, succinate, *myo*-inositol, ethanol, glycerol, amyl media, aspartate, leucine, glutamate, alanine, arginine, serine and glycine. End products detected from glucose fermentation were acetate, ethanol and presumably CO₂ and H₂. Iron(III), manganese(IV), thiosulfate and elemental sulfur, but not sulfate, sulfite, nitrate or nitrite, were used as electron acceptors in the presence of 0.2 % yeast extract. Iron(III) in the form of amorphous Fe(III) oxyhydroxide and Fe(III) citrate was also reduced in the presence of tryptone, peptone and Casamino acids, but not with chitin, xylan, pectin, formate, starch, pyruvate, acetate, benzoate, threonine, lactate, propionate, succinate, inositol, ethanol, glycerol, mannitol, aspartate, leucine, glutamate, alanine, arginine, serine or glycine. Strain Y170^T was not able to utilize molecular hydrogen and/or carbon dioxide in the presence or absence of iron(III). Chloramphenicol, streptomycin, tetracycline, penicillin and ampicillin and NaCl concentrations greater than 2 % inhibited growth. The G + C content of the DNA was 48 ± 1 mol% [SD (*n*=3); *T*_m]. 16S rRNA gene sequence analysis indicated that strain Y170^T is a member of the family *Syntrophomonadaceae*, class *Clostridia*, phylum *Firmicutes* and was most closely related to members of the genus *Thermosediminibacter* (mean similarity of 93.6 %). On the basis of the 16S rRNA gene sequence comparisons and physiological characteristics, strain Y170^T is considered to represent a novel species of a new genus, for which the name *Fervidicola ferrireducens* gen. nov., sp. nov. is proposed. The type strain is Y170^T (=KCTC 5610^T=JCM 15106^T=DSM 21121^T).

The Great Artesian Basin (GAB) of Australia underlies approximately one-fifth of the Australian continent, covering an area of 1.7×10^6 km² with a water-storage capacity of 8.7×10^{12} m³ (Habermahl, 1980), and is considered to be the world's largest geothermal subsurface aquifer. The GAB provides a vital water resource for rural semiarid communities and contains the largest onshore oil

and gas reserves in Australia. It is composed of alternating layers of water-bearing permeable sandstone aquifers and non-water-bearing impermeable mudstones and siltstones, which have a major influence on the chemical composition of GAB groundwaters, which can be bicarbonate-, chloride-, sulfate- or iron-rich. The GAB is estimated to be between 100 and 250 million years old, but contains groundwater estimated to be 2 million years old. The GAB is recharged at the eastern margin of the basin by rainwater and, after traversing some 1200 km underground at an estimated flow rate of 1–5 m year⁻¹, is discharged in

Abbreviation: GAB, Great Artesian Basin.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Y170^T is EU443728.

central Australia as mound springs. In addition, approximately 5000 man-made artesian bore wells have tapped the GAB as a much needed water resource for outback communities. The GAB water is heated due to its great depth (up to 3000 m) and the source temperatures of free-flowing bores can be as high as 100 °C, depending on bore depth. The combination of the unique chemical composition, varying temperatures and slow flow rate of the GAB groundwaters makes the GAB a conducive habitat for microbial growth and a range of thermophilic and mesophilic micro-organisms have been isolated and characterized from the GAB waters, including sulfate-reducers (Love *et al.*, 1993; Redburn & Patel, 1994), carbohydrate-fermenters (Andrews & Patel, 1996), strict aerobes (Kanso & Patel, 2003; Spanevello *et al.*, 2002) and Fe(III)-reducers (Kanso *et al.*, 2002). Recently, we have focused our studies on four visible and thriving macroscopic microbial mats spatially distributed at specific temperatures along the temperature gradient of the free-flowing bore water run-off channels formed by the New Lorne bore (registered bore number 17263). Culture-independent analyses of four microbial mats from the New Lorne bore run-off channel indicated that members of the genus *Thermus* dominated the 75 °C grey mat, *Meiothermus* dominated the 66 °C red mat, *Cyanobacteria* dominated the 57 °C green mat and *Deinococci-Thermus* and *Alphaproteobacteria* dominated the 52 °C brown mat, and that the phylogenetic diversity increased as the water temperature decreased and species spanning the domain *Bacteria*, including numerous uncultured organisms, were present (Spanevello, 2001). The GAB is very rich in reduced iron and, as Fe(III)-reducing micro-organisms play important roles in the cycling of metals and organic matter in deep subsurface environments and influence water quality and taste (Lovley, 1997), have potential applications in bioremediation, biomineral leaching and energy generation processes, are of immense evolutionary significance as they have been linked to the Last Common Ancestor (Vargas *et al.*, 1998) and together with sulfate-reducers may be responsible for bore metal corrosion, leading to bore failure causing significant water wastage and hardship to outback communities, we have initiated studies on iron-reducers of the GAB. In this report, we describe a strictly anaerobic, thermophilic, Fe(III)-reducer, designated strain Y170^T, isolated from a grey mat colonizing the run-off channel of the New Lorne bore at 75 °C. Strain Y170^T is considered to represent a novel species of a novel genus, *Fervidicola ferrireducens* gen. nov., sp. nov.

The grey microbial mat used in this study was collected from the run-off channel of the New Lorne bore situated near Blackall, some 1000 km north-west of Brisbane, QLD, Australia (24° 54' 48" S 145° 08' 18" E). The bore well was drilled initially in 1966 and has a depth of 1613 m. The temperature of the free-flowing water (flow rate of 7.6 l s⁻¹) at the bore head was 88 °C and the pH was 8.5, and the temperature at the site where the grey mat was

collected was 75 °C. Iron concentrations of the free-flowing water were found to be 0.01 mg l⁻¹. The mats were collected in sterile glass vessels and the vessels were filled to the brim with water from the run-off channel, capped, transported to the laboratory and stored at 4 °C until used. The microbial mats have been described previously (Spanevello *et al.*, 2002).

Enrichment and isolation was performed in anaerobic PL medium, with the addition of 2 g yeast extract l⁻¹. The PL medium contained (l⁻¹ deionized water): 1 g NH₄Cl, 0.6 g K₂HPO₄, 0.3 g KH₂PO₄, 0.1 g MgCl₂·6H₂O, 0.1 g CaCl₂·2H₂O, 1.0 g NaCl, 1 ml vitamin solution (Wolin *et al.*, 1963), 1 ml trace-element solution (Zeikus *et al.*, 1979), 12.0 g HEPES, 0.2 g yeast extract and 3.0 g amorphous Fe(III) oxyhydroxide (Lovley & Phillips, 1986). The pH of the medium was adjusted to 7.2 with 1 M NaOH and 1 M HCl and the medium was heated for 10 min in an autoclave at 121 °C and 1–1.5 kg cm⁻² pressure. The medium was then cooled under a stream of oxygen-free nitrogen gas, dispensed into Hungate tubes under oxygen-free nitrogen gas and sterilized for 30 min at 121 °C and at a pressure of 1–1.5 kg cm⁻². Enrichment cultures were initiated by injecting 500 µl of mat sample suspension into the PL medium (9.5 ml) followed by incubation at 70 °C for 3 days. The enrichment culture was serially diluted and incubated again under the same conditions. The tube containing the highest serial dilution was selected and the procedure was repeated at least three times before a bacterium, designated Y170^T, was isolated and deemed to be a pure culture. Culture purity was confirmed by using 16S rRNA gene sequencing and the pure culture was stored in a glycerol-PL medium (50:50) mixture at -20 °C.

Cell morphology was determined by using phase-contrast microscopy and electron microscopy (Kanso & Patel, 2003). The cells of strain Y170^T were peritrichously flagellated, slightly curved rods (1.2–12 × 0.8–1.1 µm) that occurred singly, in pairs and in chains. Spores were not observed for cultures that had been grown under suboptimal conditions (different pH and temperatures), and on glucose and xylose. In addition, cultures heated at 95 °C for 20 min failed to grow in tryptone-yeast extract-glucose (TYEG) medium after subculture (Patel *et al.*, 1985a, b), suggesting the absence of heat resistant bodies such as spores. Cells of strain Y170^T stained Gram-negative and electron micrographs of thin sections confirmed a Gram-negative cell-wall ultrastructure consisting of an electron-dense layer (inner wall layer) adjacent to the cell membrane and an electron light layer consisting of regular protein subunits (outer wall layer) (Fig. 1).

Unless otherwise indicated, all growth experiments were conducted at least twice and cultures were subcultured at least once in the same medium prior to the conduct of the experiment. Growth of strain Y170^T was tested in anaerobic TYEG medium that contained low phosphate-buffered salts (LPBS) supplemented with 0.2 % each of

Fig. 1. (a) Electron micrograph of negatively stained cells of strain Y170^T showing cell morphology and irregular cell division. Bar, 1 μ m. (b) Transmission electron micrograph of thin sections of cells of strain Y170^T. Bar, 200 nm. (c) Enlargement of (a) showing cell-wall ultrastructure, showing the presence of a cytoplasmic membrane (CM), an electron-dense cell-wall layer adjacent to the membrane (CWi) and an electron-light outer cell-wall layer composed of regular subunits (CWo). Bar, 100 nm.

tryptone, yeast extract and glucose at temperatures and pH ranging from 26 to 85 °C and 5.0 to 9.5, respectively. Growth was measured at 580 nm by inserting Hungate tubes directly into a modified cuvette holder of a Novaspec LKB spectrophotometer (Pharmacia-Biotech). Strain Y170^T grew optimally in TYEG medium at 70 °C (temperature range for growth of 55–80 °C) and a pH of 7 (pH range for growth of 6–9), with a generation time of 4.6 h. Unless indicated otherwise, all subsequent growth experiments were conducted under these conditions.

Strain Y170^T was tested for its ability to use LPBS supplemented with 0.2% tryptone (medium T), 0.2% yeast extract (medium YE) or 0.2% each of tryptone and yeast extract (medium TYE) as carbon sources. No growth was observed in medium T and growth of approximately 15 and 28% was observed in medium YE and medium TYE, respectively, compared with growth (100%) in TYEG medium (100% growth = OD 580 nm of 0.4). Strain Y170^T did not grow on 0.2% glucose (medium G) as the sole carbon source, which was fermented in the presence of 0.2% tryptone (TG medium) reaching an OD 580 nm of 0.2 (50% growth) and in the presence of 0.2% yeast extract (YEG medium) reaching an OD 580 nm of 0.4 (100% growth).

Substrate utilization tests were performed in the presence of 0.2% yeast extract. Soluble substrates were added to sterile YE medium from sterile anaerobic stock solutions to a final concentration of 0.2%. Insoluble substrates were weighed directly into Hungate tubes, YE medium was dispensed and the medium was sterilized. Growth was measured at 580 nm three times a day until the culture reached stationary phase. Growth was considered as positive when the turbidity of the culture was at least 20% higher in YE medium containing substrates, compared with YE medium that lacked the substrates. Strain Y170^T utilized tryptone, peptone, glucose, fructose, galactose, cellobiose, mannose, sucrose, xylose, mannitol, formate, pyruvate, Casamino acids and threonine, but

not arabinose, lactose, maltose, raffinose, chitin, xylan, pectin, starch, acetate, benzoate, lactate, propionate, succinate, *myo*-inositol, ethanol, glycerol, amyl media, aspartate, leucine, glutamate, alanine, arginine, serine or glycine.

End products from glucose fermentation in TYEG medium were determined as described previously (Chrisotomos *et al.*, 1996), with the exception that the oven and injector temperatures were 200 and 215 °C, respectively. End products detected from glucose fermentation were ethanol, acetate and presumably CO₂ and H₂.

To determine the substrates used by strain Y170^T with reduction of Fe(III), substrates were added to PL medium, supplemented with 2 g ammonium Fe(III) citrate l⁻¹ instead of amorphous Fe(III) oxide. The cultures were incubated for 8 days at 70 °C and Fe(III) reduction was inferred when a transformation of the reddish-brown colour of the Fe(III) to a dark precipitate [Fe(II)] and a clearing of the media were observed. Fe(III) reduction was confirmed by measuring Fe(II) accumulation using the ferozine method (Sorensen, 1982). A 0.1 ml sample was mixed with 3 ml ferozine reagent, and after 1 min the absorbance at 562 nm was determined. A culture in PL media without substrates was used as a negative control and a culture in PL media supplemented with 2 g yeast extract l⁻¹ was used as a positive control. Under sterile conditions, Fe(III) reduction can occur spontaneously at elevated temperatures in the presence of carbohydrates (Zavarzina *et al.*, 2002) and hence carbohydrates were not tested. Fe(III) was reduced in the presence of yeast extract, tryptone, peptone and Casamino acids, but not formate, acetate, propionate, pyruvate, lactate, succinate, ethanol, glycerol, benzoate, aspartate, leucine, glutamate, threonine, alanine, arginine, serine or glycine. Given that Fe(III) is reduced in the presence of substrates that could also be utilized fermentatively, an investigation to determine whether energy is conserved to support growth from the reduction of Fe(III) to Fe(II) was deemed to be impractical.

The potential for molecular hydrogen or carbon dioxide to serve as an electron donor in the presence and absence of Fe(III) was studied using 60 ml serum bottles containing 10 ml PL medium with H₂ (100, 10 and 5%, v/v)/CO₂ (balance) or CO₂ (100%) as the gas phase, and 10 ml yeast extract (0.02%)-supplemented LPBS media with CO₂ or H₂:CO₂ (80:20) as the gas phase. Strain Y170^T did not reduce Fe(III) in the presence of CO₂ (100%) or H₂/CO₂ (100, 10 and 5%, v/v) and did not grow with CO₂ or H₂:CO₂ (80:20).

Electron acceptor utilization tests for sodium thiosulfate (20 mM), sodium sulfate (20 mM), sodium sulfite (5 mM), elemental sulfur (1%), sodium nitrate (20 mM) and sodium nitrite (5 mM) were determined in YE medium without the reductant Na₂S. The reduction of sulfate, sulfite, elemental sulfur and thiosulfate was tested using the method of Ramamoorthy *et al.* (2006). Fe(NH₄)₂(SO₄)₂ (0.5%) was added to culture tubes, which were scored positive for the reduction of oxidized sulfur compounds when a black FeS precipitate was formed. The reduction of nitrate and nitrite to nitrogen (N₂) was determined by using Durham tubes. Cultures were scored as positive when the gas collected in the Durham tube was greater than that found in control tubes without nitrate and nitrite. Reduction of nitrate to nitrite was determined using Nitrite Test kits as described by the manufacturer (Aqua One). The reduction of nitrate to ammonium was subsequently tested using the same solution with the addition of 1 mg zinc powder (Merck). The strain was scored as positive for the reduction of nitrate to ammonium when no colour change occurred or negative for nitrate reduction when a pink colour change occurred. The reduction of nitrite to ammonium was tested using an Ammonia Test kit (Aqua One). Electron acceptor utilization tests for manganese(IV) were tested in PL medium in which amorphous Fe(III) oxyhydroxide was replaced with 2 g Mn(IV) I⁻¹ (Lovley & Phillips, 1988). Mn(IV) reduction was inferred by a clearing of the media and was further confirmed using the leuco crystal violet manganese oxide detection technique (Spratt *et al.*, 1994). MnO₂ concentration was determined by mixing 0.2 ml of the sample with 4 ml leuco crystal violet reagent, which was diluted, and the absorbance at 562 nm was determined. Strain Y170^T was able to reduce Mn(IV), thiosulfate and elemental sulfur, but not sulfate, sulfite, nitrate or nitrite.

Strain Y170^T was unable to grow under aerobic conditions in medium D (Brock & Freeze, 1969) or in aerobic YE medium.

Antibiotic sensitivity was determined in TYEG media to which was added antibiotics (from filter-sterilized stock solutions) to a final concentration of 10 and 100 µg ml⁻¹. Growth inhibition for each antibiotic was calculated from final turbidity measurements of cultures that had been grown in antibiotic-free TYEG medium and was expressed as percentage inhibition. Growth of strain Y170^T was

completely inhibited (100% inhibition) in the presence of 10 µg ml⁻¹ ampicillin, streptomycin, tetracycline or penicillin and 100 µg chloramphenicol ml⁻¹. Strain Y170^T was able to grow without NaCl or at concentrations less than 2%.

To determine the mol% DNA G+C content of strain Y170^T, DNA extraction and genome amplification was performed using TempliPhi Amplification kits (Amersham Biosciences). An aliquot of 0.4 µl pelleted exponential-phase cells that had been washed three times in sterile deionized H₂O and incubated at 4–4.5 min at 95 °C to extract the DNA was used per TempliPhi reaction. DNA quality was assessed by using agarose gel electrophoresis and ethidium bromide staining followed by visualization under a UVP GDAS 1200 Gel Documentation Analysis System (UVP). The mol% G+C content of the genomic DNA was determined by using the thermal denaturation method (Marmur & Doty, 1962) with a Cintra20 spectrophotometer (GBC Scientific Equipment) as described by Spanevello *et al.* (2002) and was calculated to be 48 ± 1 mol% (SD; *n*=3).

Inorganic Fe(III) present in PL medium can interfere with genomic DNA extraction and PCR (Kashefi *et al.*, 2003). Thioglycolic acid pre-treatment was used to reduce and precipitate Fe(III) prior to genomic DNA extraction. For this, 40 µl thioglycolic acid was added to exponential-phase cultures in 35 ml PL medium, mixed by inversion and allowed to settle for 5 min. The supernatant was then centrifuged at 8000 r.p.m. at 4 °C for 15 min (Sigma 4K15). The pellet was collected and resuspended in 7.5 ml TE buffer, and 10 µl thioglycolic acid was added and mixed by gentle inversion. The mixture was allowed to settle and the supernatant was centrifuged as described above. The cell pellet was washed in 3.5 ml TE buffer to remove traces of thioglycolic acid and resuspended in 460 µl TE buffer. Genomic DNA was extracted from the pre-treated cell pellet using a modification of the method of Marmur (1961), in which achromopeptidase (final concentration, 0.3 mg ml⁻¹) was used to improve cell lysis with lysozyme. The DNA was dissolved in 10 mM Tris/HCl (pH 8.0) and its quality was assessed by using agarose gel electrophoresis as described above. Aliquots of 2 µl of the DNA were used as a template for amplification of the 16S rRNA gene (Andrews & Patel, 1996). The sequencing of the gene was performed essentially as described previously (Andrews & Patel, 1996). The partial sequences that were generated were assembled using BioEdit v. 5.0.1 (Hall, 1999) and the consensus sequence of 1528 nucleotides was corrected manually for errors. The most closely related sequences in the GenBank (version 152) and the Ribosomal Database Project II (release 9.37) identified using BLAST (Altschul *et al.*, 1997) and Sequence Match program (Cole *et al.*, 2005) were extracted, aligned and manually adjusted according to the 16S rRNA secondary structure using BioEdit. Nucleotide ambiguities were omitted and evolutionary distances were calculated by using the Jukes and Cantor option (Jukes & Cantor, 1969) in TREECON (Van de

Peer *et al.*, 1997). Phylogenetic trees were constructed from evolutionary distances using the neighbour-joining method (Saitou & Nei, 1987). Tree topology was re-examined by using the bootstrap method based on 1000 resamplings (Felsenstein, 1985).

16S rRNA gene sequence analysis indicated that strain Y170^T was related to members of the family *Syntrophomonadaceae*, class *Clostridia*, phylum *Firmicutes*, and was phylogenetically positioned between members of the genus *Thermosediminibacter* (mean sequence similarity, 93.5 %) and *Thermovenabulum ferriorganovorum* (91.9 %) (Fig. 2). All strains are motile, thermophilic organisms that grow optimally near neutral pH levels, vary greatly in cell length, require yeast extract for growth and utilize fructose, sucrose and xylose, reduce manganese(IV), thiosulfate and elemental sulfur but not sulfate and, with the exception of *Thermovenabulum ferriorganovorum*, exist in deep subsurface environments (Lee *et al.*, 2005; Zavarzina *et al.*, 2002). Phenotypic characteristics shared between strain Y170^T and *Thermovenabulum ferriorganovorum*, but not possessed by *Thermosediminibacter* species, *Thermosediminibacter litoriperuensis* and *Thermosediminibacter oceani*, such as Fe(III)

reduction, a lower salinity growth range and the inability to utilize lactate, concur with the 16S rRNA gene sequence analysis that strain Y170^T represents a new genus that is intermediate between the genera *Thermosediminibacter* and *Thermovenabulum*. Similarly, strain Y170^T has various phenotypic characteristics in common with *Thermosediminibacter* species but not *Thermovenabulum ferriorganovorum*, including Gram-negative cell-wall structures, longer cell length, sensitivity to heat-shock, similar mol% DNA G + C content, the ability to utilize glucose, galactose and mannose, and the inability to form spores and reduce sulfite and nitrate as electron acceptors. In addition, strain Y170^T can be differentiated from members of the genus *Thermosediminibacter* by glucose fermentation end products, its higher DNA G + C content and its inability to utilize inositol (Table 1). Strain Y170^T can be differentiated from *Thermosediminibacter litoriperuensis* by its ability to utilize cellobiose, pyruvate and Casamino acids, but not acetate and from *Thermosediminibacter oceani* by its inability to utilize maltose. Strain Y170^T also differed from *Thermovenabulum ferriorganovorum* by its sensitivity to heat-shock and 100 µg penicillin ml⁻¹, higher G + C DNA

Fig. 2. Dendrogram showing the phylogenetic position of strain Y170^T (*Fervidicola ferrireducens* gen. nov., sp. nov.) and its closest relatives. GenBank accession numbers are given in the parentheses. Clusters of members of the same genus or species are indicated by triangles and include: *Thermoanaerobacter mathranii* DSM 11426^T (GenBank accession number Y11279), *Thermoanaerobacter siderophilus* DSM 12299^T (AF120479), *Thermoanaerobacter italicus* DSM 9252^T (AJ250846), *Thermoanaerobacter acetoethylicus* ATCC 33265^T (X69336), *Thermoanaerobacter wiegelii* DSM 10319^T (X92513), *Thermoanaerobacter kivui* ATCC 33488^T (L09160), *Thermoanaerobacter thermohydrosulfuricus* ATCC 35045^T (L09161), *Thermoanaerobacter ethanolicus* ATCC 31550^T (L09162), *Thermoanaerobacter brockii* subsp. *brockii* DSM 1457^T (L09165), *Thermoanaerobacter brockii* subsp. *finii* DSM 3389^T (L09166) and *Thermoanaerobacter thermocopriae* JCM 7501^T (L09167) for the genus *Thermoanaerobacter*, and *Caldanaerobacter subterraneus* subsp. *tengcongensis* JCM 11007^T (AF209708) and *Caldanaerobacter subterraneus* subsp. *yonseiensis* DSM 13777^T (AF212925) for the species *Caldanaerobacter subterraneus*. Bootstrap values >95 % are shown. Bar, 2 nucleotide changes per 100 nucleotides.

Table 1. Selected characteristics of strain Y170^T and closest relatives

Strains: 1, strain Y170^T (*Fervidicola ferrireducens* gen. nov., sp. nov.); 2, *Thermosediminibacter litoriperuensis* DSM 16647^T (data from Lee *et al.*, 2005); 3, *Thermosediminibacter oceani* DSM 16646^T (Lee *et al.*, 2005); 4, *Thermovenabulum ferriorganovororum* DSM 14006^T (Zavarzina *et al.*, 2002). +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4
Habitat	Microbial mats from a thermal aquifer bore outflow	Subseafloor	Subseafloor	Hot spring
Geographical location	Great Artesian Basin, Australia	Peru margin	Peru margin	Uzon caldera, Kamchatka peninsula, Russia
Cell width (µm)	0.8–1.1	0.3–0.5	0.2–0.7	0.5–0.6
Cell length (µm)	1.2–12	2–10	1.5–16	1.5–7.0
Gram stain	–	–	–	+
Cell wall type	–	–	–	+
Spores observed	–	–	–	+
Heat resistant	– (95 °C for 20 min)	– (100 °C for 10 min)	– (100 °C for 10 min)	– (121 °C for 90 min)
Salinity range (% NaCl, w/v)	0–2	0–4.5	0–6.0	0–3.5
Inhibition of growth by 100 µg penicillin ml ^{–1}	+	ND	ND	–
DNA G + C content (mol%)*	48 ^a	50 ^b	50 ^b	36 ^a
Carbohydrate fermentation				
Glucose	+	+	+	–
Galactose	+	+	+	–
Mannose	+	+	+	–
Maltose	–	–	+	+
Cellobiose	+	–	+	–
Inositol	–	+	+	ND
Starch	–	ND	ND	+
Amino acid fermentation				
Casamino acids	+	–	+	+
Organic acid fermentation				
Acetate	–	+	–	–
Formate	+	ND	ND	–
Lactate	–	+	+	–
Pyruvate	+	–	+	+
Electron acceptors				
Fe(III)	+	–	–	+
Sulfite	–	–	–	+
Nitrate	–	–	–	+
Glucose fermentation end products	Ethanol and acetate	Acetate, propionate, iso-butyrate and isovalerate	Acetate, propionate, iso-butyrate and isovalerate	ND
16S rRNA gene similarity to strain Y170 ^T (%)	100	93.7	93.5	91.9

*Determined by using: *a*, the thermal denaturation method; *b*, HPLC.

content, and its ability to utilize cellobiose and formate, but not maltose or starch.

Based on the phenotypic differences and the phylogenetic distance separating strain Y170^T from other members of the family *Syntrophomonadaceae*, we propose a new genus and novel species with the name *Fervidicola ferrireducens* gen. nov., sp. nov., to accommodate this newly described strain.

Description of *Fervidicola* gen. nov.

Fervidicola [Fer.vi.di'co.la. L. adj. *fervidus* glowing, burning; L. suff. *-cola* (from L. masc. or fem. n. *incola*) inhabitant, dweller; N.L. masc. n. *Fervidicola* inhabitant of thermal waters, a thermophilic bacterium].

Cells are strictly anaerobic, slightly curved rods. Gram-reaction is negative. Cells possess peritrichous flagella. No spores are produced. Fe(III), Mn(IV), thiosulfate and

elemental sulfur are used as electron acceptors. 16S rRNA gene analysis indicates that the genus is a member of the family *Syntrophomonadaceae*, most closely related to members of the genus *Thermosediminibacter* with a mean similarity of 93.5 %. The type species is *Fervidicola ferrireducens*.

Description of *Fervidicola ferrireducens* sp. nov.

Fervidicola ferrireducens [fer.ri.re.du'cens. L. n. *ferrum* iron; L. part. adj. *reducens* leading back, bringing back and in chemistry converting to a different oxidation state; N.L. part. adj. *ferrireducens* reducing Fe(III) to Fe(II)].

Exhibits the following properties in addition to those given in the genus description. Cells are 1.2–12 × 0.8–1.1 µm. Growth occurs between 55 and 80 °C (optimum temperature, 70 °C) and pH 5 to 9 (optimum, pH 7.0). Grows on yeast extract as a sole carbon source but not on tryptone. Yeast extract is required for growth on tryptone, peptone, fructose, glucose, galactose, cellobiose, mannose, sucrose, xylose, mannitol, formate, pyruvate, Casamino acids and threonine. No growth occurs with arabinose, lactose, maltose, raffinose, chitin, xylan, pectin, starch, acetate, benzoate, lactate, propionate, succinate, *myo*-inositol, ethanol, glycerol, amyl media, aspartate, leucine, glutamate, alanine, arginine, serine or glycine. Fe(III), Mn(IV), thiosulfate and elemental sulfur serve as electron acceptors, but not sulfate, sulfite, nitrate or nitrite. Fe(III) is reduced in the presence of yeast extract, tryptone, peptone and Casamino acids, but not chitin, xylan, pectin, formate, starch, pyruvate, acetate, benzoate, threonine, lactate, propionate, succinate, inositol, ethanol, glycerol, mannitol, aspartate, leucine, glutamate, alanine, arginine, serine, glycine or H₂ and CO₂. Sensitive to streptomycin, tetracycline, penicillin, ampicillin and chloramphenicol. Does not grow in NaCl concentrations greater than 2 %. The G + C content of the genomic DNA of the type strain is 48 ± 1 mol%. Phylogenetically related to members of the genus *Thermosediminibacter* with a mean 16S rRNA gene similarity of 93.5 %.

The type strain, Y170^T (=KCTC 5610^T=JCM 15106^T=DSM 21121^T), was isolated from a microbial mat sample from the outflow of a GAB bore (the New Lorne bore) in Queensland, Australia.

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