Fervidicella metallireducens gen. nov., sp. nov., a thermophilic, anaerobic bacterium from geothermal waters

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A strictly anaerobic, thermophilic bacterium, designated strain AeB^T, was isolated from microbial mats colonizing a run-off channel formed by free-flowing thermal water from a bore well (registered number 17263) of the Great Artesian Basin, Australia. Cells of strain AeB^T were slightly curved rods (2.5–6.0×1.0 µm) that stained Gram-negative and formed spherical terminal to subterminal spores. The strain grew optimally in tryptone–yeast extract–Casamino acids medium at 50 °C (range 37–55 °C) and pH 7 (range pH 5–9). Strain AeB^T grew poorly on yeast extract (0.2 %) and tryptone (0.2 %) as sole carbon sources, which were obligately required for growth on other energy sources. Growth of strain AeB^T increased in the presence of various carbohydrates and amino acids, but not organic acids. End products detected from glucose fermentation were ethanol, acetate, CO₂ and H₂. In the presence of 0.2 % yeast extract, iron(III), manganese(IV), vanadium(V) and cobalt(III) were reduced, but not sulfate, thiosulfate, sulfite, elemental sulfur, nitrate or nitrite. Iron(III) was also reduced in the presence of tryptone, peptone, Casamino acids and amyl media (Research Achievement), but not starch, xylan, chitin, glycerol, ethanol, pyruvate, benzoate, lactate, acetate, propionate, succinate, glycine, serine, lysine, threonine, arginine, glutamate, valine, leucine, histidine, alanine, aspartate, isoleucine or methionine. Growth was inhibited by chloramphenicol, streptomycin, tetracycline, penicillin, ampicillin and NaCl concentrations >2 %. The DNA G + C content was 35.4 ± 1 mol%, as determined by the thermal denaturation method. 16S rRNA gene sequence analysis indicated that strain AeB^T is a member of the family *Clostridiaceae*, class *Clostridia*, phylum 'Firmicutes', and is positioned approximately equidistantly between the genera *Sarcina*, *Anaerobacter*, *Caloramator* and *Clostridium* (16S rRNA gene similarity values of 87.8–90.9 %). On the basis of 16S rRNA gene sequence comparisons and physiological characteristics, strain AeB^T is considered to represent a novel species in a new genus, for which the name *Fervidicella metallireducens* gen. nov., sp. nov. is proposed; the type strain is AeB^T (=JCM 15555^T=KCTC 5667^T).

The Great Artesian Basin (GAB) underlies more than one-fifth of the Australian continent, an area over 1.7 × 10⁶ km², and is considered to be the largest geothermal subsurface aquifer in the world, with a water-storage capacity of 8.7 × 10¹² m³ (Habermehl, 1980). The GAB was formed between 100 and 250 million years ago and contains groundwaters that are estimated to be 2 million years old. It is composed of at least four alternating layers of water-bearing permeable sandstone aquifers and non-water-bearing impermeable shale, the sequence of which can be found up to 3000 m deep. The GAB groundwaters are heated by the Earth's magma due to the aquifer's great depth, and flow from the recharge areas at the edge of the

basin to the discharge areas in central Australia as mound springs at an estimated rate of 1–5 m year⁻¹. Chemically, GAB groundwaters can be bicarbonate-, chloride-, sulfate- or iron-rich depending on the surrounding geological formations. The GAB is tapped by more than 5000 free-flowing bores, with source temperatures ranging between 30 and 100 °C, which provide a vital water resource to outback communities to support agricultural, industrial and domestic activities. The varying temperatures, chemical compositions and the slow flow rate of GAB groundwaters provide a conducive environment for a wide diversity of microbial habitats. Distinct, macroscopic microbial mat communities thrive at specific temperatures along the temperature gradient created as flowing borewaters cool to ambient temperatures in open drain run-off channels, which often stretch hundreds of kilometres to rehydrate livestock. Previous culture-independent analyses

Abbreviation: GAB, Great Artesian Basin.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AeB^T is FJ481102.

of four microbial mat communities existing in the New Lorne Bore (registered bore number 17263) at temperatures between 52 and 75 °C indicated the presence of species spanning the full diversity of domain 'Bacteria', including a diverse range of as-yet-uncultured novel organisms (Spanevello, 2001). Recent culture-dependent studies of these microbial mats have focused on thermophilic metal-reducers (Ogg & Patel, 2009a, b, c; Ogg *et al.*, 2010), which have a significant influence on the geochemistry of deep subsurface aquifers through their cycling of metals, particularly Fe(III) and Mn(IV), and organic matter and thereby influence water quality and taste (Lovley, 1997). It is likely that metal-reducing microorganisms inhabiting the GAB are responsible for bore-metal corrosion, leading to bore failure causing significant water wastage and economic hardship to outback communities, who depend largely on this water resource. In this report, properties of a novel thermophilic, strictly anaerobic, Fe(III)- and Mn(IV)-reducing bacterium, designated strain AeB^T, which was isolated from brown-coloured microbial mats thriving in the New Lorne Bore run-off channel at 52 °C, are described. Strain AeB^T represents a novel species in a new genus, for which the name *Fervidicella metallireducens* gen. nov., sp. nov. is proposed.

The microbial mat sample 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 was drilled in 1966 and has a depth of 1613 m and the temperature of the free-flowing water at the bore head was 88 °C, with a pH of 8.5 and a flow rate of 7.61 l s⁻¹ at the time of sampling. Iron and manganese concentrations of the free-flowing water were found to be 0.01 mg l⁻¹. The brown-coloured microbial mats that thrived at 52 °C 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 by Spanevello *et al.* (2002).

Enrichments were performed in APL medium amended with yeast extract (0.2 %). APL medium contained [(l deionized water)⁻¹]: 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, 12.0 g HEPES, 1 ml vitamin solution (Wolin *et al.*, 1963), 1 ml trace element solution (Zeikus *et al.*, 1979), 1 ml 0.2 % resazurin, 0.2 g yeast extract and 2.0 g ammonium iron(III) citrate (brown). The pH of the medium was adjusted to 7.2 with 1 M NaOH and 1 M HCl. APL medium (9.5 ml) was distributed in McCartney bottles and autoclaved for 30 min at 121 °C and 1.0–1.5 kg cm⁻² pressure. When APL medium was rendered anoxic, the medium was designated PL medium. Anoxic conditions were achieved by heating the medium for 10 min in an autoclave at 121 °C and 1.0–1.5 kg cm⁻² pressure to remove dissolved oxygen and the medium was subsequently cooled under a stream of oxygen-free nitrogen gas. Vitamins and trace elements were added and

9.3 ml medium was dispensed into Hungate tubes under oxygen-free nitrogen gas and sterilized for 20 min at 121 °C and 1.0–1.5 kg cm⁻² pressure.

Enrichments were initiated in three different types of medium: APL medium (aerobic medium), PL medium (anaerobic medium), and PL medium minus ammonium iron(III) citrate, but amended with 3.0 g amorphous iron(III) oxyhydroxide l⁻¹ (Lovley & Phillips, 1986). All media were amended with yeast extract (0.2 %) as an electron donor and were initiated by adding 500 µl brown mat sample to 9.5 ml medium and incubating at 50 °C. Enrichment in APL medium (enrichment culture 1) was performed in an attempt to cultivate microaerophilic or facultative anaerobic Fe(III)-reducing micro-organisms and, after 3 days incubation, a slimy surface layer was observed in the upper portion of the APL medium enrichment culture, suggesting that aerobic growth had occurred, and the reddish-brown colour of the APL medium had transformed into a colourless medium containing a dark precipitate [Fe(II)], suggesting Fe(III) reduction. On inversion of enrichment culture 1, the colourless medium changed to a pink colour, signifying that, prior to the inversion, the medium had reached anoxic conditions. An attempt to isolate the Fe(III)-reducing micro-organism from the APL enrichment culture by subculturing (5 %) into anaerobically prepared PL medium amended with yeast extract (0.2 %) failed; however, Fe(III) reduction was observed when subcultured into PL medium amended with Casamino acids (0.2 %) after 3 days incubation at 50 °C. This culture was serially diluted, incubated under the same conditions and the tube containing the lowest serial dilution (10⁻⁴) was selected; the procedure was repeated at least three times before a strain, designated AeB^T, was isolated and deemed to be a pure culture. Purity of the strain was subsequently confirmed by microscopy, subcultures on other substrates, including sugars, and 16S rRNA gene sequencing. To identify the dominant aerobe within enrichment culture 1, the initial enrichment was subcultured (5 %) in 9.5 ml medium D (Brock & Freeze, 1969) and incubated at 50 °C for 2 days. Repeat serial dilutions were performed as described above and a strain designated AeBMD^T was isolated and deemed to be a pure culture. Partial 16S rRNA gene sequencing of 1327 nt revealed that strain AeBMD^T was related closely to *Anoxybacillus flavithermus* clone LK4 (GenBank accession no. EU816689; 99.1 % similarity) and so no further studies were performed on this strain.

Fe(III) reduction was observed after 2 days incubation in both enrichment culture 2 (PL medium) and enrichment culture 3 [PL medium minus ammonium iron(III) citrate, but amended with 3.0 g amorphous iron(III) oxyhydroxide l⁻¹]. Repeat serial dilutions were performed on both enrichment cultures as described above and a strain designated strain B2-1^T was isolated from enrichment culture 2 and deemed to be a pure culture. The isolation and subsequent characterization of strain B2-1^T have been reported previously (Ogg & Patel, 2009b) and the strain

was designated *Thermotalea metallivorans* gen. nov., sp. nov. Repeat subcultures in dilution of enrichment culture 3 failed to isolate a pure culture, and so PL medium fortified with 0.2% yeast extract and 1% Gelrite was inoculated with 0.1 ml of the lowest positive dilution (10^{-4} dilution) taken from enrichment culture 3. Colonies developed after 4 days incubation at 50 °C; they were picked and subcultured in PL medium minus ammonium iron(III) citrate amended with amorphous iron(III) oxyhydroxide and yeast extract (0.2%). This isolate was designated B150^T and partial 16S rRNA gene sequencing of 765 nt revealed that strain B150^T was identical (99.8%) to strain AeB^T and so no further characterization studies have been performed as yet. All pure cultures were stored in a glycerol/medium (50:50) mixture at -20 °C.

Strain AeB^T was not able to grow under aerobic conditions in medium D (Brock & Freeze, 1969). This result demonstrates the failure of the above enrichment strategy to cultivate facultative Fe(III)-reducing micro-organisms and concurs with previous enrichment attempts using this strategy (Ogg & Patel, 2009d).

Cell morphology of strain AeB^T was determined by phase-contrast microscopy and by electron microscopy (Kanso & Patel, 2003). Cells of strain AeB^T were peritrichously flagellated, slightly curved rods (2.5–6.0 × 1.0 µm) that existed singly, in pairs and in small chains. Cells of strain AeB^T stained Gram-negative and electron micrographs of thin sections confirmed a Gram-negative cell-wall ultra-structure consisting of an inner electron-dense layer

adjacent to the cell membrane and an outer electron-light layer consisting of regular protein subunits (Fig. 1).

Cells of strain AeB^T could be subcultured into tryptone–yeast extract–Casamino acids (TYECas) medium after 20 min heating at 95 °C, but not after 30 min. Spherical terminal and subterminal spores were detected in death-phase cultures. Using the method of Ogg & Patel (2009a), a primer pair, forward GGI(A/G)TICGICICA(C/T)AT(A/T/C)AA(A/G)GGITA and reverse CATIGC(G/A/T)AT(A/G)AA(C/T)TCI(G/C)(A/T)(A/G)TTIGTIGG(C/T)TT (Brown *et al.*, 1994), was used to amplify a 270 bp fragment of the sporulation *spo0A* gene. Sequencing and sequence analysis indicated that the amplicon was related to the *spo0A* genes of *Clostridium botulinum* B strain Eklund 17B (GenBank accession no. CP001056), *Clostridium acetobutylicum* ATCC 824^T, (GenBank accession no. AE001437.1) and *Clostridium butyricum* ATCC 19398^T (GenBank accession no. U09980.1) with a similarity value of 80%.

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 studies of strain AeB^T were tested in anaerobic TYECas medium (modified TYEG medium), which consisted of low-phosphate-buffered salts (LPBS) amended with 0.2% each of tryptone, yeast extract and Casamino acids. LPBS was prepared anaerobically as described previously (Patel *et al.*, 1985a, b) and contained [(1 deionized water)⁻¹]: 0.2 g MgCl₂·6H₂O, 0.9 g NH₄Cl, 0.75 g KH₂PO₄, 1.5 g K₂HPO₄, 1.0 g NaCl, 5 µl 10% FeSO₄, 9 ml trace element solution (Zeikus *et al.*, 1979), 5 ml vitamin solution (Wolin *et al.*, 1963), 1 ml 0.2% resazurin and 2 ml 10% Na₂S·9H₂O. Growth of strain AeB^T was tested at temperatures ranging from 26 to 70 °C and pH values ranging from 4.5 to 9.5. Growth was measured at 580 nm by inserting Hungate tubes directly into a modified cuvette holder of a Novaspec LKB spectrophotometer (Pharmacia Biotech). Strain AeB^T grew optimally in TYECas medium at 50 °C (range 37–55 °C) and pH 7 (range pH 5–9), with a generation time of 46.4 min under these conditions. Unless indicated otherwise, all subsequent growth experiments were conducted under these conditions.

Strain AeB^T was tested for its ability to grow in LPBS amended 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 and compared with growth in the complete TYECas medium. After 5 h incubation, growth of approximately 25, 50 and 100% was observed in the three media, respectively (OD₅₈₀ of 0.22=100%). Furthermore, Casamino acids could not be used as the sole carbon source (medium Cas), but were utilized in the presence of tryptone (medium TCas) or yeast extract (medium YECas). Under these conditions, growth reached levels of 75 and 85% in TCas and YECas, respectively, after 5 h incubation. When the yeast extract

Fig. 1. Electron micrographs of cells of strain AeB^T. (a) Negatively stained cells showing a peritrichously flagellated cell. F, Flagella. Bar, 500 nm. (b) Transmission electron micrograph of a thin section showing cell-wall ultrastructure revealing the presence of a cytoplasmic membrane (CM), an electron-dense wall layer adjacent to the membrane (CWi) and an electron-light outer cell-wall layer composed of regular subunits (CWo). Bar, 100 nm.

concentration in YECas medium was lowered to 0.02, 0.05 or 0.1 %, approximately 45, 50 and 70 % growth, respectively, was observed (compared with growth with 0.2 % YECas medium), indicating that growth of strain AeB^T was influenced by the components in yeast extract in a dose-dependent manner.

Substrate-utilization tests were performed in the presence of 0.02 % yeast extract. Soluble substrates were added from sterile anaerobic stock solutions to a final concentration of 0.2 % to sterile YE (0.02 %) medium. Insoluble substrates were weighed directly into Hungate tubes, YE (0.02 %) medium was dispensed and the medium was sterilized. Growth was measured hourly at 580 nm until stationary phase was reached. Cultures containing no added substrates were used as negative controls and tubes containing Casamino acids (0.2 %) were used as positive controls. Growth was observed with peptone, glucose (approximate generation time of 36.2 min), sucrose, fructose, maltose, mannitol, starch, pectin, pyruvate, amyl media, Casamino acids (approximate generation time of 157.2 min), methionine, serine and threonine, but not galactose, mannose, lactose, cellobiose, rhamnose, arabinose, raffinose, inositol, glycerol, ethanol, formate, acetate, propionate, lactate, aspartate, succinate, glycine (both in the presence and absence of uric acid), adenine, guanine, uric acid, caffeine, purine, valine, leucine, alanine, methionine, isoleucine, arginine or histidine.

End products from glucose fermentation were determined in a culture grown in YE medium amended with glucose (0.2 %) as described previously (Chrisostomos *et al.*, 1996; Ogg & Patel, 2009a). End products from glucose fermentation were ethanol, acetate, CO₂ and H₂.

To determine the substrates used by strain AeB^T with reduction of Fe(III), substrates were added to PL medium and cultures were incubated for up to 8 days at 50 °C. Fe(III) reduction was inferred when transformation of the reddish-brown colour of the Fe(III) to a dark precipitate [Fe(II)] and clearing of the medium were observed. Fe(III) reduction was confirmed by measuring Fe(II) accumulation using the ferrozine method (Sørensen, 1982). A 0.1 ml sample was mixed with 3 ml ferrozine reagent and, after 1 min, A₅₆₂ was determined. A culture containing PL medium lacking a substrate was used as a negative control and a culture containing PL medium amended with 2 g Casamino acids l⁻¹ was used as a positive control. Fe(III) reduction can occur spontaneously in the presence of carbohydrates under sterile conditions (Zavarzina *et al.*, 2002) and, hence, most carbohydrates were not tested. Fe(III) was reduced in the presence of yeast extract, tryptone, peptone and amyl media, but not with starch, xylan, chitin, glycerol, ethanol, pyruvate, benzoate, lactate, acetate, propionate, succinate, serine, lysine, threonine, arginine, glutamate, glycine, valine, leucine, histidine, alanine, aspartate, isoleucine or methionine. Given that Fe(III) was only reduced in the presence of substrates that could be fermented, an investigation to determine whether

energy is conserved to support growth from the reduction of Fe(III) to Fe(II) was deemed to be equivocal.

The ability of strain AeB^T to utilize H₂ and CO₂ in the presence and absence of Fe(III) was tested in 60 ml serum bottles that contained 15 ml PL medium and H₂/CO₂ gas mixture (5 : 95 or 10 : 90, v/v) or CO₂ (100 %). No growth or Fe(III) reduction was noted.

Electron acceptor-utilization tests for sodium thiosulfate (20 mM), sodium sulfate (20 mM), sodium sulfite (10 mM), elemental sulfur (1 %), sodium nitrate (20 mM) and sodium nitrite (5 mM) were tested in YE medium as described by Ramamoorthy *et al.* (2006) and Ogg & Patel (2009a). Reduction of 3 g amorphous iron(III) oxyhydroxide l⁻¹ (Lovley & Phillips, 1986), 2 g MnO₂ l⁻¹ (Lovley & Phillips, 1988), 4 mM vanadium(V) and 5 mM cobalt(III) was tested in PL medium amended with yeast extract (0.2 %), but lacking ammonium iron(III) citrate. Fe(III) reduction was determined as described above. Mn(IV) reduction was determined as described previously by Spratt *et al.* (1994) and Ogg & Patel (2009a). V(V) reduction was inferred from a medium colour change to green and/or a green precipitate formed in the medium and was confirmed using the vanadate assay as described by Carpentier *et al.* (2003). For this, 250 µl 1 % (w/v) diphenylcarbazide in acetone was added to 250 µl 2 M H₂SO₄, which was combined with 500 µl diluted sample and the A₃₂₀ was measured after 15 min. Co(III) reduction was scored as positive when a medium colour change from purple [Co(III)] to clear was observed. In the presence of yeast extract (0.2 %), strain AeB^T was able to reduce amorphous iron(III) oxyhydroxide, Mn(IV), V(V) and Co(III), but not elemental sulfur, sulfate, thiosulfate, sulfite, nitrate or nitrite.

Antibiotic sensitivity was determined by adding antibiotics from filter-sterilized stock solutions to sterilized TYECas medium to final concentrations of 10 and 100 µg ml⁻¹. Growth inhibition of each antibiotic was calculated from final turbidity measurements of cultures that had been grown in antibiotic-free TYECas medium and growth was expressed as percentage inhibition. Strain AeB^T was inhibited completely in the presence of ampicillin, streptomycin, tetracycline, penicillin and chloramphenicol (all at 10 µg ml⁻¹). The effect of NaCl on growth of strain AeB^T was determined; NaCl was added directly to Hungate tubes at concentrations of 0, 0.01 and 1–5 %, the TYECas medium was dispensed and the medium was sterilized. Strain AeB^T was able to grow without NaCl, but not at concentrations >2 %. Reduced growth was observed at NaCl concentrations >0.1 %.

The DNA G+C content of strain AeB^T was determined by the thermal denaturation method (Marmur & Doty, 1962) in a Cintra20 spectrophotometer (GBC Scientific Equipment) with DNA that had been amplified using a TempliPhi Amplification kit (Amersham Biosciences) as described by Ogg & Patel (2009a). The G+C content of the genomic DNA was calculated to be 35.4 ± 1 mol%.

16S rRNA gene amplification, sequencing and analysis were performed using the method of Ogg & Patel (2009a). Phylogenetic analysis of the consensus 16S rRNA gene sequence, comprising 1423 nt, indicated that strain AeB^T was a member of the family *Clostridiaceae*, class *Clostridia*, phylum 'Firmicutes', and is positioned approximately equidistantly between the genera *Sarcina*, *Anaerobacter*, *Caloramator* and *Clostridium* (16S rRNA gene sequence similarity values of 87.8–90.9%) (Fig. 2). The closest relatives to strain AeB^T included *Clostridium cylindrosporium* DSM 605^T (90.9% similarity), which is known to inhabit various soils and chicken intestines (Andreesen *et al.*, 1985), and *Caloramator viterbiensis* DSM 13723^T (90.7% similarity), which inhabits hot-spring environments (Seyfried *et al.*, 2002). Phenotypically, strain AeB^T could be differentiated from both of these strains by its morphology, negative Gram stain, G+C content, optimal growth conditions and nutritional spectrum (Table 1). Interestingly, strain AeB^T possessed more phenotypic characteristics in common with *Caloramator viterbiensis* than with *Clostridium cylindrosporium*, including thermophilic growth, carbohydrate fermentation, the inability to reduce sulfate, elemental sulfur and nitrate, and the utilization of yeast extract, glucose, sucrose, fructose, starch, serine and threonine, but not arabinose, ethanol or organic acids. However, strain AeB^T could be further differentiated from *Caloramator viterbiensis* by its ability to reduce Fe(III) and its inability to utilize galactose, mannose, lactose, cellobiose, glycerol, aspartate, leucine and histidine. Strain AeB^T could be further differentiated from *Clostridium cylindrosporium* by its ability to ferment carbohydrates and serine, but not glycine, uric acid or guanine. Based on the phenotypic differences and the large phylogenetic distance separating strain AeB^T from other members of the family *Clostridiaceae*, a novel species in a new genus, *Fervidicella metallireducens* gen. nov., sp. nov., is proposed to accommodate this newly described strain.

Description of *Fervidicella* gen. nov.

Fervidicella (Fer.vi.di.cel'la. L. adj. *fervidus* glowing, hot, burning; L. fem. n. *cella* a storeroom, chamber, and, in

biology, a cell; N.L. fem. n. *Fervidicella* a glowing cell, a thermophile).

Cells are strictly anaerobic, slightly curved rods. Gram reaction is negative. Cells possess peritrichous flagella. Spherical terminal to subterminal spores are formed. 16S rRNA gene analysis indicates that the genus is a member of the family *Clostridiaceae*, approximately equidistant between the genera *Sarcina*, *Anaerobacter*, *Caloramator* and *Clostridium* (87.8–90.9% 16S rRNA gene sequence similarity). The type species and the sole member of this genus is *Fervidicella metallireducens*.

Description of *Fervidicella metallireducens* sp. nov.

Fervidicella metallireducens (me.tal'li.re.du'cens. L. n. *metallum* metal; L. part. adj. *reducens* leading back, bringing back, and, in chemistry, converting to a different oxidation state; N.L. part. adj. *metallireducens* reducing metal).

Cells are 2.5–6.0 × 1.0 µm. Growth occurs between 37 and 55 °C (optimum temperature 50 °C) and at pH 5–9 (optimum pH 7). Grows on yeast extract and tryptone as sole carbon sources. Yeast extract or tryptone is required for growth with other substrates. Utilizes peptone, glucose, sucrose, fructose, maltose, mannitol, starch, pectin, pyruvate, amyl media, Casamino acids, methionine, serine and threonine, but not galactose, mannose, lactose, cellobiose, rhamnose, raffinose, arabinose, inositol, glycerol, ethanol, formate, acetate, propionate, lactate, aspartate, succinate, glycine, adenine, guanine, uric acid, caffeine, purine, leucine, alanine, isoleucine, arginine, histidine or H₂/CO₂. End products from glucose fermentation are ethanol, acetate, CO₂ and H₂. Fe(III), Mn(IV), V(V) and Co(III) are reduced in the presence of yeast extract, but not elemental sulfur, sulfate, sulfite, thiosulfate, nitrate or nitrite. Fe(III) is also reduced in the presence of tryptone, peptone, Casamino acids and amyl media, but not starch, xylan, chitin, glycerol, ethanol, pyruvate, benzoate, lactate, acetate, propionate, succinate, glycine, serine, lysine,

Fig. 2. Dendrogram showing the phylogenetic position of *Fervidicella metallireducens* AeB^T and its closest relatives. GenBank accession numbers are given in parentheses. Bootstrap values >95% are shown. Bar, 5 nucleotide changes per 100 nt.

Table 1. Selected characteristics of *Fervidicella metallireducens* AeB^T and closely related strains

Strains: 1, *Fervidicella metallireducens* AeB^T; 2, *Clostridium cylindrosporum* DSM 605^T (Andreesen *et al.*, 1985); 3, *Caloramator viterbiensis* DSM 13723^T (Seyfried *et al.*, 2002). +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3
Isolation source	Microbial mats from a thermal aquifer bore outflow	Soil and chicken intestines	Hot springs
Geographical location	Great Artesian Basin, Australia	Various locations	Bagbaccio Spring area, Italy
Morphology	Slightly curved rods	Straight rods	Straight to slightly curved rods
Cell width (µm)	1.0	0.8	0.4–0.6
Cell length (µm)	2.5–6.0	3.3	2.0–3.0
Gram stain	–	+*	+
Cell-wall ultrastructure	–	–†	+
Sporulation	–	+	–
Motility	+	+	–
Growth temperature (°C):			
Optimum	50	40–45	58
Range	30–55	19 to ND	33–64
Growth pH:			
Optimum	7.0	7.5–8.0‡	6.0–6.5
Range	6–9	6.7–8.5	5.0–7.8
DNA G + C content (mol%)	35.4	27.9	32
Nutritional characteristics			
Carbohydrate fermentation:			
Fructose	+	–	+
Starch	+	–	+
Glucose	+	–	+
Galactose	–	ND	+
Mannose	–	ND	+
Lactose	–	ND	+
Cellobiose	–	ND	+
Alcohol fermentation:			
Glycerol	–	ND	+
Amino acid fermentation:			
Glycine	–	+ †§	ND
Uric acid	–	+	ND
Guanine	–	+	ND
Aspartate	–	ND	+
Serine	+	–	+
Leucine	–	ND	+
Histidine	–	ND	+
Fe(III) reduction	+	ND	–
16S rRNA gene sequence similarity to strain AeB ^T (%)	100	91.0	90.7

*Only actively growing cultures stain Gram-positive.

†Data from Dürre *et al.* (1981).

‡pH optimum changes with different substrates.

§In the presence of uric acid.

threonine, arginine, glutamate, valine, leucine, histidine, alanine, aspartate, isoleucine, methionine or H₂/CO₂. Sensitive to chloramphenicol, streptomycin, tetracycline, penicillin, ampicillin and NaCl concentrations >2 %.

The type strain is AeB^T (=KCTC 5667^T=JCM 15555^T), isolated from a microbial mat sample from the outflow of a

Great Artesian Basin bore (the New Lorne Bore) in Queensland, Australia. The G + C content of the genomic DNA of the type strain is 35.4 ± 1 mol%. Phylogenetically related to *Clostridium cylindrosporum* DSM 605^T (90.9 % 16S rRNA gene sequence similarity) and *Caloramator viterbiensis* DSM 13723^T (90.7 % 16S rRNA gene sequence similarity).

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