Thermotalea metallivorans gen. nov., sp. nov., a thermophilic, anaerobic bacterium from the Great Artesian Basin of Australia aquifer

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A strictly anaerobic, thermophilic bacterium, designated strain B2-1^T, was isolated from microbial mats colonizing a runoff channel formed by free-flowing thermal water from a Great Artesian Basin, Australia, bore well (registered number 17263). The cells of strain B2-1^T were slightly curved rods (3.0–3.5×0.6–0.7 µm) which stained Gram-negative. The strain grew optimally in tryptone-yeast extract-glucose medium at 50 °C (temperature growth range 30–55 °C) and a pH of 8 (pH growth range 6.5–9). Strain B2-1^T grew poorly on yeast extract (0.2 %) and/or tryptone (0.2 %), which were obligately required for growth on other energy sources, including a range of other carbohydrates and organic acids, but not amino acids. The end-products of glucose fermentation were ethanol and acetate. In the presence of 0.2 % yeast extract, iron(III), manganese(IV) and elemental sulfur were reduced but sulfate, thiosulfate, sulfite, nitrate and nitrite were not reduced. Growth was inhibited by chloramphenicol, streptomycin, tetracycline, penicillin, ampicillin, sodium azide and by NaCl concentrations greater than 4 % (w/v). The DNA G + C content was 48 ± 1 mol% as determined by the thermal denaturation method. 16S rRNA gene sequence analysis indicated that strain B2-1^T was a member of the family *Clostridiaceae*, class *Clostridia*, phylum *Firmicutes* and was most closely related to *Geosporobacter subterraneus* DSM 17957^T (89.9 % similarity). On the basis of 16S rRNA gene sequence comparisons and physiological characteristics, strain B2-1^T is considered to represent a novel species of a new genus, for which the name *Thermotalea metallivorans* gen. nov., sp. nov. is proposed. The type strain is B2-1^T (=KCTC 5625^T=JCM 15105^T=DSM 21119^T).

The Great Artesian Basin (GAB) underlies approximately 22 % of the arid and subarid regions of the Australian continent, an area of over 1.7×10^6 km², with a water storage capacity of 8.7×10^{12} m³, and is considered to be the world's largest geothermal subsurface aquifer (Habermahl, 1980). The GAB was formed between 100 and 250 million years ago and contains groundwaters which are estimated to be 2 million years old. It is composed of alternating layers of water-bearing permeable sandstone aquifers and non-water-bearing impermeable shale and includes two regions that contain the largest onshore oil and gas reserves in Australia. These geological formations have an immense influence on the chemical composition of GAB groundwaters, which can be bicarbonate-, chloride-, sulfate- or iron-rich. The GAB subsurface waters 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 and are heated by the Earth's magma due to the aquifer's great depth. The GAB is tapped by more than 5000 free-flowing bores which provide vital water to outback communities where it is used for agricultural, industrial and domestic purposes. These bores can be found up to 3000 m deep, with source temperatures ranging from 30 to 100 °C depending on the bore depth.

The unique chemical composition, combined with varying temperatures and the slow flow rate of GAB groundwaters, provides a conducive environment for the growth of a wide diversity of microbial life. Distinct thriving macroscopic microbial mat communities can be seen colonizing specific temperatures along the temperature gradient of runoff channels formed by the free-flowing bores. Culture-independent analyses of four microbial mat communities existing in the New Lorne bore (registered bore number 17263) have indicated that members of the genus *Thermus* dominated the 75 °C grey mat, members of the genus *Meiothermus* dominated the 66 °C red mat, *Cyanobacteria* dominated the 57 °C green mat and members of the *Deinococcus–Thermus* group and class *Alphaproteobacteria*

Abbreviations: GAB, Great Artesian Basin; TYEG, tryptone-yeast extract-glucose medium.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B2-1^T is EU443727.

dominate the 52 °C brown mat (Spanevello, 2001). Identical phylotypes were discovered in adjacent communities and the phylogenetic diversity was found to increase as the water temperature decreased. Furthermore, species spanning the full diversity of the domain *Bacteria* were identified, including a diverse range of as-yet-uncultured, novel organisms.

Numerous thermophilic and mesophilic micro-organisms have been characterized from the GAB waters and they include sulfate-reducers (Love *et al.*, 1993; Redburn & Patel, 1994), carbohydrate-fermenters (Andrews & Patel, 1996), strict aerobes (Kanso & Patel, 2003; Spanevello *et al.*, 2002) and metal-reducers (Kanso *et al.*, 2002). Metal-reducing micro-organisms are known to have a significant impact on the geochemistry of deep subsurface aquifers through their cycling of metals [particularly Fe(III) and Mn(IV)] and organic matter, and thereby have an influence on water quality and taste (Lovley, 1997). Our findings of metal-reducers (Kanso *et al.*, 2002) in the GAB environment has led to a concern that their colonization of, and growth on, the metal casing of bores could lead to corrosion, causing significant water leakage and therefore economic loss and hardship to the communities that rely on the aquifer waters.

In this report, we describe a novel, strictly anaerobic, thermophilic, metal-reducing micro-organism, designated strain B2-1^T, inhabiting the 52 °C brown microbial mat of a GAB bore runoff channel. The microbial mat sample used in this study was collected from the runoff 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 is 1613 m deep. 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. The 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. The vessels were filled to the brim with water from the runoff channel, capped, transported to the laboratory and stored at 4 °C until required. The microbial mats have been described by Spanevello *et al.* (2002).

Enrichment and isolation were performed in anaerobic PL medium. 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, 2.0 g yeast extract and 2.0 g ammonium Fe(III) citrate (brown). The pH of the medium was adjusted to 7.2 with 1 M NaOH and 1 M HCl and heated for 10 min in an autoclave at 121 °C and under 1–1.5 kg cm⁻² pressure. The medium was subsequently 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 1–1.5 kg cm⁻² pressure. Enrichment cultures were initiated by injecting

500 µl mat sample suspension into PL medium (9.5 ml) followed by incubation at 50 °C for 2 days. The enrichment culture was serially diluted, incubated under the same conditions and the tube containing the lowest serial dilution was selected. This procedure was repeated at least three times before a strain, designated B2-1^T, was isolated and deemed to be a pure culture. The strain's purity was subsequently confirmed by 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 phase-contrast light microscopy and by electron microscopy (Kanso & Patel, 2003). The cells of strain B2-1^T were peritrichously flagellated, slightly curved rods (3.0–3.5 × 0.6–0.7 µm) and existed singly and rarely in long chains. Spores were not observed under the microscope from cultures that had been grown under suboptimal conditions (pH and temperatures), after storage at 4 °C for 5 days or with substrates that included glucose and xylose. In addition, cultures heat-treated at 95 °C for 10 min did not grow after subculture into tryptone-yeast extract-glucose (TYEG) medium, suggesting the absence of heat-resistant bodies such as spores. Cells of strain B2-1^T stained Gram-negative and electron micrographs of thin sections confirmed a Gram-negative cell-wall ultrastructure 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).

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 performance of the experiment. The growth of strain B2-1^T was tested in anaerobic TYEG medium which consisted of low phosphate buffered salts (LPBS) amended with 0.2 % each of tryptone, yeast extract and glucose at temperatures ranging from 26 to 70 °C and at pH values ranging from 5.0 to 9.5. Anaerobic TYEG medium was prepared as described previously (Patel *et al.*, 1985a, b). Growth was measured at 580 nm by inserting Hungate tubes directly into a modified cuvette holder of a Novaspec LKB spectrophotometer (Pharmacia-Biotech Pty. Ltd). Strain B2-1^T grew optimally in TYEG medium at 50 °C (temperature growth range, 30–55 °C) and a pH of 8 (pH growth range, 6–9) with a generation time of 6.7 h. Unless indicated otherwise, all subsequent growth experiments were conducted using these conditions.

Strain B2-1^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. Compared with growth of the novel strain in TYEG medium (an OD₅₈₀ 0.3 = 100 %), growth of approximately 28 %, 20 % and 40 % was obtained in LPBS, YE and TYE, respectively. No growth of strain B2-1^T was observed with 0.2 % glucose (medium G) as the sole carbon source, but glucose (0.2 %) was fermented in the presence of 0.2 % yeast extract (YEG

Fig. 1. Electron micrographs of cells of strain B2-1^T. (a) Negatively stained cells showing a peritrichously flagellated cell. 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, 50 nm.

medium) and 0.2% tryptone (TG medium) reaching approximately 75% and equal maximum growth, respectively compared with the maximum growth in TYEG medium. No growth of strain B2-1^T was observed when yeast extract concentrations in the YEG medium were lowered to 0.005% or 0.01% and approximately 65% growth was observed with 0.1% yeast extract compared with growth in 0.2% yeast extract. This indicates that components in yeast extract were required for glucose fermentation in a dose-dependent manner.

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 three times a day at 580 nm until the stationary phase was reached. Growth was taken as positive if the turbidity of the culture was at least 20% higher in YE medium containing substrates when compared to YE medium lacking the substrates. Growth was observed with glucose, sucrose, maltose, fructose, arabinose, mannose, cellobiose, *myo*-inositol, mannitol, Casamino acids, serine and leucine but not with lactose, galactose, raffinose, xylose, ribose, chitin, starch, amylopectin, casein, xylan, acetate, propionate, lactate, formate, pyruvate, ethanol, glycerol, amyl media, arginine, alanine, glycine, threonine, glutamate, isoleucine or methionine.

The metabolic profile of strain B2-1^T was further investigated using GP2 Biolog MicroPlates (Biolog Inc.). For this, 6 ml exponential phase cells of strain B2-1^T grown in TYEG medium were centrifuged at 6000 r.p.m. at 4 °C for 8 min and resuspended in 10 ml inoculating fluid (IF). The cell-containing solution was again centrifuged and this process was repeated twice. The washed cells of strain B2-1^T were resuspended in 10 ml IF containing 3 drops of thioglycolic acid and were inoculated into the wells of the GP2 Biolog MicroPlates as recommended by the manufac-

turer. The MicroPlate was placed inside a humidified plastic sealed container and incubated at 50 °C for 5 h. The absorbances in the wells of the MicroPlates were read and the results transformed into positive, borderline and negative scores using Biolog MicroStation system software. The results of the metabolic analysis are provided in the species description. Ambiguities exist between the substrates metabolized by strain B2-1^T and those utilized for growth in the presence of yeast extract. Such results include the positive metabolism results obtained for ribose, xylose, acetic acid and propionic acid which provided no increased growth for strain B2-1^T in the presence of yeast extract. This suggests that these substrates are metabolized by strain B2-1^T in a reaction where no energy generation for increased cellular growth is yielded, such as an intermediate pathway, or perhaps an additional substrate other than yeast extract is required for their utilization. Conversely, the negative metabolism result for the substrates maltose and mannitol, which provide an increased growth of strain B2-1^T in the presence of yeast extract, suggests that yeast extract is absolutely required for the metabolism of these substrates.

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

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 lacking the reductant Na₂S. The reduction of sulfate, sulfite, elemental sulfur and thiosulfate was tested using the method of Ramamoorthy *et al.* (2006). Tubes were scored positive for the reduction of oxidized sulfur compounds if a black FeS precipitate formed after the addition of 0.5% Fe(NH₄)₂(SO₄)₂ to the culture tubes. The

reduction of nitrate and nitrite to nitrogen (N₂) was determined by assessing nitrogen gas formation using a Durham tube inserted into media tubes prior to sterilization. Cultures were scored positive for the reduction of nitrate or nitrite to nitrogen if gas levels in the Durham tube were greater than those found in a control tube with no added oxidized nitrogen compounds. Reduction of nitrate to nitrite was determined using Aqua One (Australia) nitrite test kits using the protocols described by the manufacturer. Additionally, the reduction of nitrate to ammonium was subsequently tested in the same solution with the addition of 1 mg zinc powder (Merck) and was scored positive if no colour change was observed and scored negative if a pink colour change occurred. The reduction of nitrite to ammonium was tested using Aqua One (Australia) ammonia test kits using the protocols described by the manufacturer and with uninoculated media as a control. Utilization of amorphous Fe(III) oxyhydroxide (Lovley & Phillips, 1986) and Mn(IV) (Lovley & Phillips, 1988) were tested at concentrations of 2 g l⁻¹ in PL medium minus ammonium Fe(III) citrate. The reduction of Fe(III) was inferred when a transformation of the reddish-brown colour of the Fe(III) oxide to a dark precipitate [Fe(II)] and a clearing of the media was observed. This was confirmed using the ferozine method (Sorensen, 1982). A 0.1 ml sample was mixed with 3 ml ferozine reagent and the absorbance at 562 nm was determined after 1 min. 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). The concentration of MnO₂ was determined by mixing a 0.2 ml sample with 4 ml leuco crystal violet reagent, which was diluted, and the absorbance at 562 nm was determined. Strain B2-1^T was able to reduce ammonium Fe(III) citrate, amorphous Fe(III) oxyhydroxide, Mn(IV) and elemental sulfur, but not sulfate, thiosulfate, sulfite, nitrate or nitrite.

Strain B2-1^T was unable to grow under aerobic conditions in medium D (Brock & Freeze, 1969) or in aerobic YE medium.

Antibiotic sensitivity was determined by adding antibiotics from filter-sterilized stock solutions to sterilized TYEG medium to final concentrations of 10 and 100 µg ml⁻¹. Growth inhibition of each antibiotic was calculated from the final turbidity measurements of cultures that had been grown in antibiotic-free TYEG medium and growth was expressed as % inhibition. Strain B2-1^T was completely inhibited (100 % inhibition) in the presence of 10 µg ml⁻¹ ampicillin, streptomycin, tetracycline and penicillin, 100 µg chloramphenicol ml⁻¹ and 250 µg sodium azide ml⁻¹. The effect of NaCl on the growth of strain B2-1^T was determined by adding NaCl directly to Hungate tubes at concentrations of 0–5 % (w/v), the TYEG medium was dispensed and sterilized. Strain B2-1^T was able to grow without NaCl, but not at concentrations higher than 4 %. Reduced growth was observed at NaCl concentrations above 1 %.

To determine the G + C content of the DNA of strain B2-1^T, 2 l of actively growing culture in TYEG medium was used for genomic DNA preparation. Genomic DNA was prepared using a modification of the method of Marmur (1961) in which achromopeptidase (Sigma; final concentration 0.3 mg ml⁻¹) was used to improve cell lysis with lysozyme. The spooled DNA was dissolved in 10 mM Tris/HCl (pH 8.0) and the quality was assessed by agarose gel electrophoresis, ethidium bromide staining and visualization under a UVP GDAS 1200 Gel Documentation Analysis System (UVP Inc.). The G + C content of the genomic DNA was determined by the thermal denaturation method (Marmur & Doty, 1962) using a Cintra20 spectrophotometer (GBC Scientific Equipment) as described by Spanevello *et al.* (2002) and was calculated to be 48 ± 1 mol%.

Inorganic Fe(III) present in PL medium is known to interfere with genomic DNA extraction and PCR (Kashefi *et al.*, 2003). Thioglycollic acid pre-treatment was used to reduce Fe(III) concentrations and precipitate Fe(III) prior to genomic DNA extraction. For this, 40 µl thioglycollic acid was added to 35 ml of culture at the exponential phase in PL medium. The solution was mixed by inversion, allowed to settle for 5 min and the supernatant was centrifuged at 8000 r.p.m. at 4 °C for 15 min (Sigma 4K15). The cell pellet was collected, resuspended in 7.5 ml TE buffer, 10 µl thioglycollic acid was added and the tube was mixed by gentle inversion. The mixture was allowed to settle, the supernatant was centrifuged again as described and the cell pellet was washed in 3.5 ml TE buffer to remove traces of thioglycollic acid. The pellet was then resuspended in 460 µl TE buffer. Genomic DNA was extracted from the pre-treated cell pellet using the modified Marmur's method described above. Aliquots of 2 µl DNA were used as a template for the 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 v5.0.1 (Hall, 1999) and the consensus sequence of 1627 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 the 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). The tree topology was re-examined by the bootstrap method with 1000 replications (Felsenstein, 1985).

16S rRNA sequence analysis indicated that strain B2-1^T is related to members of the family *Clostridiaceae*, class *Clostridia*, phylum *Firmicutes*, and is most closely related to the uncultured bacterial clone 47mm70 which was

retrieved from a South African gold mine deep borehole (GenBank accession no. AY796047; similarity value 99.2 %). Closely related uncultured organisms also include GAB clone R82, from the red mat (AY407695; similarity value 89.3 %) and GAB clone Y36, from the grey mat (AF407679; similarity value 89.0 %) mentioned above. The most closely related type strains of recognized species to strain B2-1^T include *Geosporobacter subterraneus* DSM 17957^T (similarity value 89.9 %), *Caminicella sporogenes* DSM 14501^T (similarity value 88.2 %), *Clostridium caminithemale* DSM 15212^T (similarity value 86.8 %) and members of the genus *Alkaliphilus* (mean similarity 86.4 %) (Fig. 2). Although all of the species closely related to strain B2-1^T originate from deep subsurface environments, few organisms shared phenotypic characteristics with strain B2-1^T. The closest relative to strain B2-1^T, *G. subterraneus*, grows optimally over a similar pH range to strain B2-1^T and also utilizes glucose, fructose, cellobiose, Casamino acids and serine, but not ethanol, glycerol, starch, acetate, propionate, formate, lactate, threonine, glycine or cysteine, and does not reduce sulfate, sulfite, thiosulfate, nitrate or nitrite (Klouche *et al.*, 2007). However, strain B2-1^T can be differentiated from *G. subterraneus* by its Gram-negative cell-wall ultrastructure, motility, inability to form spores, temperature optima and range, DNA G+C content, end products from glucose fermentation, its ability to utilize mannose and mannitol, but not xylose, ribose, alanine, arginine, glutamate, glutamine or isoleucine, and its ability to reduce elemental sulfur (Table 1). Similarly, strain B2-1^T can be differentiated from *Caminicella sporogenes* (Alain *et al.*, 2002) and *Clostridium caminithemale* (Brisbarre *et al.*, 2003) by its smaller cell length, inability to form spores, lower

optimal NaCl concentration for growth, greater DNA G+C content, the end products of glucose fermentation and by its ability to utilize cellobiose and serine. Strain B2-1^T is further differentiated from *Caminicella sporogenes* by its ability to reduce Fe(III), but not thiosulfate, to utilize fructose and by its inability to grow at pH 5 or below. Similarly, strain B2-1^T can be further differentiated from *Clostridium caminithemale* by its Gram-negative cell-wall ultrastructure, its ability to reduce sulfur and to utilize mannitol, but not ribose, glycerol, arginine, glutamate or isoleucine. Strain B2-1^T differs from members of the genus *Alkaliphilus*, which are mesophiles (and hence were not included in Table 1) and features a lower DNA G+C content and is unable to ferment glucose (Cao *et al.*, 2003; Takai *et al.*, 2001).

Based on the phenotypic differences and the large phylogenetic distance separating strain B2-1^T from other members of the family *Clostridiaceae*, it is proposed that the strain should be placed in a new genus, *Thermotalea* gen. nov., as *Thermotalea metallivorans* sp. nov.

Description of *Thermotalea* gen. nov.

Thermotalea (Ther.mo.ta'le.a. Gr. adj. *thermos* hot; L. fem. n. *talea* a slender staff, rod; N.L. fem. n. *Thermotalea* a rod-shaped thermophile).

Cells are strictly anaerobic, slightly curved rods. Gram-reaction is negative. Cells possess peritrichous flagella. No spores are produced. Fe(III), Mn(IV) and elemental sulfur are reduced. 16S rRNA gene analysis indicates that the genus is a member of the family *Clostridiaceae* and is most closely related to the genus *Geosporobacter* with sequence similarities of 89.9 %. The type species is *Thermotalea metallivorans*.

Fig. 2. A dendrogram showing the phylogenetic position of *Thermotalea metallivorans* sp. nov. B2-1^T and its closest relatives. GenBank accession numbers are given in parentheses. Bootstrap values ≥ 95 are shown. Bar, 2 nucleotide changes per 100 nucleotides.

Table 1. Selected characteristics of strain B2-1^T and related members of the family *Clostridiaceae*

Strains: 1, B2-1^T; 2, *Geosporobacter subterraneus* DSM 17957^T (Klouche *et al.*, 2007); 3, *Caminicella sporogenes* DSM 14501^T (Alain *et al.*, 2002); 4, *Clostridium caminithermale* DSM 15212^T (Brisbarre *et al.*, 2003). +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4
Habitat	Microbial mats from a thermal aquifer bore outflow	Deep subsurface aquifer water	Deep-sea hydrothermal vent chimney	Deep-sea hydrothermal vent chimney
Geographical location	GAB, Australia	Paris Basin, France	East Pacific Rise	Atlantic Ocean Ridge
Cell width (µm)	0.6–0.7	0.5	0.5–0.7	0.4–0.5
Cell length (µm)	3.0–3.5	3.0–5.0	3–10	5–9
Gram stain	–	+	–	+
Cell wall type	–	+	–	+
Sporulation	–	+	+	+
Motility	+	–	+	+
Temperature (°C)				
Optimum	50	42	60	45
Range	30–55	25–55	45–65	20–58
pH				
Optimum	8.0	7.3	7.5	6.6
Range	6.5–9.0	5.6–8.4	4.5–8.0	5.8–8.2
DNA G + C content (mol%)	48*	42.2†	24.2*	33.1†
Nutritional characteristics				
Carbohydrate fermentation:				
Cellobiose	+	+	–	–
Fructose	+	+	–	+
Mannitol	+	–	ND	–
Mannose	+	–	ND	+
Ribose	–	+	–	+
Xylose	–	+	–	–
Alcohol fermentation:				
Glycerol	–	–	ND	+
Amino acid fermentation:				
Alanine	–	+	ND	–
Arginine	–	+	ND	+
Glutamate	–	+	ND	+
Glutamine	–	+	ND	–
Isoleucine	–	+	–	+
Serine	+	+	–	–
Reduction of electron acceptors				
Fe(III)	+	ND	–	ND
Thiosulfate	–	–	+	–
Elemental sulfur	+	–	+	–
16S rRNA gene sequence similarity to strain B2-1 ^T (%)	100	89.9	88.2	86.8
Products from glucose fermentation‡	Ethanol, acetate	Acetate, formate, H ₂ , CO ₂ , (ethanol)	Butyrate, acetate, ethanol, H ₂ , CO ₂	Acetate, propionate, H ₂ , CO ₂

*Determined by the thermal denaturation method.

†Determined by HPLC.

‡Trace products shown in parentheses.

Description of *Thermotalea metallivorans* sp. nov.

Thermotalea metallivorans (me.ta.li.vo'rans. L. n. *metallum* metal; L. v. *vorare* to devour; N.L. part. adj. *metallivorans* metal-devouring).

Cells are 3.0–3.5 × 0.6–0.7 µm. Growth occurs between 30 and 55 °C (optimum temperature 55 °C) and pH 6.5 to 9

(optimum pH 8.0). Grows on yeast extract and tryptone as sole carbon sources. Yeast extract or tryptone is required for growth with other substrates. Grows with glucose, sucrose, maltose, fructose, arabinose, mannose, cellobiose, *myo*-inositol, mannitol, Casamino acids, serine, and leucine, but not with lactose, galactose, raffinose, xylose, ribose, chitin, starch, acetate, propionate, lactate, formate,

pyruvate, ethanol, glycerol, amyl media, arginine, alanine, glycine, threonine, glutamate, isoleucine, methionine, amylopectin, casein or xylan. Metabolizes mannan, Tween 40, Tween 80, L-arabinose, D-arabitol, cellobiose, D-fructose, α -D-glucose, *myo*-inositol, lactulose, D-mannose, methyl α -D-galactoside, methyl β -D-glucoside, palatinose, ribose, sedoheptulosan, sucrose, D-tagatose, D-xylose, acetic acid, γ -hydroxybutyric acid, D-malic acid, L-malic acid, succinic acid monomethyl ester, propionic acid, N-acetyl-L-glutamic acid, L-asparagine, L-serine, putrescine, adenosine 5'-monophosphate, uridine 5'-monophosphate, D-fructose 6-phosphate, α -D-glucose 1-phosphate, D-glucose 6-phosphate and DL- α -glycerol phosphate, but not α -cyclodextrin, β -cyclodextrin, dextrin, glycogen, inulin, N-acetyl-D-glucosamine, N-acetyl- β -D-mannosamine, amygdalin, arbutin, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, α -D-lactose, maltose, maltotriose, D-mannitol, melezitose, melibiose, methyl β -D-galactoside, 3-methyl D-glucose, methyl α -D-glucoside, methyl α -D-mannoside, D-psicose, raffinose, L-rhamnose, D-salicin, D-sorbitol, stachyose, trehalose, turanose, xylitol, α -hydroxybutyric acid, β -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α -ketoglutaric acid, α -ketovaleric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, pyruvic acid methyl ester, pyruvic acid, succinamic acid, succinic acid, L-alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-glutamic acid, glycyl L-glutamic acid, L-pyroglutamic acid, 2,3-butanediol, glycerol, adenosine, 2'-deoxyadenosine, inosine, thymidine, uridine and thymidine 5'-monophosphate. End products from glucose fermentation are ethanol and acetate. Fe(III), Mn(IV) and elemental sulfur are reduced in the presence of yeast extract, but not sulfate, sulfite, thiosulfate, nitrate or nitrite. Sensitive to chloramphenicol, streptomycin, tetracycline, penicillin, ampicillin, sodium azide and to NaCl concentrations greater than 4% (w/v). Phylogenetically related to *Geosporobacter subterraneus* DSM 17957^T (16S rRNA gene sequence similarity value 89.9%).

The type strain, B2-1^T (=KCTC 5625^T=JCM 15105^T=DSM 21119^T), was isolated from a microbial mat sample from the outflow of a GAB bore (New Lorne Bore) in Queensland, Australia. The G+C content of the genomic DNA of the type strain is 48 ± 1 mol%.

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