

# *Fervidobacterium gondwanense* sp. nov., a New Thermophilic Anaerobic Bacterium Isolated from Nonvolcanically Heated Geothermal Waters of the Great Artesian Basin of Australia

K. T. ANDREWS AND B. K. C. PATEL\*

*Faculty of Science and Technology, Griffith University, Nathan, Brisbane, Queensland, Australia 4111*

A new thermophilic, carbohydrate-fermenting, obligately anaerobic bacterial species was isolated from a runoff channel formed from flowing bore water from the geothermally heated aquifer of the Great Artesian Basin of Australia. The cells of this organism were nonsporulating, motile, gram negative, and rod shaped and generally occurred singly or in pairs. The optimum temperature for growth was 65 to 68°C, and no growth occurred at temperatures below 44°C or above 80°C. Growth was inhibited by 10 µg of lysozyme per ml, 10 µg of penicillin per ml, 10 µg of tetracycline per ml, 10 µg of phosphomycin per ml, 10 µg of vancomycin per ml, and NaCl concentrations greater than 0.2%. The optimum pH for growth was 7.0, and no growth occurred at pH 5.5 or 8.5. The DNA base composition was 35 mol% guanine plus cytosine, as determined by thermal denaturation. The end products of glucose fermentation were lactate, acetate, ethanol, CO<sub>2</sub>, and H<sub>2</sub>. Sulfur, but not thiosulfate, sulfite, or sulfate, was reduced to sulfide. Phase-contrast microscopy of whole cells and an electron microscopic examination of thin sections of cells revealed the presence of single terminal spheroids, a trait common in members of the genus *Fervidobacterium*. However, a phylogenetic analysis of the 16S rRNA sequence revealed that the new organism could not be assigned to either of the two previously described *Fervidobacterium* species. On the basis of these observations, we propose that the new organism should be designated a new *Fervido-bacterium* species, *Fervidobacterium gondwanense*. The type strain of this species is strain AB39 (= Australian Collection of Microorganisms strain ACM 5017).

The most favored ecosystems for isolating thermophilic bacteria are naturally occurring volcanic marine and terrestrial hot springs (19). However, some thermophilic bacteria have been isolated from man-made heated environments, such as hot water storage tanks (15), and from mesophilic environments, such as lake sediments (14). Waters of the large deep subsurface aquifers, which are heated geothermally because of the temperature increase of 2.13°C/100 m of depth (3), are also an important potential source of phylogenetically novel thermophilic bacteria (2, 9, 13). The Great Artesian Basin of Australia is one of the largest deep subsurface aquifers and covers approximately one-fifth of the Australian continent. Some 5,000 bore holes have been sunk into the Great Artesian Basin in order to tap this water resource, and the water is distributed on the surface by runoff channels. The water temperature at the bore source can be as high as 100°C, and temperature gradients form in the runoff channels (5). Microbiological investigations of samples collected directly from bore source and runoff channels have revealed that there is great microbial diversity (2, 9, 13). In this paper we describe the isolation and characterization of a new species of the genus *Fervidobacterium*. The results of a preliminary study of the fatty acid composition of this organism have been described previously (13).

## MATERIALS AND METHODS

**Bacterial cultures.** Strain AB39<sup>T</sup> (T = type strain) was isolated from a runoff channel from a bore hole containing geothermal water from the Great Artesian Basin. *Fervidobacterium nodosum* Rt17-B1<sup>T</sup> (= ATCC 35602<sup>T</sup>) was obtained from H. W. Morgan (Thermophile Research Unit, Waikato University, Hamilton, New Zealand), and *Fervidobacterium islandicum* H21<sup>T</sup> (= DSM 5733<sup>T</sup>) was obtained from B. Ollivier (ORSTOM Laboratoire de Microbiologie, Marseille,

France), who had obtained it from the Deutsche Sammlung von Mikroorganismen und Zellkulturen. All three strains were routinely cultured by using previously described methods (11, 12).

**Sample source and collection.** Samples were collected directly from bore sources and from runoff channels of the Great Artesian Basin of Australia. Water was collected directly in sterile containers from the bore source or a mixture of water and sediments, or microbial mats were collected from the runoff channels by scooping material into sterile glass containers. In all cases, the containers were completely filled and capped tightly. Temperatures were determined in situ during sample collection. A total of 44 samples, whose temperatures ranged from 31 to 88°C, were collected. The samples were transported to our laboratory at ambient temperatures and stored until they were used.

**Enrichment and isolation.** To initiate enrichment cultures, 0.1- to 0.5-ml portions of samples were inoculated into 10-ml portions of prereduced Trypticase-yeast extract-glucose (TYEG) medium, and the preparations were incubated at 68°C without agitation for up to 3 days, during which time they were examined for growth with a microscope (11, 12). Positive enrichment cultures were subcultured at least three times in the same medium prior to culture purification. Cultures were purified by using the end point dilution technique and TYEG medium supplemented with 2% (wt/vol) agar. A single colony from the most dilute tubes was picked, and the process of end point dilution was repeated at least twice before the culture was considered pure and used in subsequent characterization tests. The pure culture was stored in a glycerol-TYEG medium (50:50) mixture at -20°C.

**pH, temperature, and NaCl concentration ranges for growth.** All experiments were performed in duplicate. Growth was measured by determining the change in turbidity at 660 nm and also by determining the change in pH. To measure changes in absorbance, Bello tubes were inserted directly into a Novaspec LKB spectrophotometer (Pharmacia) which had been zeroed with uninoculated medium or water. To determine the pH range for growth, prereduced TYEG medium was adjusted to pH values between 5.0 and 10.0 by injecting appropriate volumes of sterile HCl or NaOH. TYEG medium adjusted to the optimal pH for growth was used to determine the temperature range for growth. TYEG medium at pH 7.0 and an incubation temperature of 65°C was used to determine the NaCl requirement.

**Antibiotic susceptibility.** Antibiotics were added from filter-sterilized stock solutions to sterile prereduced TYEG medium under a stream of oxygen-free nitrogen gas to give final concentrations of 10 and 100 µg/ml.

**Carbohydrate utilization and end product formation.** Carbohydrate utilization was determined in prereduced TYE medium (TYEG medium lacking glucose). Soluble carbohydrates (prepared as 10% filter-sterilized stock solutions) were dispensed into tubes, and insoluble carbohydrates (final concentration, 0.5%) were weighed directly in tubes before prereduced TYE medium was dispensed.

\* Corresponding author. Phone: 61-7-3875 7695. Fax: 61-7-3875 7800. Electronic mail address: B.Patel@sct.gu.edu.au.

Growth was measured spectrophotometrically and also by determining the change in pH.

The fermentation end products were determined by gas-liquid chromatography. A Shimadzu model GC8 gas chromatograph equipped with a thermal conductivity detector was used for CO<sub>2</sub> and H<sub>2</sub> analysis. The gases were separated on a Carboxphere (80/100) column by using N<sub>2</sub> at a flow rate of 8 ml/min as the carrier gas. A Shimadzu model GC14 gas chromatograph equipped with a flame ionization detector was used to analyze volatile fatty acids and lactate. The acids were separated on a Chromosorb 101 (80/100) column by using N<sub>2</sub> at a flow rate of 12.5 ml/min as the carrier gas and H<sub>2</sub> and air at flow rates of 18 and 250 ml/min, respectively, as flame gases. The oven temperature and the injector temperature were 180 and 200°C, respectively. The amounts of the gases and acids were determined as described previously (12), except that the Delta computer software analysis package (Digitals Solutions, Ltd., Brisbane, Queensland, Australia) was used to integrate the peaks.

Reduction of thiosulfate, sulfite, and sulfate (final concentration, 20 mM) and reduction of sulfur (final concentration, 1%) to sulfide were performed in TYEG medium (pH 7.0) at an incubation temperature of 65°C. Sulfide production in the culture supernatant in the early stationary phase was determined as described previously (9).

**Light microscopy and electron microscopy.** Light microscopy and electron microscopy were performed as described previously (12).

**G+C content.** DNA was isolated and purified by using a slight modification of the method of Marmur (10) in which an RNase treatment step was included after cell lysis. DNA was resuspended in 0.1 × SSC (1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and the thermal denaturation temperature was determined with a Gilford model 250 spectrophotometer equipped with a thermoprogrammer. DNA from a reference strain of *Escherichia coli* (ACM 1803) (Australian Collection of Microorganisms, The University of Queensland, St. Lucia, Australia) was purified by using the procedure described above, and this DNA was used as a standard when the DNA base composition was calculated.

**16S rRNA sequence studies.** Partially purified DNA was used for 16S rRNA gene amplification as described previously (9, 17). The amplified 16S rRNA gene products from five 50-μl tubes were pooled, electrophoresed on a 0.8% agarose gel, and purified by using QIAEX (QIAGEN GmbH, Hilden, Germany) essentially as described by the manufacturer except that the final elution of DNA was in sterile distilled water instead of Tris-EDTA buffer. The sequence was determined with an ABI automated DNA sequencer by using a Prism dideoxy terminator cycle sequencing kit and the protocols recommended by the manufacturer (Applied Biosystems, Inc.). The primers used for sequencing have been described previously (17). The 16S rRNA gene sequence which we determined was manually aligned with reference sequences of various members of the domain *Bacteria* by using editor ae2. Reference sequences were obtained from the Ribosomal Database Project (8) and GenBank databases. Positions of sequence and alignment uncertainty were omitted from the analysis. A phylogenetic analysis was performed by using various programs implemented as part of the PHYLIP package (4), as described below. The pairwise evolutionary distances based on 1,100 unambiguous nucleotides were determined by using the method of Jukes and Cantor (7), and dendrograms were constructed from evolutionary distances by using the neighbor-joining method. A transversion analysis was performed by using the program DNAPARS. Tree topology (determined by using 1,000 bootstrapped data sets) was reexamined by running a script consisting of the following programs: SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE. A program available on TREECON (20) was also used for phylogenetic analysis.

**Nucleotide sequence accession number.** The nucleotide sequence of the 16S rRNA gene of strain AB39<sup>T</sup> has been deposited in the EMBL database under accession number Z49117.

## RESULTS

**Enrichment and isolation.** Of the 44 samples examined, 21 gave rise to positive enrichment cultures after 48 h of incubation at 68°C. Eight of these enrichment cultures contained organisms that had conventional rod and filament morphologies, whereas the remaining 13 contained rods that had single terminal swellings. The organisms that exhibited the latter morphotype were isolated from bore source samples, as well as from sediment and microbial mat samples. The temperatures of the sites from which these samples were obtained ranged from 31 to 75°C. Organisms that exhibited this morphotype were not found in enrichment cultures prepared with samples that had been collected from sites where the temperatures were 76 to 88°C. Pure isolates were obtained from enrichment cultures by using the end point dilution technique and were stored at -20°C. A pure culture that was obtained from en-

richment cultures initiated from a 66°C microbial mat sample and designated strain AB39<sup>T</sup> was studied further.

Growth of strain AB39<sup>T</sup> was inhibited in TYEG medium culture tubes when the N<sub>2</sub> gas phase was replaced with air or in TYEG medium which lacked a reductant, indicating that strain AB39<sup>T</sup> was an obligate anaerobe.

**Morphology and cell structure.** Strain AB39<sup>T</sup> cells were rods and occurred singly, in pairs, or in short chains. They were motile, gram negative, 0.5 to 0.6 μm in diameter, and 4 to 40 μm long. Protuberances or swellings were usually present at the ends of the bacteria and were observed at all growth temperatures. Only one swelling was present in each cell. The swellings ranged from 1 to 4 μm in diameter (Fig. 1a). Occasionally, giant spheres consisting of membrane-bound structures containing two or more cells and ranging in diameter from 5 to 8 μm were also observed. Phase-contrast microscopy of cultures grown at different temperatures and pH values did not reveal any spores.

Electron microscopy of thin sections of strain AB39<sup>T</sup> revealed a typical gram-negative two-layer cell wall structure (Fig. 1c). The outer layer protruded to form the characteristic swollen structure, whereas the inner layer remained tightly associated with the cell membrane (Fig. 1b and c).

**Optimal growth conditions.** NaCl was not required for growth, and concentrations of NaCl greater than 0.2% were inhibitory. Strain AB39<sup>T</sup> grew optimally at 65 to 68°C, and no growth occurred at temperatures of 44°C or below or above 80°C. The optimum pH for growth was 7.0, and no growth occurred at pH 5.5 or 8.5. The generation time of strain AB39<sup>T</sup> in TYEG medium at pH 7.0 and 65°C was 79 min.

**Substrate utilization, sulfide production, and fermentation end products.** The substrates that supported growth of strain AB39<sup>T</sup> included D-cellobiose, amylopectin, maltose, starch, dextrin, xylose, glucose, pyruvate, lactose, fructose, and mannose (Table 1). Carboxymethyl cellulose and galactose were fermented slowly, whereas Casamino Acids, gelatin, L-sorbose, chitin, dextran, arabinose, ribose, raffinose, and cellulose did not support growth.

The end products formed during glucose fermentation included ethanol (2 mM), acetate (7 mM), lactate (15 mM), CO<sub>2</sub>, and H<sub>2</sub>.

Sulfur, but not thiosulfate, sulfite, or sulfate, was reduced to sulfide.

**Antibiotic susceptibility.** Growth of strain AB39<sup>T</sup> was completely inhibited by 10 μg of streptomycin per ml, 10 μg of penicillin per ml, 10 μg of novobiocin per ml, 10 μg of phosphomycin per ml, 10 μg of tetracycline per ml, 10 μg of vancomycin per ml, 100 μg of polymyxin B per ml, 100 μg of chloramphenicol per ml, or 100 μg of rifampin per ml.

**DNA base composition.** The DNA base composition of strain AB39<sup>T</sup> was 35 mol% G+C. The *E. coli* reference DNA used had a melting temperature of 76.6°C, corresponding to a G+C content of 51.2 mol%.

**16S rRNA sequence analysis.** Using eight primers, we determined the sequence of 1,478 bases from position 27 to position 1540 (*E. coli* numbering of Winker and Woese [21]) of the 16S rRNA gene of strain AB39<sup>T</sup>. The G+C content of this 16S rRNA gene was 59.63 mol%. A phylogenetic analysis performed with representatives of the domain *Bacteria* revealed that strain AB39<sup>T</sup> is a member of the order *Thermotogales*, and an analysis performed with members of the order *Thermotogales* indicated that strain AB39<sup>T</sup> is closely related to the type strains of the two previously described *Fervidobacterium* species, *F. nodosum* Rt17-B1 and *F. islandicum* H21 (levels of similarity, 94 and 95%, respectively). Figure 2 is a dendrogram that was generated by the neighbor-joining method from the

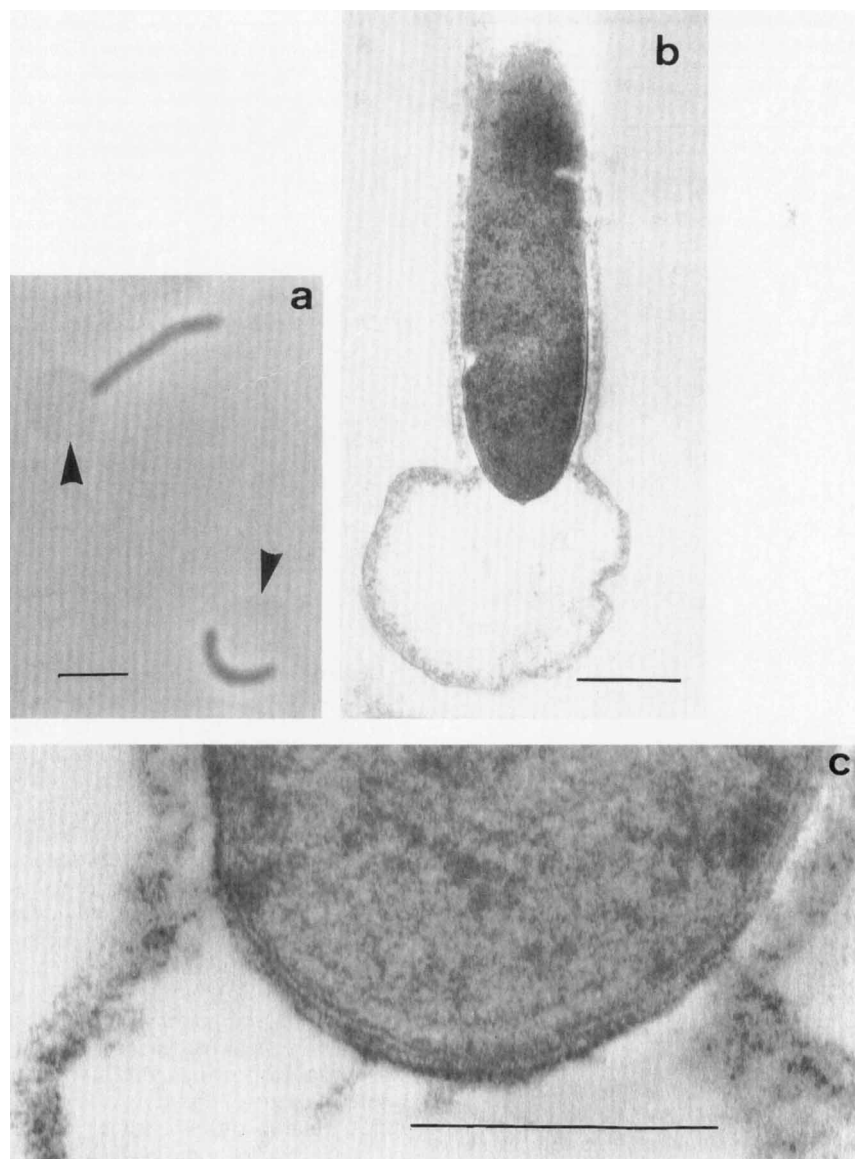


FIG. 1. (a) Phase-contrast micrograph of strain AB39<sup>T</sup> showing the presence of spheroid structures (arrowheads). Bar = 2  $\mu$ m. (b and c) Electron micrographs of a thin section of a whole cell showing the spheroid structure as part of the outer cell wall (b) and the inner cell wall associated with the cell membrane (c). Bars = 0.2  $\mu$ m.

evolutionary distance matrix and shows this relationship. A bootstrap analysis of the data revealed a modest relationship between strain AB39<sup>T</sup> and *F. islandicum* H21<sup>T</sup> (73%), although the confidence level obtained for *F. nodosum* and the other two *Fervidobacterium* species was 100%.

#### DISCUSSION

Numerous genera and species of gram-negative, thermophilic, motile, anaerobic, carbohydrate-fermenting, rod-shaped bacteria have been described. However, as strain AB39<sup>T</sup> produces terminal protuberances or swellings, its morphology is clearly similar to the morphology of members of the order *Thermotogales*. In a previous study Patel et al. found high levels of saturated, normal fatty acids (78% of the total fatty acids) and that fatty acids with even numbers of carbon atoms predominated; these findings also indicated that strain AB39<sup>T</sup> is a member of the order *Thermotogales* (13). In addition, the pro-

tuberances of strain AB39<sup>T</sup> cells closely resemble the spheroids of *Fervidobacterium* strains (12) rather than the sheath-like structures called "togas" that are characteristic of *Thermotoga*, *Petrotoga*, and *Geotoga* species (1, 16, 19). Further evidence of the relationship of strain AB39<sup>T</sup> to *F. nodosum* Rt17-B1<sup>T</sup> and *F. islandicum* H21<sup>T</sup> was also obtained from a phylogenetic analysis of the 16S rRNA gene.

*Fervidobacterium* species, including *F. nodosum* and *F. islandicum*, have been isolated previously only from volcanic hot springs (6, 12). This paper is the first report of the isolation of a *Fervidobacterium* strain from a nonvolcanic, geothermally heated water source (viz., the Great Artesian Basin of Australia). The presence of *Fervidobacterium* isolates in the aquifer was not rare, as morphologically similar strains were isolated from another 12 of the 44 enrichment samples examined. As *Fervidobacterium* strains were isolated from water samples collected from bore holes that were up to 1,500 m deep, we

TABLE 1. Differential characteristics of members of the genus *Fervidobacterium*<sup>a</sup>

Characteristic	<i>F. gondwanense</i> AB39 <sup>Tb</sup>	<i>F. nodosum</i> Rt17-B1 <sup>Tc</sup>	<i>F. islandicum</i> H21 <sup>Td</sup>
Habitat	Geothermal artesian waters of Australia	Volcanic hot springs of New Zealand	Volcanic hot springs of Iceland
Cell diam (µm)	0.5–0.6	0.5–0.55	0.6
Cell length (µm)	4–40	1–2.5	1–4
Temp range (°C)	>45–<80	47–80	50–80
Optimum temp (°C)	65–68	70	65
pH range	6.0–8.0	6.0–8.0	6.0–8.0
Optimum pH	7.0	7.0	7.2
NaCl concn range (%)	0–0.6	<1	<1
Optimum NaCl concn (%)	0.1	NR <sup>e</sup>	0.2
Generation time (min)	79	105	150
G+C content (mol%)	35	33.7	41
Utilization of the following substrates <sup>f</sup> :			
Carboxymethyl cellulose	+	–	–
Lactose	+	+	–
Starch	+	+	–
Dextrin	+	+	–
Fructose	+	–	–
Xylose	+	–	–
Arabinose	–	–	+
Pyruvate	+	–	+
Galactose	+	+	–

<sup>a</sup> All three species are spheroid-structure-bearing bacteria.

<sup>b</sup> Data from this study.

<sup>c</sup> Data from reference 12.

<sup>d</sup> Data from reference 6.

<sup>e</sup> NR, not reported.

<sup>f</sup> Data from this study. All three *Fervidobacterium* species utilized glucose, mannose, maltose, amylopectin, and cellobiose but not cellulose, Casamino Acids, gelatin, sorbose, xylan, dextran, or chitin. +, growth; –, no growth.

speculate that the primary habitat of these bacteria is the geothermal waters of the deep aquifer and that they are seeded into the runoff channels, where they adapt to the new environment. The water of the Great Artesian Basin aquifer is meteoric in origin, and the rate of water flow underground is approximately 5 m/year (5); therefore, this water could provide a conducive environment for microbial growth. The modest confidence value (73%) between *F. nodosum* or *F. islandicum* and strain AB39<sup>T</sup> indicates that there may be *Fervidobacterium* species that remain to be identified. Some *Fervidobacterium* isolates obtained from the Great Artesian Basin that are currently being investigated in our laboratory differ markedly in their ability to reduce thiosulfate or sulfur. Sequencing of the 16S rRNA genes of these isolates for phylogenetic identification is currently in progress.

Strain AB39<sup>T</sup> can be differentiated from *F. nodosum* Rt17-B1<sup>T</sup> and *F. islandicum* H21<sup>T</sup> on the basis of its fast growth rate and its greater nutritional versatility. Strain AB39<sup>T</sup> also has a lower G+C content (35 mol%) than *F. islandicum* H21<sup>T</sup> (41 mol%) (Table 1). Phylogenetically, strain AB39<sup>T</sup> is more closely related to *F. islandicum* H21<sup>T</sup> (level of similarity, 95%) than to *F. nodosum* Rt17-B1<sup>T</sup> (level of similarity, 94%). Recently, Stackebrandt and Goebel (18) concluded that strains belonging to the same genus that exhibit less than 97% 16S rRNA sequence similarity should be considered members of different species. Thus, it is clear that strain AB39<sup>T</sup> is a member of a new *Fervidobacterium* species, for which we propose the name *Fervidobacterium gondwanense*.

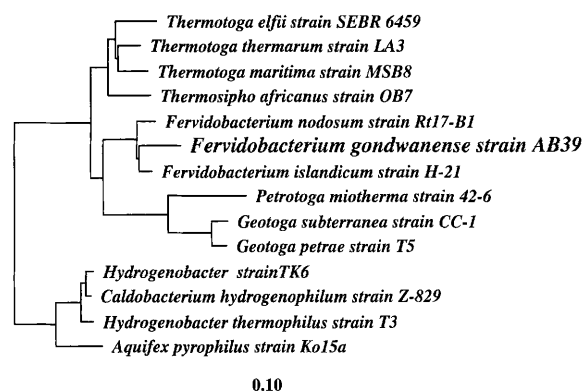


FIG. 2. Dendrogram showing the positions of strain AB39<sup>T</sup>, members of the order *Thermotogales*, and related bacteria. The dendrogram was constructed by using the neighbor-joining method and Jukes-Cantor evolutionary distance matrix data obtained from a comparison of 1,100 unambiguous nucleotides as implemented in PHYLIP (4). The sequences used in the analysis were obtained from the Ribosomal Database Project, version 4.1 (10), and from the GenBank database. The GenBank accession numbers for the *Hydrogenobacter thermophilus*, *Caldobacterium hydrogenophilum*, and *Hydrogenobacter* sp. strain T3 sequences are Z30214, Z30242, and Z30189, respectively. Bar = evolutionary distance.

**Description of *Fervidobacterium gondwanense* sp. nov.** *Fervidobacterium gondwanense* (Gond.wa.nen'se. M.L. neut. adj. *gondwanense*, pertaining to the large land mass known as Gondwana, which included Australia, Africa, India, and South America before they separated). Straight motile rods that are 0.5 to 0.6 by 4 to 40 µm and occur singly, in pairs, or in chains. Gram negative. Cells produce terminal spheroid structures which are protuberances of the outer cell wall. Rotund bodies are formed rarely. No spores are produced. Creamish brown colonies that are 1 mm in diameter develop in TYEG medium containing 2.5% agar after 48 h of incubation at 65°C.

Chemoorganotrophic, strictly anaerobic bacterium which grows at temperatures of >45°C or <80°C and at pH 6.0 to 8.0. The optimum temperature is 65 to 68°C, and the optimum pH is 7.0. Utilizes glucose, mannose, maltose, starch, amylopectin, cellobiose, carboxymethyl cellulose, lactose, dextrin, fructose, xylose, galactose, and pyruvate but not cellulose, Casamino Acids, gelatin, sorbose, ribose, raffinose, arabinose, dextran, xylan, or chitin. Produces acetate, ethanol, lactate, CO<sub>2</sub>, and H<sub>2</sub> during glucose fermentation. Reduces sulfur but not thiosulfate, sulfite, or sulfate. A member of the domain *Bacteria*. Growth is inhibited by streptomycin, penicillin G, polymyxin B, phosphomycin, tetracycline, vancomycin, chloramphenicol, and rifampin, partially inhibited by sodium azide and neomycin, and not inhibited by D-cycloserine.

The G+C content of the DNA is 35 mol%, as determined by thermal denaturation.

Phylogenetically related to members of the order *Thermotogales*, particularly *F. nodosum* Rt17-B1<sup>T</sup> and *F. islandicum* H21<sup>T</sup>.

Isolated from nonvolcanic geothermal waters of the Great Artesian Basin, an aquifer in Australia.

The type strain is strain AB39 (= Australian Collection of Microorganisms strain ACM 5017).

#### ACKNOWLEDGMENTS

This work was supported in part by the Australian Research Council. We thank the Water Resources Commission staff for help with sample collection. We also thank L. Sly for the use of the Gilford spectrophotometer and for providing the *E. coli* type strain and D. Stenzel for assistance with electron microscopy.

## REFERENCES

1. Davey, M. E., W. A. Wood, R. Key, K. Nakamura, and D. A. Stahl. 1993. Isolation of three species of *Geotoga* and *Petrotoga*: two new genera representing a new lineage in the bacterial line of descent distantly related to the "Thermotogales." *Syst. Appl. Microbiol.* **16**:191-200.
2. Denman, S., K. Hampson, and B. K. C. Patel. 1991. Isolation of strains of *Thermus aquaticus* from the Australian Artesian Basin and a simple and rapid procedure for the preparation of their plasmids. *FEMS Microbiol. Lett.* **82**:73-78.
3. Driscoll, F. G. 1986. Groundwater and wells, 2nd ed. Johnson Division, St. Paul, Minn.
4. Felsenstein, J. 1993. PHYLIP (phylogenetic inference package), version 3.51c. Department of Genetics, University of Washington, Seattle.
5. Habermehl, M. A. 1980. The Great Artesian Basin, Australia. *BMR (Bur. Miner. Resour.) J. Aust. Geol. Geophys.* **5**:9-38.
6. Huber, R., C. R. Woese, T. A. Langworthy, J. K. Kristjansson, and K. O. Stetter. 1990. *Fervidobacterium islandicum* sp. nov., a new extremely thermophilic eubacterium belonging to the "Thermotogales." *Arch. Microbiol.* **154**:105-111.
7. Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules, p. 21-132. In H. N. Munro (ed.), *Mammalian protein metabolism*. Academic Press, New York.
8. Larsen, N., G. J. Olsen, B. L. Maidak, M. J. McCaughey, R. Overbeek, T. J. Macke, T. L. Marsh, and C. R. Woese. 1993. The Ribosomal Database Project. *Nucleic Acids Res.* **21**:3021-3023.
9. Love, C. A., B. K. C. Patel, P. D. Nichols, and E. Stackebrandt. 1993. *Desulfotomaculum australicum*, sp. nov., a thermophilic sulfate-reducing bacterium isolated from the Great Artesian Basin of Australia. *Syst. Appl. Microbiol.* **16**:244-251.
10. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from bacteria. *J. Mol. Biol.* **3**:208-218.
11. Patel, B. K. C., H. W. Morgan, and R. M. Daniel. 1985. A simple and efficient method for preparing and dispensing anaerobic media. *Biotechnol. Lett.* **7**:277-288.
12. Patel, B. K. C., H. W. Morgan, and R. M. Daniel. 1985. *Fervidobacterium nodosum* gen. nov. and spec. nov., a new chemoorganotrophic, caldoactive, anaerobic bacterium. *Arch. Microbiol.* **141**:63-69.
13. Patel, B. K. C., J. H. Skerratt, and P. D. Nichols. 1991. The phospho ester-linked fatty acid composition of thermophilic bacteria. *Syst. Appl. Microbiol.* **14**:311-316.
14. Phillips, W. E., and J. J. Perry. 1976. *Thermomicrobium fosteri* sp. nov., a hydrocarbon-utilizing obligate thermophile. *Int. J. Syst. Bacteriol.* **26**:220-225.
15. Ramaley, R. F., and K. Bitzinger. 1975. Types and distribution of obligate thermophilic bacteria in man-made and natural gradients. *Appl. Microbiol.* **30**:152-155.
16. Ravot, G., M. Magot, M. L. Fardeau, B. K. C. Patel, G. Prensier, A. Egan, J. L. Garcia, and B. Ollivier. 1995. *Thermotoga elfi* sp. nov., a novel thermophilic bacterium from an African oil-producing well. *Int. J. Syst. Bacteriol.* **45**:308-314.
17. Redburn, A. C., and B. K. C. Patel. 1993. Phylogenetic analysis of *Desulfotomaculum thermobenzoicum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. *FEMS Microbiol. Lett.* **113**:81-86.
18. Stackebrandt, E., and B. M. Goebel. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **44**:846-849.
19. Stetter, K. O., G. Fiala, G. Huber, R. Huber, and A. Seegerer. 1990. Hyperthermophilic microorganisms. *FEMS Microbiol. Rev.* **75**:117-124.
20. Van de Peer, Y., and R. De Wachter. 1992. TREECON: a software package for the construction and drawing of evolutionary trees. *Comput. Appl. Biosci.* **9**:177-182.
21. Winker, S., and C. R. Woese. 1991. A definition of the domains *Archaea*, *Bacteria* and *Eucarya* in terms of small subunit ribosomal RNA characteristics. *Syst. Appl. Microbiol.* **14**:305-310.