Description of *Thermoanaerobacter brockii* subsp. *lactiethylicus* subsp. nov., Isolated from a Deep Subsurface French Oil Well, a Proposal To Reclassify *Thermoanaerobacter finnii* as *Thermoanaerobacter brockii* subsp. *finnii* comb. nov., and an Emended Description of *Thermoanaerobacter brockii*

**J.-L. CAYOL** 1 B. OLLIVIER,1,* B. K. C. PATEL,1,2 G. RAVOT,1 M. MAGOT,3 E. AGERON,4 P. A. D. GRIMONT,4 AND J.-L. GARCIA1

Laboratoire de Microbiologie ORSTOM, Université de Provence, 13331 Marseille Cedex 3,3 Sanofi Recherche, Centre de Labège, 31 676 Labège Cedex,2 and Unité des Entérobactéries, Institut Pasteur, 75724 Paris Cedex 15,4 France, and Faculty of Science and Technology, Griffith University, Nathan, Brisbane, 4111 Australia4

A strictly anaerobic, thermophilic, gram-positive, spore-forming eubacterium designated strain SEBR 5268T (T = type strain) was isolated from an oil field at a depth of 2,100 m, where the temperature was 92°C. The cells of this organism were gram-positive, straight, motile rods (0.5 by 2 to 3 μm) with peritrichous flagella. The cells occurred singly or in pairs during the logarithmic growth phase, but were pleomorphic and filamentous (length, 15 μm) in old cultures. Growth occurred at temperatures of 40 to 75°C, and optimum growth occurred at temperatures between 55 and 60°C. The fermentable substrates included glucose, fructose, galactose, mannose, cellobiose, maltose, sucrose, lactose, D-xylose, D-ribose, mannitol, pyruvate, and starch. The products of fermentation of glucose were lactate, acetate, ethanol, H2, and CO2. The DNA base composition was 35 mol% G+C. The results of 16S rRNA sequence comparisons indicated that strain SEBR 5268T was closely related to *Thermoanaerobacter brockii* and *Thermoanaerobacter finnii*, and these three organisms exhibited levels of ribosomal DNA sequence homology of 98 to 99%. The results of DNA-DNA hybridization studies performed with the three organisms confirmed this close affiliation, and as base pairing values of >70% were obtained, these organisms belong to the same species. Therefore, we propose that *T. finnii* should be reclassified as a subspecies of *T. brockii*, *Thermoanaerobacter brockii* subsp. *finnii* comb. nov. This automatically creates *Thermoanaerobacter brockii* subsp. *brockii*. We also propose that strain SEBR 5268T should be classified as a member of a new subspecies of *T. brockii*, *Thermoanaerobacter brockii* subsp. *lactiethylicus*. The latter differs from *T. brockii* subsp. *brockii* and *T. brockii* subsp. *finnii* by its 16S rRNA sequence, DNA sequence diversity, lower temperature optimum, G+C content, and carbohydrate utilization spectrum. Strain SEBR 5268T has been deposited in the Deutsche Sammlung von Mikroorganismen as strain DSM 9801T.

In the past two decades, workers have performed intensive studies to isolate thermophilic, anaerobic, carbohydrate-fermenting eubacteria from marine and terrestrial volcanic hot springs (24, 41), and have studied these organisms with a view toward using these microbes and their enzymes for biotechnological applications (24). Because of the large number of new isolates, the taxonomy of this group of bacteria has been revised recently, mainly on the basis of 16S rRNA sequence data (9).

Oil fields represent a new and exciting ecosystem because of their physicochemical conditions. Some oil-bearing reservoirs are deep seated and include halophilic and thermal environments which are conducive to the growth of thermophilic bacteria. To date, only a few reports describing thermophilic bacteria obtained from such environments have been published, and the strains described include methanogens (11, 18, 29), sulfate reducers (8, 28), elemental sulfur reducers (9), and fermentative bacteria (12, 30, 33). During microbiological investigations of oil fields, we isolated several fermentative thermophilic strains that belong to the genus *Thermoanaerobacter* and are capable of using thiosulfate as an electron acceptor in the presence of H2, amino acids, peptides, or carbohydrates (12-14). In this paper we describe the isolation, characterization, and distribution of thermophilic, anaerobic, fermentative bacteria belonging to the genus *Thermoanaerobacter* obtained from oil fields.

**MATERIALS AND METHODS**

**Origins of strains.** Strain SEBR 5268T (T = type strain) was isolated from a French oil field, whereas strains SEBR 7311 and SEBR 7312 were isolated from African oil fields in Cameroon. The in situ temperature of the wells was 92°C, but the temperatures were 51 to 53°C by the time that the samples were collected at the wellhead. The method of sampling used has been described elsewhere (3). *Thermoanaerobacter finnii* AKO-1T (= DSM 3389) and *Thermoanaerobacter brockii* HTDS1T (= DSM 14577) were obtained from the Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany. All strains were routinely cultured in the glucose-based growth medium described below.

**Culture medium.** A glucose-based medium was used to culture the strains. This medium contained (per liter) 1.0 g of NH4Cl, 0.3 g of K2HPO4, 0.3 g of KH2PO4, 1.3 g of MgCl2 ⋅ 6H2O, 0.1 g of CaCl2 ⋅ 2H2O, 2.0 g of KCl, 2.0 g of Na2CO3, 0.5 g of CaCO3, 5.0 g of D-(+)-Trypsin (BioMerieux, Craponne, France), 5.0 g of yeast extract (Difco Laboratories, Detroit, Mich.), 10.0 g of glucose, 1 ml of 0.1% resazurin, and 10 ml of a trace element solution (1). Unless indicated otherwise, the anaerobic technique of Hungate was used throughout.
this study (17, 25, 27). Portions (20 ml) of the anaerobic medium were dispensed to 50 ml serum bottles under a stream of oxygen-free N2,CO2 (80:20). The medium dispensed in this way was sterilized for 45 min at 110°C. Just prior to inoculation, 0.2 ml of 2% Na2S + 9H2O, 1.0 ml of 10% NaHCO3, 0.2 ml of a vitamin solution (51), and 0.1 ml of a 0.2% sodium dithionite solution (from sterilized anaerobic stock solutions) were injected into each bottle. For enrichment cultures, 2 ml of a sample was injected, and the preparation was incubated at 60°C for 24 h without shaking. Pure cultures were obtained by repeatedly using the agar shake dilution method with glucose-based medium that was supplemented with 2% Noble agar.

Nutritional characterization. Basal medium containing 1 g of yeast extract per liter but no glucose was used for nutritional characterization. This medium was prepared as described above, and 5 ml portions were distributed into Hungate tubes. Just prior to inoculation, 0.05 ml of a 2% Na2S + 9H2O sterile anaerobic stock solution and 0.25 ml of a 10% NaHCO3 sterile anaerobic stock solution were injected into each tube. Substrates were injected from sterile anaerobic stock solutions to give final concentrations of 20 mM, unless indicated otherwise.

pH, temperature, and sodium chloride ranges for growth. In the pH studies, prerreduced growth medium in anaerobic tubes was adjusted to the desired pH with NaHCO3 or Na2CO3 sterile anaerobic stock solutions. In the salt range studies, sodium chloride was weighed directly in tubes to give the desired final concentrations, and then the basal glucose medium was dispensed into these tubes at 4°C. Basal medium containing the optimum sodium chloride concentration and the optimum pH were used in the temperature range studies.

Analytical techniques. Volatile fatty acids and alcohols were analyzed as described previously (7). Bacterial growth was monitored by measuring the optical density at 660 nm with a model UV-160A spectrophotometer (Shimadzu Corp., Kyoto, Japan). DNA was isolated from exponentially growing cells, and the guanosine-plus-cytosine (G+C) content was determined by using high-performance liquid chromatography as described previously (6, 26). Phase-contrast microscopy was performed as described previously (7).

16S rRNA sequence studies. Semipurified DNA was extracted for amplification of the 16S rRNA gene by using the following protocol. A 20 ml culture was centrifuged, and the resulting pellet was resuspended in 50 ml of lysis buffer (50 mM Tris-HCl [pH 7.2], 50 mM EDTA, 3% sodium dodecyl sulfate) and transferred into a microcentrifuge tube with a pipette. The suspension was microvanned at the high power setting for four cycles, each of which consisted of 15 s with the heat on and 5 s with the heat off with the lid of the tube open. Then 350 ml of lysis buffer was added, the lid was closed, the preparation was incubated at 80°C for 15 min, and the suspension was vortexed with 400 ml of phenol-chloroform (1:1). The preparation was then centrifuged at 13,000 × g for 15 min to separate the phases. The aqueous phase (approximately 200 ml) was removed; we were careful to avoid any material from the interface when we did this. Then 10 ml of isopropanol and 5 ml of 5 M sodium acetate (pH 5.8) were added to the aqueous phase, and the preparation was vortexed. The suspension was centrifuged at 13,000 × g, and the resulting pellet was washed with cold 80% ethanol, placed in a desiccator to evaporate the residual ethanol, resuspended in 50 ml of sterile distilled water, and stored at −20°C until it was used. Amplification of the 16S rRNA gene from the semipurified DNA followed by purification of the amplified product was performed as described previously (23, 34). The purified product was sequenced directly with an ABI automated DNA sequencer by using a Prism dye terminator cycle sequencing kit and the protocols recommended by the manufacturer (Applied Biosystems, Ltd., Foster City, Calif.). The primers used for sequencing have been described previously (34).

The 2a2 editor was used to align the 16S ribosomal DNA sequence obtained from the sequencing data with the sequences of various members of the bacterial phylum obtained from the Ribosomal Database Project (version 4.0) (21). Positions of sequence and alignment uncertainty were omitted from the analysis, and pairwise evolutionary distances based on 1,320 unambiguous nucleotides were computed by using the method of Jukes and Cantor (19). Dendrograms were constructed from evolutionary distances by using the neighbor-joining method, a transversion analysis was performed by using the program DNAF, and tree topology was examined by using 100 bootstraped data sets by running the program Boot. For DNA base composition, the following sequence of events during the analysis: SEGBOOT, DNADIST, FITCH, and CONSENSE. All programs are available as part of the PHYLIP package (15). Programs available in the Molecular Evolutionary Genetic Analysis (MEGA) package, version 1 (20), were also used in the analysis. All of the programs except the MEGA programs were run on a Sun Sparc workstation; the MEGA programs were run on a Toshiba model T3100SX 386 laptop IBM-compatible computer.

DNA was extracted and purified as described elsewhere (5). The exact procedures used for in vitro labelling of DNA with tritium-labelled nucleotides and for hybridization experiments (S1 nuclease-trichloroacetic acid procedure) have been described previously (16). The temperature at which 50% of the DNA became hydrolyzed by the S1 nuclease was determined as described by Croua et al. (10). The difference between the Tm of a homoduplex and the Tm of a heteroduplex (ΔTm) provided an estimate of the level of divergence between two DNAs (4).

RESULTS

Enrichment and isolation. After incubation at 60°C for 48 h, three enrichment cultures were found to be positive for growth. Microscopic examination revealed bacterial populations composed of nonsporulating rods. Enrichment cultures were maintained by repeatedly transferring 1% inocula into fresh anaerobic glucose-based medium. Colonies (diameter, 4 mm) developed in the roll tubes after incubation at 60°C for 48 h. All of the colonies were smooth, uniformly round, mucoid, nonpigmented, and flat, indicating that the populations were homogeneous. Three strains that were very similar to each other were isolated and were designated strains SEBR 5268T, SEBR 7311, and SEBR 7312. SEBR 5268T was obtained from enrichment cultures that had been grown under an N2,CO2 (80:20) gas phase, whereas strains SEBR 7311 and SEBR 7312 were obtained from enrichment cultures in modified basal medium containing 1% NaCl and 20 mM thiosulfate with H2,CO3 (2 × 10−3 Pa) as the gas phase. Only limited genotypic studies were performed with strains SEBR 7311 and SEBR 7312, whereas strain SEBR 5268T was studied in greater detail.

Cellular features. Cells of strain SEBR 5268T were straight rods (Fig. 1) that were 0.5 μm in diameter and 2 to 3 μm long during the exponential growth phase. They occurred singly and in pairs in young cultures. However, pleomorphic forms, including filaments up to 15 μm long, developed in old cultures. Strain SEBR 5268T was motile and possessed peritrichous flagella (data not shown). Spores were never observed on rich medium containing glucose and in the presence of thiosulfate, but were observed after 48 h of incubation at 60°C (Fig. 1) in a medium containing r-xylose and thiosulfate. In addition, growth was observed after the culture was pasteurized at 90°C for 20 min on glucose-thiosulfate medium. Cells from all phases of growth were gram positive. Electron microscopy also revealed a gram-positive type of cell wall (data not shown).

Growth and nutritional properties. Strain SEBR 5268T required yeast extract to ferment carbohydrates. The doubling time of this isolate in glucose medium was about 2 h at 60°C.

The relationship between strain SEBR 5268T growth and temperature is shown in Fig. 2. The optimum temperature for growth was between 55 and 60°C. Strain SEBR 5268T did not grow at 37 or 80°C. Growth occurred in the presence of NaCl concentrations up to 4.5%. Growth was detected at pH values between 5.6 and 8.8. The energy sources used by strain SEBR 5268T were glucose, fructose, galactose, mannose, cellobiose, maltose, sucrose, lactose, r-xylose, r-ribose, mannitol, pyruvate, starch, and yeast extract, but no growth was observed on r-arabinose, cellulose, r-rhamnose, glyceral, ribitol, galactitol, sorbose, or melibiose. The fermentation products during growth on glucose were lactate, acetate, ethanol, H2, and CO2. Strain SEBR 5268T did not grow on cellulose and did not grow by reducing sulfate, nitrate, or fumarate. Thiosulfate, sulfite, and elemental sulfur were reduced to sulfide.

DNA base composition. The DNA base composition of strain SEBR 5268T was 35 mol% G+C.

16S rRNA sequence analysis. Comparisons of partial 16S rRNA gene sequences (500 nucleotides) revealed that strains SEBR 5268T, SEBR 7311, and SEBR 7312 were closely related as they exhibited a level of sequence similarity of 99% (data not shown). Therefore, the complete sequence of only strain SEBR 5268T (1,507 bases) was determined. This sequence

Nucleotide sequence accession number. The 16S rRNA sequence of strain SEBR 5268T has been deposited in the GenBank data library under accession number U14300.
FIG. 1. Phase-contrast photomicrograph of strain SEBR 5268T showing terminal spores. Bar = 10 μm.

FIG. 2. Effect of temperature on the growth rate of strain SEBR 5268T.

(positions 32 to 1,541; Escherichia coli numbering of Winker and Woese [40]) was aligned with the sequences of representatives of the various phyla of the domain Bacteria, and a phylogenetic analysis was performed. This analysis revealed that strain SEBR 5268T was a member of the low-G+C-content, gram-positive phylum. Additional sequence alignment and evolutionary distance analyses performed with members of this phylum indicated that the closest relatives of strain SEBR 5268T were T. finnii, T. brockii, and strain Gluc 1 (Table 1). Isolation of strain Gluc 1 has been described, but detailed characteristics of this isolate have not been published yet (38). The 16S rRNA sequence of strain Gluc 1 was obtained from the Ribosomal Database Project (21). Figure 3 is a dendrogram which was generated by the neighbor-joining method (15) from a Jukes-Cantor evolutionary distance matrix (19) (Table 1) and shows these relationships.

DNA relatedness. Strains SEBR 5268T, SEBR 7311, and SEBR 7312 were closely related to both T. finnii and T. brockii. Most of the similarity values were >70% with a ΔT_m of 0°C; the only exception was T. brockii and strain SEBR 7311 (ΔT_m, 1°C) (Table 2).

DISCUSSION

Isolation of members of various physiological groups of thermophilic bacteria from deep subsurface environments has been reported previously, and the organisms isolated include methanogens and sulfur- or sulfate-reducing bacteria (11, 28, 37). To date, no formal characterization of fermentative bacteria isolated from such environments has been published, although short descriptions of strains SEBR 5268T and Gluc 1 have appeared previously (12, 38). Our formal description of strain SEBR 5268T obtained from an oil field in this paper extends the physiological diversity and taxonomic diversity of thermophiles found in deep subsurface environments.

Strain SEBR 5268T did not grow at temperatures above 75°C, although the in situ temperature of the oil well from which it was isolated was 92°C, indicating that this microbe may colonize the cooler parts of the reservoir. However, we cannot eliminate the possibility that thermophilic anaerobic heterotrophs may have been introduced during sample collection. Interestingly, strain SEBR 5268T cells sporulated when they were grown with D-xylose as an electron donor and thiosulfate as an electron acceptor; thus, this organism is able to survive but not necessarily grow at temperatures higher than 75°C.

Strain SEBR 5268T has been reported to utilize thiosulfate as an electron acceptor and to produce sulfide (12). When yeast extract was used as a growth substrate, H_2 was oxidized to H_2S by this isolate in the presence of thiosulfate. H_2 oxidation took place mainly after the exponential growth phase. These physiological features (H_2 consumption and sulfide production) make strain SEBR 5268T a potential biocorrosive agent in oil petroleum fields in the presence of thiosulfate (12–14). The diversity and role in pipeline corrosion and biofouling of fermentative bacteria similar to strain SEBR 5268T are currently being examined by workers in our laboratories.

Strain SEBR 5268T is a sporulating, anaerobic, rod-shaped thermophile which ferments a variety of sugars and produces ethanol, acetate, lactate, CO_2, and H_2. In this respect strain SEBR 5268T is similar to numerous thermoanaerobes belonging to the domain Bacteria. However, phylogenetically, strain SEBR 5268T is clearly related to Thermoanaerobacter species, including T. finnii and T. brockii (average level of similarity,
anaerobacter ethanolicus, obacter 786 CAYOL ET AL. INT. J. SYST. BACTERIOL.

higher DNA G+C content. In contrast to our isolate, from the seven previously validly described
nucleotides were used.
of its optimum growth temperature
lowest optimum growth temperature among the

Thermoanaerobacter kivuii
Thermoanaerobacter thermohydrosulfuricus
Thermoanaerobacter ethanolicus
Thermoanaerobacter acetoethylicus
Thermoanaerobacter sp. strain Gluc 1
Thermoanaerobacter brockii DSM 1457T
Thermoanaerobacter thermosulfurigenes
Thermoanaerobacterium lactoethylicum
Thermoanaerobacterium thermosaccharolyticum
Thermoanaerobacterium xylanolyticum

98.4%), as well as Thermoanaerobacter acetoethyllicus, Thermoanaerobacter ethanolicus, and Thermoanaerobacter thermohydrosulfuricus (average level of similarity, 95.8%). On the basis of its optimum growth temperature (55 to 60°C), which is the lowest optimum growth temperature among the Thermoanaerobacter strains that have been examined, and phenotypic characteristics, strain SEBR 5268T can be clearly differentiated from the seven previously validly described Thermoanaerobacter species (9, 22, 32, 35, 39) (Table 3). Strain SEBR 5268T differs from T. brockii (22, 42) and T. ethanolicus (39) by its higher DNA G+C content. In contrast to our isolate, T. brockii does not use mannose and D-xylose, while T. ethanolicus does not use mannitol, whereas strain SEBR 5268* does. Recent studies revealed that T. ethanolicus and T. thermohydrosulfuricus are poor H₂ users in the presence of thiosulfate compared with strain SEBR 5268T (13). Strain SEBR 5268T is not related to T. acetoethyllicus as the latter organism is a gram-negative nonsporulating bacterium that cannot produce lactate from fermentation of glucose (2, 32). Furthermore, T. acetoethyllicus does not use pyruvate and D-xylose, whereas strain SEBR 5268T does. Although strain Gluc 1, a partially characterized isolate obtained from a 6,779-m-deep Swedish bore hole, is morphologically and phylogenetically similar to strain SEBR 5268T does. However, strain SEBR 5268T differs from T. finnii in its optimum temperature and pH for growth and uses melibirose. Furthermore, when T. finnii is cultured under the same growth conditions as strain SEBR 5268T, it produces ethanol, lactate, CO₂, and H₂ from fermentation of glucose (38), whereas strain SEBR 5268T produces acetate in addition to these products. The phenotypically most closely related Thermoanaerobacter species is T. finnii. However, strain SEBR 5268T produces equal amounts of lactate and ethanol, which may affect the end product profile, as has been reported for T. ethanolicus (39).

TABLE 1. Evolutionary distance matrix determined from a comparison of the 16S rRNA sequences of carbohydrate-fermenting thermoanaerobes by using the method of Jukes and Cantor*

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<th>Strain</th>
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<th>Thermoanaerobacter sp. strain SEBR 5268T</th>
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<th>Thermoanaerobacter ethanolicus ATCC 31550T</th>
<th>Thermoanaerobacter thermohydrosulfuricus DSM 567T</th>
<th>DSM 786CAYOL ET AL. INT. J. SYST. BACTERIOL.</th>
<th>Thermoanaerobacter sp. strain Gluc 1</th>
<th>Thermoanaerobacter brockii DSM 1457T</th>
<th>Thermoanaerobacter thermosulfurigenes</th>
<th>Thermoanaerobacterium lactoethylicum ATCC 33743T</th>
<th>Thermoanaerobacterium thermosaccharolyticum TAM 13577T</th>
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* See Materials and Methods. The sequences used in this analysis were obtained from the Ribosomal Database Project, version 4.0 (21). Only 1,320 unambiguous nucleotides were used.

A comparison of the 16S ribosomal DNA sequence of strain SEBR 5268T with the sequences of Thermoanaerobacter species

TABLE 2. Levels of DNA relatedness for T. brockii, T. finnii, and three new strains obtained from oil wells

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* Unless indicated otherwise, the ΔTm was 0°C.

The ΔTm was 1°C.
cies also revealed that strain SEBR 5268T is more closely related to \textit{T. brockii} and \textit{T. finnii} (level of similarity, 98.3\%) than to \textit{T. thermohydrodsulfuricus} (level of similarity, 95.7\%), \textit{T. ethanolicus} (level of similarity, 95.6\%), \textit{Thermoanaerobacter kivui} (level of similarity, 96.3\%), or \textit{Thermoanaerobacter thermocorpiae} (level of similarity, 94.7\%). It has been proposed that if the level of 16S rRNA similarity is more than 97\%, it should not be used to distinguish taxonomically related strains (36), and therefore DNA-DNA hybridization experiments were performed. Our DNA-DNA hybridization data confirmed that strain SEBR 5268T could not be classified as a member of a new \textit{Thermoanaerobacter} species. \textit{T. brockii} and \textit{T. finnii} are also closely related to each other (level of similarity, 98.9\%). On the basis of our DNA-DNA hybridization data, it is evident that these organisms should not be distinguished at the species level (Table 2). This is in contrast to the results of Schmid et al. (35), who obtained values low enough to place \textit{T. finnii} and \textit{T. brockii} strains in separate species. This discrepancy in results can be explained by the fact that two different methods were used for DNA-DNA hybridization experiments. The DNA-DNA hybridization method has been refined, and now the data include ΔT \textit{m} values for improved sensitivity; the ΔT \textit{m} should be >5°C to differentiate species. The ΔT \textit{m} value obtained in most hybridization experiments performed with \textit{T. brockii}, \textit{T. finnii}, and strain SEBR 5268T was 0°C; the only exception was the value obtained in the experiment performed with \textit{T. brockii} strain and strain SEBR 7311 (ΔT \textit{m}, 1°C), which indicated that these organisms should be considered strains rather than members of distinct species. DNA-DNA hybridization studies performed with two other oil field \textit{Thermoanaerobacter} strains (SEBR 7311 and SEBR 7312) also gave similar results. On the basis of the results described above, we propose that \textit{T. finnii} should be reclassified in the species \textit{T. brockii} since the latter bacterium was described first (42). Therefore, \textit{T. finnii} becomes a new subspecies of \textit{T. brockii}, \textit{T. brockii} subsp. \textit{finnii} comb. nov. We also propose that strain SEBR 5268T should be classified as a member of a new subspecies of \textit{T. brockii}, \textit{T. brockii} subsp. \textit{lactethylicus}. The description of the genus \textit{Thermoanaerobacter} is the description given previously by Lee et al. (22).

Our results also indicate that \textit{T. brockii} strains are widely distributed in nature as they have now been isolated from soil, lake sediments, volcanic hot springs, and subsurface terrain, such as oil fields and deep bore holes. As these organisms are sporulators, their distribution in such a diverse range of ecosystems is perhaps not surprising.

**Emendation of the species description of \textit{Thermoanaerobacter brockii} Zeikus, Hegge, and Anderson 1979; Lee, Jain, Lee, Lowe, and Zeikus 1993.** \textit{Thermoanaerobacter brockii} (brock'i). M. L. gen. n. \textit{brockii}, of Brock, named for Thomas Dale Brock, who performed pioneering studies on the physiological ecology of extreme thermophiles). Rods are 0.4 to 1.0 by 1 to 20 μm. Cells occur singly, in pairs, in short chains, and in filaments. Gram positive. Heat-resistant terminal endospores are formed. Colonies are circular and 0.2 to 4 mm in diameter. Thermophilic. The optimum growth temperature is 55 to 70°C; the temperature range for growth is 35 to 85°C. The optimum pH is 6.5 to 7.5. Obligate anaerobe. Chemooorganotrophic. Ferments hexoses and pyruvate. The end products of glucose fermentation are ethanol, lactate, acetate, H₂, and CO₂. Reduces thiosulfate to hydrogen sulfide. The G+C content of the DNA is 30 to 35 mol%. Isolated from the sediment of lakes, hot springs, and oil wells.

**Description of \textit{Thermoanaerobacter brockii} subsp. \textit{brockii} Zeikus, Hegge, and Anderson 1979; Lee, Jain, Lee, Lowe, and Zeikus 1993.** \textit{Thermoanaerobacter brockii} subsp. \textit{brockii} (brock'i). M. L. gen. n. \textit{brockii}, of Brock, named for Thomas Dale Brock, who performed pioneering studies on the physiological ecology of extreme thermophiles). Short rods are 1.0 by 2 to 20 μm. Cells frequently vary in length (minicells) and occur in pairs, chains, and filaments. Gram positive. Round, heat-resistant terminal endospores are formed. Cytochrome pigments and catalase are absent. Colonies are circular, 0.2 to 0.3 mm in diameter, flat, mucoid, and nonpigmented. Monolayer cell wall architecture without an outer wall membrane. Growth is inhibited by penicillin, cycloserine, streptomycin, tetracycline, and chloramphenicol. Thermophilic. The optimum growth temperature is 65 to 70°C; the temperature range for growth is >35 to <85°C. The pH range for growth is 5.5 to 9.5; the optimum pH is 7.5. Obligate anaerobe. Chemooorganotrophic. Ferments glucose, maltose, sucrose, lactose, cellobiose, starch, and pyruvate. Does not use xylose, cellulose, arabinose, mannos, lactate, tartrate, ethanol, tryptone, Casamino Acids, and pectin. The end products of glucose fermentation are ethanol, lactate, acetate, H₂, and CO₂. Reduces thiosulfate to hydrogen sulfide. The G+C content of the DNA is 30 to 31 mol%. Isolated from a thermal spring sediment in Yellowstone National Park.

### TABLE 3. Salient features of \textit{Thermoanaerobacter} species and subspecies

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Cell size (μm)</th>
<th>Spore formation</th>
<th>Gram reaction</th>
<th>Flagellation</th>
<th>G+C content (mol%)</th>
<th>Temp (°C) Range</th>
<th>Optimum pH</th>
<th>Optimum Substrates usedf</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{T. acetethylicus}</td>
<td>1.5-2.5 x 0.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>31-40</td>
<td>40-80</td>
<td>65</td>
<td>NR</td>
</tr>
<tr>
<td>\textit{T. ethanolicus}</td>
<td>4-8 x 0.3-0.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>32</td>
<td>37-78</td>
<td>69</td>
<td>5.8-8.5 NR</td>
</tr>
<tr>
<td>\textit{T. thermohydrodsulfuricus}</td>
<td>2-13 x 0.3-0.6</td>
<td>+</td>
<td>Variable</td>
<td>Peritrichous</td>
<td>35-37</td>
<td>28-78</td>
<td>67-69</td>
<td>6.9-7.5 NR</td>
</tr>
<tr>
<td>\textit{T. brockii} subsp. \textit{brockii}</td>
<td>2-20 x 0.8-1.0</td>
<td>+</td>
<td>Positive</td>
<td>NR</td>
<td>30-31</td>
<td>40-80</td>
<td>65-70</td>
<td>7.5 NR</td>
</tr>
<tr>
<td>\textit{T. brockii} subsp. \textit{finnii}</td>
<td>1-4 x 0.4-0.6</td>
<td>+</td>
<td>Positive</td>
<td>NR</td>
<td>32</td>
<td>40-75</td>
<td>63</td>
<td>6.5-6.8 +</td>
</tr>
<tr>
<td>\textit{T. brockii} subsp. \textit{lactethylicus}</td>
<td>2-3 x 0.5</td>
<td>+</td>
<td>Positive</td>
<td>Peritrichous</td>
<td>35</td>
<td>37-75</td>
<td>55-60</td>
<td>7.3 +</td>
</tr>
</tbody>
</table>

* +, positive; –, negative; 2, variable.

f Data from references 22 and 32.

NR, not reported.

Data from references 22 and 39.

Data from reference 22.

Data from references 22 and 42.

Data from reference 35.

Data from this study.

Average cell dimensions during the exponential growth phase.
The type strain is HTD4 (DSM 1457 = ATCC 33075). Description of Thermoanaerobacter brockii subsp. finnii Schmid, Giesel, Schobert, and Sahm 1986. Thermoanaerobacter brockii subsp. finnii (fin’i.i. M. L. gen. n. finnii, of Finn, named for Robert K. Finn, who made important contributions to the development of the ethanol vacuum fermentation process). Short rods are 0.4 to 0.6 by 1 to 4 μm. Cells occur singly, in pairs, and in short chains and are motile. Occasionally cocccoid cells are found. Heat-resistant terminal endospores are occasionally formed. Colonies are circular, 1 to 3 mm in diameter, smooth, and white. Contains peptidoglycan of the meso-diaminopimelic acid type. Susceptible to penicillin G and tetracycline. The type strain is SEBR 5268. The type strain is HTD4.

The type strain is AKO-1 (DSM 3389). Description of Thermoanaerobacter brockii subsp. lactiethylicus subsp. nov. Thermoanaerobacter brockii subsp. lactiethylicus (lac. ti. e. thy’ ii. cus. L. n. lacticum, lactic acid; M. L. n. ethylicus, ethyl alcohol; lactiethylicus, referring to the production of both lactic acid and ethanol). Cells are straight rods (0.5 by 2 μm) that are motile by means of peritrichous flagella and occur singly or in pairs in young cultures. Pleomorphic filamentous cells (length, 15 μm) occur in old cultures. Gram positive. Spores are formed in medium containing d-xylose as an electron donor and thiosulfate as an electron acceptor. Colonies in roll tubes are 4 mm in diameter after 2 days of incubation at 50°C; smooth, uniformly round, mucoid, nonpigmented, and flat. The optimum temperature is 55 to 60°C (range, 40 to 75°C). Tolerates up to 4% NaCl. The optimum sodium chloride concentration for growth is 1%. Obligately anaerobic. Ferments glucose, fructose, galactose, mannose, cellobiose, maltose, sucrose, lactose, xylose, ribose, mannitol, and pyruvate. The fermentation products from glucose and xylose fermentation are ethanol and CO₂, as well as minor amounts of L-lactate and acetate. Reduces thiosulfate to hydrogen sulfide. The G+C content of the DNA is 38 mol%. Isolated from sediment sludge from Lake Kivu in East Africa. The type strain is SEBR 5268 (DSM 9801).

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REFERENCES