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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*

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A strictly anaerobic, thermophilic, gram-positive, spore-forming eubacterium designated strain SEBR 5268^T (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, H_2, and CO_2. The DNA base composition was 35 mol% G+C. The results of 16S rRNA sequence comparisons indicated that strain SEBR 5268^T 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 5268^T 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 5268^T has been deposited in the Deutsche Sammlung von Mikroorganismen as strain DSM 9801^T.

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 (37), 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 H_2, 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.

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MATERIALS AND METHODS

Origins of strains. Strain SEBR 5268^T (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-1^T (= DSM 3389) and *Thermoanaerobacter brockii* HTD4^T (= DSM 1457^T) 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 NH_4Cl, 0.3 g of K_2HPO_4, 0.3 g of KH_2PO_4, 1.3 g of $MgCl_2 \cdot 6H_2O$, 0.1 g of $CaCl_2 \cdot 2H_2O$, 2.0 g of KCl, 2.0 g of NaCl, 0.5 g of CH_3COONa, 5.0 g of bio-Trypcase (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 into 50-ml serum bottles under a stream of oxygen-free N_2-CO_2 (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% $Na_2S \cdot 9H_2O$, 1 ml of 10% $NaHCO_3$, 0.2 ml of a vitamin solution (31), 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 and 1 g of bio-Trypcase 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% $Na_2S \cdot 9H_2O$ sterile anaerobic stock solution and 0.25 ml of a 10% $NaHCO_3$ 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, prereduced growth medium in anaerobic tubes was adjusted to the desired pH with $NaHCO_3$ or Na_2CO_3 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 as described above. Basal glucose 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 guanine-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 μl 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 microwaved 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 μl 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 μl of phenol-chloroform (1:1). The preparation was then centrifuged at 13,000 × *g* for 15 min to separate the phases. The top aqueous phase (approximately 200 μl) was removed; we were careful to avoid any material from the interface when we did this. Then 10 μl of isopropanol and 5 μl 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 μl 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 PCR product was sequenced directly with an ABI automated DNA sequencer by using a Prism dideoxy 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 ae2 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 DNAPARS, and tree topology was examined by using 100 bootstrapped data sets by running the script file DBOOT. For DBOOT we used the following sequence of events during the analysis: SEQBOOT, 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 relatedness. 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 reassociated DNA became hydrolyzable by the S1 nuclease (T_m) was determined as described by Crosa et al. (10). The difference between the T_m of a homoduplex and the T_m of a heteroduplex (ΔT_m) provided an estimate of the level of divergence between two DNAs (4).

Nucleotide sequence accession number. The 16S rRNA sequence of strain SEBR 5268^T has been deposited in the GenBank data library under accession number U14330.

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 5268^T, SEBR 7311, and SEBR 7312. SEBR 5268^T was obtained from enrichment cultures that had been grown under an N_2-CO_2 (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 H_2-CO_2 (2×10^5 Pa) as the gas phase. Only limited genotypic studies were performed with strains SEBR 7311 and SEBR 7312, whereas strain SEBR 5268^T was studied in greater detail.

Cellular features. Cells of strain SEBR 5268^T 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 5268^T 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 D-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 5268^T 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 5268^T growth and temperature is shown in Fig. 2. The optimum temperature for growth was between 55 and 60°C. Strain SEBR 5268^T 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 5268^T were glucose, fructose, galactose, mannose, cellobiose, maltose, sucrose, lactose, D-xylose, D-ribose, mannitol, pyruvate, starch, and yeast extract, but no growth was observed on L-arabinose, cellulose, L-rhamnose, glycerol, ribitol, galactitol, sorbose, or melibiose. The fermentation products during growth on glucose were lactate, acetate, ethanol, H_2, and CO_2. Strain SEBR 5268^T 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 5268^T was 35 mol% G+C.

16S rRNA sequence analysis. Comparisons of partial 16S rRNA gene sequences (500 nucleotides) revealed that strains SEBR 5268^T, 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 5268^T (1,507 bases) was determined. This sequence

FIG. 1. Phase-contrast photomicrograph of strain SEBR 5268^T showing terminal spores. Bar = 10 μm.

(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 5268^T 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 5268^T 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 5268^T, 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).

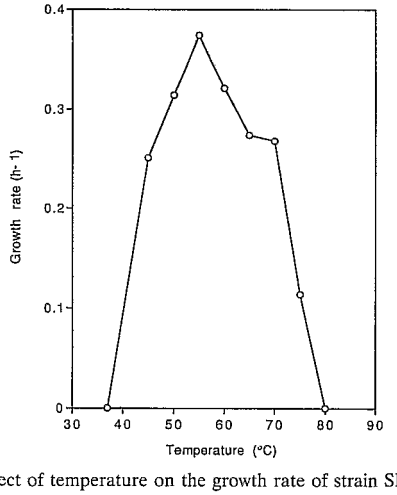

FIG. 2. Effect of temperature on the growth rate of strain SEBR 5268^T.

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 5268^T and Gluc 1 have appeared previously (12, 38). Our formal description of strain SEBR 5268^T obtained from an oil field in this paper extends the physiological diversity and taxonomic diversity of thermophiles found in deep subsurface environments.

Strain SEBR 5268^T 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 5268^T 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 5268^T 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 5268^T 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 5268^T are currently being examined by workers in our laboratories.

Strain SEBR 5268^T 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 5268^T is similar to numerous thermoanaerobes belonging to the domain *Bacteria*. However, phylogenetically, strain SEBR 5268^T is clearly related to *Thermoanaerobacter* species, including *T. finnii* and *T. brockii* (average level of similarity,

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[a]

Strain	Evolutionary distance												
	Escherichia coli	*Thermoanaerobacter* sp. strain SEBR 5268^T	*Thermoanaerobacter acetoethylicus* ATCC 33265T	*Thermoanaerobacter ethanolicus* ATCC 31550^T	*Thermoanaerobacter thermohydrosulfuricus* DSM 567^T	*Thermoanaerobacter kivuii* DSM 2030^T	*Thermoanaerobacter finnii* DSM 3389^T	*Thermoanaerobacter* sp. strain Gluc 1	*Thermoanaerobacter brockii* DSM 1457^T	*Thermoanaerobacter thermocopriae* IAM 13577^T	*Thermoanaerobacterium thermosaccharolyticum* ATCC 7956^T	*Thermoanaerobacterium lactoethylicum* ZE-1	*Thermoanaerobacterium thermosulfurigenes* ATCC 33743^T
Thermoanaerobacter sp. strain SEBR 5268^T	27.6												
Thermoanaerobacter acetoethylicus ATCC 33265T	28.4	4.0											
Thermoanaerobacter ethanolicus ATCC 31550^T	28.3	4.4	0.8										
Thermoanaerobacter thermohydrosulfuricus DSM 567^T	28.1	4.3	2.3	1.9									
Thermoanaerobacter kivuii DSM 2030^T	26.7	3.7	4.3	4.4	3.6								
Thermoanaerobacter finnii DSM 3389^T	27.8	1.7	3.8	4.2	3.6	3.8							
Thermoanaerobacter sp. strain Gluc 1	26.9	0.9	3.0	3.4	3.3	3.0	0.8						
Thermoanaerobacter brockii DSM 1457^T	27.1	1.7	3.6	4.0	3.6	3.6	1.1	0.8					
Thermoanaerobacter thermocopriae IAM 13577^T	27.7	5.3	6.2	6.2	5.9	6.1	5.2	4.4	5.2				
Thermoanaerobacterium thermosaccharolyticum ATCC 7956^T	26.0	13.9	14.2	14.1	13.8	14.4	13.9	13.8	14.0	15.3			
Thermoanaerobacterium lactoethylicum ZE-1	26.0	14.1	13.7	13.6	13.3	14.2	14.1	13.8	14.1	15.3	2.4		
Thermoanaerobacterium thermosulfurigenes ATCC 33743^T	26.3	14.1	13.6	13.5	13.2	14.3	13.9	13.8	13.9	15.5	2.1	1.0	
Thermoanaerobacterium xylanolyticum DSM 7097^T	26.3	14.4	13.8	13.5	13.2	14.2	14.2	14.0	14.2	15.8	2.5	1.5	1.0

[a] 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.

98.4%), as well as *Thermoanaerobacter acetoethylicus*, *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 5268^T can be clearly differentiated from the seven previously validly described *Thermoanaerobacter* species (9, 22, 32, 35, 39) (Table 3). Strain SEBR 5268^T 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^T does. Recent studies revealed that *T. ethanolicus* and *T. thermohydrosulfuricus* are poor H_2 users in the presence of thiosulfate compared with strain SEBR 5268^T (13). Strain SEBR 5268^T is not related to *T. acetoethylicus* as the latter organism is a gram-negative nonsporulating bacterium that cannot produce lactate from fermentation of glucose (2, 32). Furthermore, *T. acetoethylicus* does not use pyruvate and D-xylose, whereas strain SEBR 5268^T 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 5268^T, it produces ethanol, lactate, CO_2, and H_2 from fermentation of glucose (38), whereas strain SEBR 5268^T produces acetate in addition to these products. The phenotypically most closely related *Thermoanaerobacter* species is *T. finnii*. However, strain SEBR 5268^T differs from *T. finnii* in its optimum temperature and pH for growth and uses melibiose. Furthermore, when *T. finnii* is cultured under the same growth conditions as strain SEBR 5268^T, it produces ethanol as the major end product of glucose metabolism, whereas strain SEBR 5268^T produces equal amounts of lactate and ethanol. However, it is possible that varying culture conditions may affect the end product profile, as has been reported for *T. ethanolicus* (39).

A comparison of the 16S ribosomal DNA sequence of strain SEBR 5268^T with the sequences of *Thermoanaerobacter* spe-

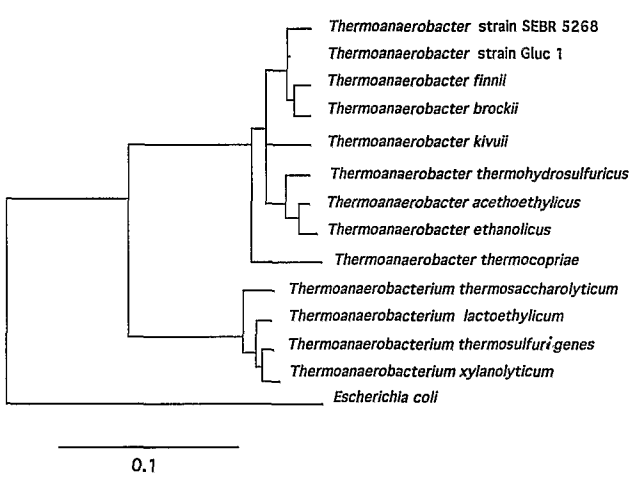

FIG. 3. Dendrogram showing the position of strain SEBR 5268^T among representatives of the genus *Thermoanaerobacter* and related genera. The dendrogram was derived from the evolutionary distance matrix shown in Table 1.

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

Source of unlabelled DNA	% Relatedness to ^{3}H-labelled DNA from[a]:		
	T. brockii	*T. finnii*	SEBR 5268^T
T. brockii	100	97	76
T. finnii	89	100	85
SEBR 5268^T	85	76	100
SEBR 7311	96[b]	75	89[b]
SEBR 7312	81	78	86

[a] Unless indicated otherwise, the ΔT_m was 0°C.
[b] The ΔT_m was 1°C.

TABLE 3. Salient features of *Thermoanaerobacter* species and subspecies

Taxon	Cell size (μm)	Spore formation	Gram reaction	Flagellation	G+C content (mol%)	Temp (°C)		Optimum pH	Substrates used[a]			
						Range	Optimum		Melibiose	Mannose	Xylose	Mannitol
T. acetoethylicus[b]	1.5–2.5 × 0.6	−	Negative	Peritrichous	31	40–80	65	NR[c]	NR	+	−	NR
T. ethanolicus[d]	4–8 × 0.3–0.8	−	Variable	Peritrichous	32	37–78	69	5.8–8.5	NR	+	+	−
T. thermohydrosulfuricus[e]	2–13 × 0.3–0.6	+	Variable	Peritrichous	35–37	28–78	67–69	6.9–7.5	NR	+	+	±
T. brockii subsp. *brockii*[f]	2–20 × 0.8–1.0	+	Positive	NR	30–31	40–80	65–70	7.5	NR	−	−	NR
T. brockii subsp. *finnii*[g]	1–4 × 0.4–0.6	+	Variable	NR	32	40–75	65	6.5–6.8	+	+	+	+
T. brockii subsp. *lactiethylicus*[h]	2–3 × 0.5[i]	+	Positive	Peritrichous	35	37–75	55–60	7.3	−	+	+	+

[a] +, positive; −, negative; ±, variable.
[b] Data from references 2 and 32.
[c] NR, not reported.
[d] Data from references 22 and 39.
[e] Data from reference 22.
[f] Data from references 22 and 42.
[g] Data from reference 35.
[h] Data from this study.
[i] Average cell dimensions during the exponential growth phase.

cies also revealed that strain SEBR 5268^T is more closely related to *T. brockii* and *T. finnii* (level of similarity, 98.3%) than to *T. thermohydrosulfuricus* (level of similarity, 95.7%), *T. ethanolicus* (level of similarity, 95.6%), *Thermoanaerobacter kivui* (level of similarity, 96.3%), or *Thermoanaerobacter thermocopriae* (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 5268^T could not be classified as a member of a new *Thermoanaerobacter* species. *T. brockii* and *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 *T. finnii* and *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_m values for improved sensitivity; the ΔT_m should be >5°C to differentiate species. The ΔT_m value obtained in most hybridization experiments performed with *T. brockii*, *T. finnii*, and strain SEBR 5268^T was 0°C; the only exception was the value obtained in the experiment performed with a *T. brockii* strain and strain SEBR 7311 (ΔT_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 *Thermoanaerobacter* strains (SEBR 7311 and SEBR 7312) also gave similar results. On the basis of the results described above, we propose that *T. finnii* should be reclassified in the species *T. brockii* since the latter bacterium was described first (42). Therefore, *T. finnii* becomes a new subspecies of *T. brockii*, *T. brockii* subsp. *finnii* comb. nov. We also propose that strain SEBR 5268^T should be classified as a member of a new subspecies of *T. brockii*, *T. brockii* subsp. *lactiethylicus*. The description of the genus *Thermoanaerobacter* is the description given previously by Lee et al. (22).

Our results also indicate that *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 *Thermoanaerobacter brockii* Zeikus, Hegge, and Anderson 1979; Lee, Jain, Lee, Lowe, and Zeikus 1993. *Thermoanaerobacter brockii* (brock'i.i. M. L. gen. n. *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. Chemoorganotrophic. Ferments hexoses and pyruvate. The end products of glucose fermentation are ethanol, lactate, acetate, H_2, and CO_2. 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 *Thermoanaerobacter brockii* subsp. *brockii* Zeikus, Hegge, and Anderson 1979; Lee, Jain, Lee, Lowe, and Zeikus 1993. *Thermoanaerobacter brockii* subsp. *brockii* (brock'i.i. M. L. gen. n. *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. Chemoorganotrophic. Ferments glucose, maltose, sucrose, lactose, cellobiose, starch, and pyruvate. Does not use xylose, cellulose, arabinose, mannose, lactate, tartrate, ethanol, tryptone, Casamino Acids, and pectin. The end products of glucose fermentation are ethanol, lactate, acetate, H_2, and CO_2. 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.

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'ni.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 coccoid cells are found. Heat-resistant terminal endospores are 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. Thermophilic. The optimum growth temperature is 65°C; the temperature range for growth is 40 to 75°C. The optimum pH is 6.5 to 6.8. Obligate anaerobe. Chemoorganotrophic. Ferments glucose, fructose, galactose, mannose, cellobiose, maltose, sucrose, lactose, xylose, ribose, mannitol, and pyruvate. The end products of glucose and xylose fermentation are ethanol and CO_2, as well as minor amounts of L-lactate and acetate. Reduces thiosulfate to hydrogen sulfide. The G+C content of the DNA is 32 mol%. Isolated from sediment sludge from Lake Kivu in East Africa.

The type strain is AKO-1 (= DSM 3389).

Description of *Thermoanaerobacter brockii* subsp. *lactiethylicus* subsp. nov. *Thermoanaerobacter brockii* subsp. *lactiethylicus* (lac. ti. e. thy' li. 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 60°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, D-ribose, D-xylose, cellobiose, lactose, maltose, sucrose, mannitol, and pyruvate. The fermentation products from glucose are lactate, acetate, ethanol, H_2, and CO_2. Reduces thiosulfate to hydrogen sulfide. The G+C content of the DNA is 35 mol%. Phylogenetically related to *Thermoanaerobacter* species in the low-G+C-content subbranch of the gram-positive bacteria. The habitat is geothermal oil samples.

The type strain is SEBR 5268 (= DSM 9801).

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FICHE DESCRIPTIVE

Auteur(s) : Cayol J.L., **Ollivier B.**, Patel B.K.C., Ravot G., Magot M., Ageron E., Grimont P.A.D., **Garcia J.L.**

Titre original : 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 amended description of *Thermoanaerobacter brockii*

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Titre en Français : Description de *Thermoanaerobacter brockii* subsp. *lactiethylicus* subsp. nov., isolé d'un puits de pétrole français, une proposition de reclassement de *Thermoanaerobacter finnii* en *Thermoanaerobacter brockii* subsp. *finnii* comb. nov., et une description modifiée de *Thermoanaerobacter brockii*

Mots-clés matières (10 au plus) : *Thermoanaerobacter* - réduction du thiosulfate - puits de pétrole - Anaérobiose - Taxonomie

Résumé en Français : (150 mots maximum)

Plan de classement : Monde végétal et Animal - Fermentations