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Thermotoga hypogea sp. nov., a Xylanolytic, Thermophilic Bacterium from an Oil-Producing Well

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A new thermophilic, xylanolytic, strictly anaerobic, rod-shaped bacterium, strain SEBR 7054^T, was isolated from an African oil-producing well. Based on the presence of an outer sheath (toga) and 16S rRNA sequence analysis data, this organism was identified as a member of the genus *Thermotoga*. Strain SEBR 7054^T possessed lateral flagella, had a G+C content of 50 mol%, produced traces of ethanol from glucose but no lactate, and grew optimally in the presence of 0 to 0.2% NaCl at 70°C. Its phenotypic and phylogenetic characteristics clearly differed from those reported for the five previously validly described *Thermotoga* species. Therefore, we propose that strain SEBR 7054^T is a member of a new species of the genus *Thermotoga*, *Thermotoga hypogea* sp. nov. The type strain of *T. hypogea* is SEBR 7054 (= DSM 11164).

Members of the order *Thermotogales* are rod-shaped bacteria that have a characteristic outer sheathlike structure called a toga. This order includes the following five genera: *Thermotoga* (18, 22, 23, 34, 44), *Thermosipho* (19, 37), *Fervidobacterium* (1, 20, 32), *Geotoga*, and *Petrotoga* (9). It represents, along with the *Aquificales*, the deepest phylogenetic branch in the domain *Bacteria* (45). The genus *Thermotoga* includes all of the hyperthermophiles (optimum temperature for growth, around 80°C) of the order *Thermotogales* (18, 21, 22) and the thermophiles (optimum temperature for growth, 65 to 70°C) recently isolated from oil fields (23, 34).

Stetter et al. (39) provided evidence of the presence of *Thermotoga* strains in oil fields. The isolation of *Thermotoga elfii* and *Thermotoga subterranea* from such ecosystems was reported soon thereafter (23, 34). In contrast to *Thermotoga maritima* and *Thermotoga neapolitana*, *T. elfii* and *T. subterranea* were not able to grow at temperatures above 75°C and reduced thiosulfate but not sulfur, which led to speculation that thiosulfate rather than sulfur may be an important electron acceptor in oil field ecosystems (34). The presence of thiosulfate is also thought to increase biocorrosion of oil field installations (8, 27). We therefore initiated intensive studies to isolate *Thermotoga* strains from oil field subsurface ecosystems that grow at temperatures above 80°C and use thiosulfate as an electron acceptor. Our studies have focused on xylanolytic extremophilic microorganisms since thermostable xylanases have a potential use in paper primary-pulp manufacturing (33). In this paper, we describe the first isolation from oil field water of a xylanolytic *Thermotoga* species able to grow at temperatures up to 90°C. The phenotypic and phylogenetic characteristics of the new strain are consistent with its placement in a new species of the genus *Thermotoga*, *Thermotoga hypogea* sp. nov.

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MATERIALS AND METHODS

Sample collection and sample source. Strain SEBR 7054^T was isolated from an oil-producing well in Cameroon in central Africa. The in situ temperature was 66°C, and the concentration of sodium chloride was 12 g/liter. A 1-liter sample was collected at the wellhead as described elsewhere (3), transported to our laboratory, and stored at 4°C until it was used.

Other strain. *Thermotoga thermarum* DSM 5069 was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

Culture media. Enrichment was performed by using MB medium containing 1 g of NH_4Cl, 0.3 g of K_2HPO_4, 0.3 g of KH_2PO_4, 0.5 g of $MgCl_2$, 0.1 g of $CaCl_2$, 10 g of NaCl, 0.2 g of KCl, 0.5 g of cysteine-HCl, 2 g of yeast extract (Difco Laboratories, Detroit, Mich.), 2 g of bio-Trypticase (bioMérieux, Marcy l'Etoile, France), 10 g of xylan, 10 ml of the trace mineral element solution of Balch et al. (2), 1 mg of resazurin, and 1 liter of distilled water. The pH was adjusted to 8.0 with 10 M KOH, and the medium was boiled under a stream of O_2-free N_2 gas and cooled to room temperature. Then 5- and 20-ml aliquots were dispensed into Hungate tubes and serum bottles, respectively, under a stream of N_2-CO_2 (80:20, vol:vol), and the vessels were autoclaved for 45 min at 110°C. Prior to inoculation, $Na_2S \cdot 9H_2O$, Na_2CO_3, and thiosulfate were injected from sterile stock solutions to obtain final concentrations of 0.04%, 0.2%, and 20 mM, respectively. For isolation, MBX medium (MB medium in which xylan was replaced by 20 mM xylose) was used.

Enrichment and isolation. A 2-ml sample of well water was inoculated into 20 ml of medium, which was incubated at 70°C without agitation to initiate an enrichment culture. The culture was purified by repeated use of the Hungate roll tube method with MBX medium solidified with 4% Phytagel as described by Deming and Baross (11).

pH, temperature, and NaCl concentration ranges for growth. The pH, temperature, and NaCl concentration ranges for growth were determined by using MB medium containing 1 g of yeast extract per liter and 5 g of bio-Trypticase per liter but no xylan. The pH of the medium in Hungate tubes was adjusted by injecting $NaHCO_3$ or Na_2CO_3 from 10% sterile anaerobic stock solutions. Mineral oil baths were used to obtain incubation temperatures of >50°C. For studies of NaCl requirements, NaCl was weighed directly in the tubes before the medium was dispensed. The strain was subcultured at least once under the same experimental conditions before the growth rates were determined.

Substrate utilization tests. Substrates were tested at a final concentration of 20 mM in the absence or presence of sodium thiosulfate in MB medium which was modified to contain 1 g of yeast extract per liter and 1 g of bio-Trypticase per liter. *T. thermarum* was cultured in the same medium containing 3 g of NaCl per liter. To test for electron acceptors, sodium thiosulfate, sodium sulfate, and elemental sulfur (Prolabo, Paris, France) were added to the medium at final concentrations of 20 mM, 20 mM, and 2% (wt/vol), respectively.

Light microscopy and electron microscopy. Light microscopy was performed as previously described (5). For electron microscopy, exponentially grown cells were negatively stained with 1% sodium phosphotungstic acid (pH 7.2). To prepare thin sections, exponentially grown cells were centrifuged, fixed for 1 h in 3% (wt/vol) glutaraldehyde in HCl-cacodylate buffer (pH 6.0) containing 0.7 M sucrose, and postfixed overnight in 1% (wt/vol) osmium tetraoxide in 0.75 M

sucrose. Then the cells were washed, embedded in 2% agarose, and stained with 4% uranyl acetate. The agar was cut into small cubes, dehydrated in acetone, and embedded in Araldite. Thin sections were stained with 5% uranyl acetate for 20 min and with 2% lead citrate for 10 min. Micrographs were taken with a Hitachi model H600 electron microscope at an accelerating voltage of 75 kV.

Analytical techniques. Unless otherwise indicated, duplicate culture tubes were used throughout the analytical studies. Growth was measured by inserting tubes directly into a model UV-160A spectrophotometer (Shimadzu Corp., Kyoto, Japan) and measuring the optical density at 580 nm. Sulfide was determined photometrically as collodial CuS by using the method of Cord- Ruwisch (7). H_2, CO_2, sugars, alcohols, and volatile and nonvolatile fatty acids were measured as described previously (13, 14). CO_2 was measured in MB medium that lacked xylan but contained 20 mM glucose. In this medium, N_2-CO_2 and bicarbonate buffer were replaced by N_2 and phosphate buffer, respectively. L-Alanine was determined enzymatically with an L-alanine dehydrogenase kit (catalog no. A-7653; Sigma). Cell-free supernatant was deproteinized with an equal volume of 1 M sodium perchlorate, and the mixture was centrifuged and neutralized with $KHCO_3$. Fifty microliters of the sample was mixed with 200 μl of Tris HCl (pH 10.0). Then L-alanine was determined by using the manufacturer's instructions. Gram staining was performed with a Sigma kit.

Enzyme assays. For xylanolytic activity measurements, cells were harvested in the late exponential or early stationary phase. Cells were collected by centrifugation at 13,000 rpm (Biofuge 13; Heraeus, Les Ulis, France) for 10 min at 4°C. Reducing sugars were quantified with dinitrosalicylic acid (31). Xylanolytic activity was assayed in the supernatant and in resuspended cells by measuring the release of reducing sugars from xylan. Each assay mixture consisted of 0.5% (wt/vol) xylan supplemented with 100 mM acetate buffer (pH 6.5) and enough enzyme so that the final volume was 0.2 ml. The reaction mixture was incubated for 30 min at 70°C. The assay was terminated by adding dinitrosalicylic acid, and the xylose released from xylan was measured at 540 nm. Controls with substrate and no enzyme were included. One unit of xylanase activity was defined as the amount of enzyme required to liberate 1 μmol of xylose per min at 70°C.

Determination of G+C content. The G+C content of DNA was determined at the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. DNA was isolated and purified by chromatography on hydroxyapatite, and its G+C content was determined by using high-performance liquid chromatography as described by Mesbah et al. (30). Nonmethylated lambda DNA (Sigma) was used as the standard.

16S rRNA sequence studies. The methods used for purification and extraction of DNA and amplification and sequencing of the 16S rRNA gene have been described previously (1, 26, 38). The 16S rRNA gene sequence was manually aligned with reference sequences of various members of the domain *Bacteria* by using the editor ae2 (28). Reference sequences were obtained from the Ribosomal Database Project (28) and the EMBL and GenBank databases. Positions of sequence and alignment uncertainty were omitted from the analysis. A phylogenetic analysis was performed by using the various programs implemented as part of the PHYLIP package (15), as described below. Pairwise evolutionary distances based on 1,121 unambiguous nucleotides were computed by the method of Jukes and Cantor (24), and dendrograms were constructed from these distances by the neighbor-joining method.

Nucleotide sequence accession number. The 16S rRNA sequence of strain SEBR 7054^T has been deposited in the GenBank database under accession no. U89768.

RESULTS

Enrichment and isolation. Enrichment cultures were positive after incubation at 70°C for 3 days, and H_2S was detected from thiosulfate reduction. Microscopic examination revealed the presence of rod-shaped bacteria with an outer sheathlike structure characteristic of members of the order *Thermotogales*. Colonies that were 1 mm in diameter developed in Phytagel roll tubes after 7 days of growth at 70°C. Single colonies were picked, and serial dilution in Phytagel roll tubes was repeated at least twice before the culture was considered pure. Several axenic cultures containing cells with the typical outer polar sheathlike structures characteristic of members of the *Thermotogales* were obtained. One strain, strain SEBR 7054^T, was used for further characterization.

Morphology. Strain SEBR 7054^T was a rod-shaped bacterium. The cells were 0.5 to 1 by 2 to 3 μm and occurred singly or in pairs (Fig. 1a). No motility was observed by microscopy, but the cells possessed laterally inserted flagella (Fig. 1b) and were surrounded by a characteristic toga, a sheathlike structure that ballooned over the cell ends (Fig. 1c). Electron microscopy of thin sections of strain SEBR 7054^T revealed a typical toga cell wall ultrastructure with a spongy periplasm layer (Fig. 1d). The cells stained gram negative.

Optimum growth conditions. Strain SEBR 7054^T did not grow in oxidized medium (oxidation was indicated by the pink color of the resazurin). It grew at temperatures ranging from 56 to 90°C, and optimum growth occurred at 70°C at pH 7.0 (Fig. 2a). Growth occurred at initial pH values between 6.1 and 9.1 at 70°C, and the optimum pH was 7.3 to 7.4. At the end of growth, the pH had decreased by 0.2 to 0.3 U. The isolate grew in MB medium in the presence of NaCl concentrations ranging from 0 to 1.5%, and the optimum NaCl concentration was between 0 and 0.2% at pH 7.0 and 70°C (Fig. 2b). *T. thermarum* grew in the same medium containing 0.3% NaCl but did not grow in the presence of 1% NaCl.

Substrates used for growth. Yeast extract or bio-Trypticase was required for growth on carbohydrates, and these compounds were fermented by strain SEBR 7054^T. Yeast extract could not be replaced by Casamino Acids (1 g/liter), vitamins (42), or a mixture of Casamino Acids and vitamins. Strain SEBR 7054^T grew on the following substrates (at a concentration of 20 mM unless indicated otherwise): D-fructose, D-galactose, D-glucose, DL-lactose, DL-maltose, D-mannose, D-sucrose, D-xylose, and xylan (10 g/liter). It could not utilize D-arabinose, D-ribose, L-sorbose, L-xylose, and fatty acids (acetate, butyrate, lactate, and propionate). Acetate, L-alanine, CO_2, H_2, and traces of ethanol were produced during xylose fermentation irrespective of the presence of thiosulfate (Table 1); a similar profile of metabolites was produced by glucose fermentation. In all cases, about 1 mol of CO_2 was produced per mol of acetate produced. The level of carbon recovery ranged from 50 to 80%. Low carbon recovery values were obtained with other thermophilic anaerobes (43). In addition, in the case of *T. maritima* (18) unidentified products of sugar metabolism were detected by thin-layer chromatography. *T. thermarum* grew on sucrose and xylose, but not on D-fructose, D-galactose, DL-lactose, D-mannose, D-arabinose, and L-sorbose. *T. thermarum* also produced traces of ethanol during glucose fermentation.

Effect of added electron acceptors. Strain SEBR 7054^T reduced thiosulfate but not sulfate or sulfur to sulfide. The presence of thiosulfate increased biomass and improved the utilization of glucose but not the utilization of xylose (Table 1). In addition, it altered the concentration of metabolites during glucose and xylose oxidation, indicating that thiosulfate modified the metabolic pathway of strain SEBR 7054^T. The changes observed were as follows: (i) thiosulfate was reduced to sulfide with a concomitant decrease in H_2 to barely detectable levels; and (ii) the ratio of acetate produced to sugar consumed increased in the presence of thiosulfate, whereas the ratio of L-alanine produced to sugar consumed decreased (Table 1). *T. thermarum* also reduced thiosulfate to sulfide.

Sensitivity to rifampin. Strain SEBR 7054^T grew in the presence of rifampin (final concentration, 100 μg/ml).

Xylanase production in batch culture. Strain SEBR 7054^T grew with xylan as an energy source. We found that at the end of growth, xylanase activity was present with xylan as the substrate and cell-free culture medium or resuspended cells as the enzyme source; the xylanase activities obtained with cell-free culture medium and resuspended cells were estimated to be 93 and 81 U/liter, respectively.

G+C content. The G+C content of isolate SEBR 7054^T was 50 mol%.

16S rRNA sequence analysis. Using 12 primers, we determined 1,497 bases of the 16S rRNA gene sequence of strain SEBR 7054^T. A phylogenetic analysis revealed that strain SEBR 7054^T was a member of the order *Thermotogales* and

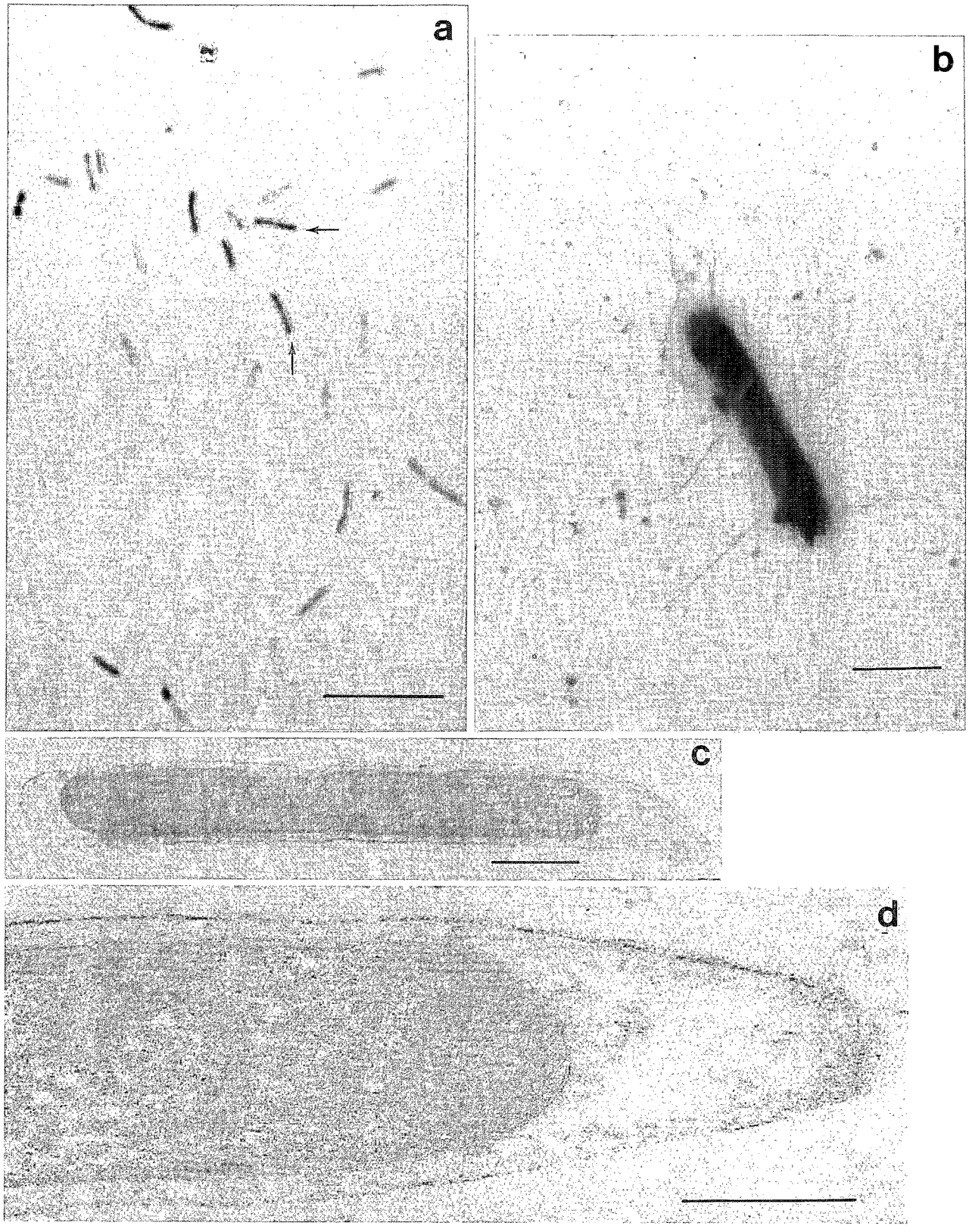

FIG. 1. (a) Phase-contrast micrograph of strain SEBR 7054^T, showing the toga (arrows), a characteristic structure of *Thermotoga* species. Bar = 10 μm. (b) Electron micrograph of a negatively stained culture of strain SEBR 7054^T, showing laterally inserted flagella. Bar = 2 μm. (c) Electron micrograph of a thin section of strain SEBR 7054^T, showing the typical outer sheathlike structure (toga) of the genus *Thermotoga*. Bar = 0.5 μm. (d) Electron micrograph of a thin section of strain SEBR 7054^T, showing the thin electron-dense layer of the cell wall, presumably a peptidoglycan layer covering a spongy periplasmic layer that expands at both ends. Bar = 0.2 μm.

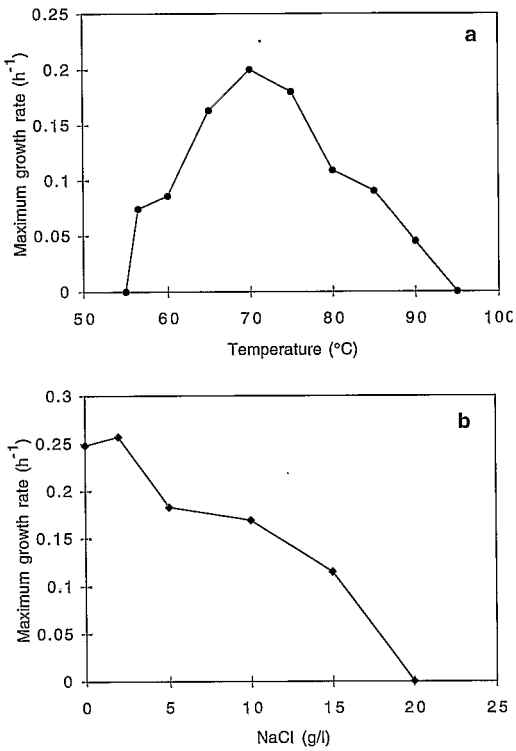

FIG. 2. Effect of temperature (a) and NaCl (b) on the growth of strain SEBR 7054^T.

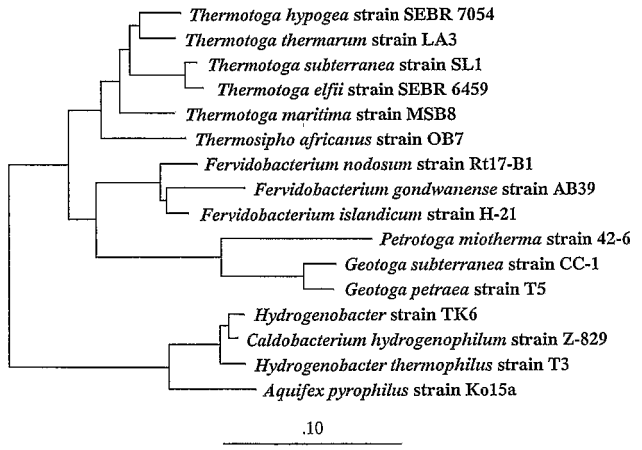

FIG. 3. Dendrogram showing the position of strain SEBR 7054^T among the members of the order *Thermotogales* and related bacteria. Bar = evolutionary distance of 0.10.

that its closest relatives were *T. elfii* (level of similarity, 94%), *T. maritima* (level of similarity, 93.7%), and *T. thermarum* (level of similarity, 95.9%). Figure 3 is a dendrogram generated by the neighbor-joining method (15) from the Jukes-Cantor evolutionary similarity matrix (24).

DISCUSSION

Hyperthermophilic members of the *Archaea* (25, 39) and thermophilic members of the *Bacteria* (13, 23, 25, 34, 39) inhabit oil field ecosystems, suggesting that these environments are suitable ecological niches from which new thermophilic and hyperthermophilic anaerobes can be isolated. Studies of these organisms may be useful in the development of microbe-enhanced oil recovery processes (4, 29). In addition, members of the order *Thermotogales* and *Thermoanaerobacter* species, which have the ability to reduce thiosulfate during growth on carbohydrates (14, 36), may be involved in biocorrosion of oil field facilities (8, 10, 12, 16, 27).

Strain SEBR 7054^T is an anaerobic, rod-shaped thermophile with an outer sheathlike structure (toga) similar to that described for the members of the genera *Thermosipho*, *Geotoga*, *Petrotoga*, and *Thermotoga*. However, the outer sheathlike structure is marginally wider than the cell, and therefore strain SEBR 7054^T is not a member of *Fervidobacterium* species, which possess a toga that is six to eight times broader than the cell (1, 20, 32). Strain SEBR 7054^T also differs from *Thermosipho* species, whose cells resemble sausages strung together within a sheathlike structure (19). On the basis of its optimum temperature for growth and sodium chloride requirements, isolate SEBR 7054^T clearly differs from *Petrotoga* and *Geotoga* species (9).

The phylogenetic analysis of 16S rRNAs indicated that strain SEBR 7054^T is a member of the genus *Thermotoga*. However, the sequence of the 16S rRNA gene of strain SEBR 7054^T differs enough (average difference, 5%) from the sequences of the five previously described *Thermotoga* species to warrant inclusion of this organism in the genus as a new species. However, as recently proposed by Vandamme et al. (41), a polyphasic approach in which phylogenetic, genomic, and phenotypic traits are examined should be used to define new species. Strain SEBR 7054^T differs phenotypically from the five previously described *Thermotoga* species in numerous ways (Table 2). In particular, it differs from its closest phylogenetic relative (level of similarity, 95.9%), *T. thermarum*, by having (i) a higher DNA G+C content (50 instead of 40 mol%), (ii) a different temperature range for growth, and (iii) a different substrate range for growth (strain SEBR 7054^T uses D-fructose,

TABLE 1. Fermentation of xylose and glucose in the presence or absence of thiosulfate by strain SEBR 7054^T

Culture conditions[a]	Amt of substrate utilized (mM)	Maximum optical density at 580 nm	Amt of end products formed (mM)					Ratio of acetate produced to sugar consumed	Ratio of L-alanine produced to sugar consumed
			H_2[b]	Ethanol	H_2S	Acetate	L-Alanine		
Xylose	12.9	0.40	19.0	1.0	0.2	8.9	2.4	0.69	0.18
Xylose + thiosulfate	12.0	0.66	1.8	1.0	7.5	13.7	1.3	1.14	0.10
Glucose	7.0	0.35	9.4	1.0	0.2	5.0	1.7	0.71	0.24
Glucose + thiosulfate	13.0	0.92	0.9	1.6	15.1	19.8	1.0	1.52	0.07

[a] Sodium thiosulfate was added at a final concentration of 20 mM. The results shown are the results after 143 h of growth at 70°C. Uninoculated controls did not exhibit H_2S production after 1 week of incubation at 70°C in the presence or absence of thiosulfate.
[b] Amount of H_2 produced in millimolar equivalents.

TABLE 2. Characteristics that differentiate members of the genus *Thermotoga*[a]

Characteristic	*T. hypogea*[b]	*T. elfii*[c]	*T. subterranea*[d]	*T. maritima*[e]	*T. neapolitana*[f]	*T. thermarum*[f]
Type strain	SEBR 7054 (= DSM 11164)	DSM 9442	DSM 9912	DSM 3109	DSM 4359	DSM 5069
Source	African oil well	African oil well	Paris oil well	Submarine thermal vent	Submarine thermal vent	Hot spring
Temp range (°C)	56–90	50–72	50–75	55–90	55–90	55–84
Optimum temp (°C)	70–75	66	70	80	80	70
pH range	6.1–9.1	5.5–8.7	6.0–8.5	5.5–9	5.5–9	5.5–9
Optimum pH	7.3–7.4	7.5	7.0	6.5	7	7
NaCl concn range (%)	0–1.5	0–2.4	0–2.4	0.25–3.75	0.25–6.0	0.2–0.55
Optimum NaCl concn (%)	0–0.2	1.2	1–2	2.7	2.0[g]	0.35
G+C content (mol%)	50	40	40	46	41	40
Reduction of S^0	−	−	−	+	+	−
Reduction of $S_2O_3^{2-}$	+	+	+	+[h]	+[h]	+[b]
Flagella	Lateral	Peritrichous	ND[i]	One subpolar	−	Lateral
Substrates used	bio-Trypticase, yeast extract, fructose, galactose, glucose, lactose, maltose, mannose, sucrose, xylan, xylose	bio-Trypticase, arabinose, fructose, glucose, lactose, maltose, ribose, sucrose, xylose	Glucose, maltose[j]	Yeast extract, galactose, glucose, glycogen, maltose, ribose, starch, sucrose, xylose	Galactose, glucose, glycogen, lactose, maltose, ribose, starch, sucrose, xylose	Yeast extract, glucose, maltose, starch, sucrose, xylose[k]
Diagnostic fermentation product(s) from glucose[l]	Ethanol, L-alanine	L-Alanine[m]	ND	Lactate, L-alanine[m]	Lactate, L-alanine[m]	ND

[a] All *Thermotoga* species are characterized by the presence of outer sheaths, termed togas.
[b] Data from this study.
[c] Data from reference 34.
[d] Data from reference 23.
[e] Data from reference 18.
[f] Data from reference 44.
[g] Data from reference 6.
[h] Data from reference 36.
[i] ND, not determined.
[j] Other substrates were not tested.
[k] Data for sucrose and xylose were determined in this study.
[l] All species that were tested produced acetate, H_2, and CO_2.
[m] Data from reference 35.

DL-lactose, D-mannose, and D-galactose, whereas *T. thermarum* does not) (Table 2).

The ability of *Thermotoga* sp. strain SEBR 7054^T to grow optimally at 70°C reflects its habitat temperature (66°C), but its ability to grow at temperatures up to 90°C is surprising. It is possible that oil fields are physicochemically heterogeneous and that *Thermotoga* sp. strain SEBR 7054^T may grow in hot spots within oil fields.

On the basis of ecological studies (44), *Thermotoga* isolates were separated into two different groups, with *T. maritima* and *T. neapolitana* representing the marine isolates and *T. thermarum* representing terrestrial isolates that are able to grow at low levels of salinity. Strain SEBR 7054^T, together with recent isolates from oil field water (*T. elfii* and *T. subterranea*), represents a third ecological group originating from subsurface ecosystems and adapted to levels of salinity intermediate between those of marine species and those of terrestrial species. In this respect, microbiological studies of subsurface ecosystems improved our knowledge of the ecological distribution of the *Thermotogales*.

Thermotoga sp. strain SEBR 7054^T was similar to *T. elfii* and *T. subterranea* in its ability to reduce thiosulfate but not sulfur to hydrogen sulfide. This adds weight to the hypothesis that thiosulfate may be a more important electron acceptor than sulfur in oil field ecosystems (34). However, this is not an exclusive trait of all oil fields since *Thermotoga* sp. strain SEBR 2665, an isolate from the Paris Basin oil field, can use both thiosulfate and sulfur as electron acceptors (36).

Our results provide evidence that the use of thiosulfate by strain SEBR 7054^T causes a shift in the flow of electrons, favoring H_2S production. This channels the electrons partially away from L-alanine to acetate, thereby increasing the acetate concentration. Such a shift in metabolism in the presence of thiosulfate has been reported previously for *Thermoanaerobacter brockii* grown on glucose or xylose (14). Under these conditions, acetate production increased and there was a concomitant decrease in lactate and ethanol production (14). It was therefore hypothesized that thiosulfate reduction might be an important feature in thermal ecosystems with regard to organic matter oxidation. It is noteworthy that *Thermoanaerobacter* and *Thermotoga* strains are common inhabitants of oil well water (13, 17, 23, 39). Therefore, the presence of thiosulfate could drastically change the fate of organic matter available in such ecosystems.

The production of L-alanine as an end product of glucose fermentation has been reported for *Thermotoga* species, and it has been hypothesized that this is an ancestral metabolism (35). We show for the first time that L-alanine is also produced from xylose fermentation. In addition, *Thermotoga* sp. strain SEBR 7054^T and *T. thermarum* produce ethanol as an end product of glucose fermentation, and this is the first report of this trait in members of *Thermotoga* species. The physiological data provided in this report may be useful for initiating evolutionary studies of enzymes involved in the production of L-alanine and ethanol dehydrogenases. *Thermotoga* sp. strain SEBR 7054^T; *T. maritima*, and *T. neapolitana* have the highest growth temperature (90°C) in the domain *Bacteria*. We provide evidence of xylanolytic activity in *Thermotoga* sp. strain SEBR 7054^T; therefore, it should be useful to compare the thermostabilities of xylanases in our isolate, *T. maritima*, and *T. neapolitana* since thermostability is a desirable property for enzymes in many industrial applications (33, 40).

The G+C content of *Thermotoga* sp. strain SEBR 7054^T (50 mol%) differs substantially from the values reported for the five previously described *Thermotoga* species (40 to 46 mol%).

Isolate SEBR 7054^T is a xylanolytic anaerobe that is sufficiently phenotypically and phylogenetically distinct from the five previously described *Thermotoga* species to be proposed as a member of a new species of the genus *Thermotoga*, *T. hypogea* sp. nov. In addition, in this paper we provide further phenotypic characterization of *T. thermarum* with regard to its substrate range for growth and provide evidence that this organism is able to reduce thiosulfate to sulfide, which indicates that this function is quite widespread in the *Thermotogales*, as previously suggested (36).

Description of *Thermotoga hypogea* sp. nov. *Thermotoga hypogea* (hy. po.ge'a. Gr. pron. *hypos*, under; Gr. n. *ge*, earth; L. fem. adj. *hypogea*, under the earth, referring to the site of isolation). Round colonies (diameter, 1 mm) are present after 7 days of incubation at 70°C. Cells are rods (0.5 to 1 by 2 to 3 μm), and each cell has an outer sheathlike structure (toga). The cells stain gram negative, occur singly or in pairs, and possess laterally inserted flagella. Chemoorganotrophic and obligately anaerobic member of the domain *Bacteria*. The optimum temperature for growth is 70°C at pH 7.0; the temperature range is 56 to 90°C. The optimum pH is 7.3 to 7.4 at 70°C; growth occurs between pH 6.1 and pH 9.1. The optimum NaCl concentration for growth is between 0 and 0.2% at 70°C and pH 7.0; growth occurs in the presence of NaCl concentrations ranging from 0 and 1.5%. Uses D-glucose, DL-fructose, D-galactose, DL-lactose, DL-maltose, D-mannose, D-sucrose, D-xylose, and xylan but not D-arabinose, D-ribose, L-sorbose, L-xylose, acetate, butyrate, lactate, or propionate. Ferments yeast extract and bio-Trypticase. Requires yeast extract or bio-Trypticase for growth on carbohydrates. Yeast extract cannot be replaced by Casamino Acids, a vitamin solution, or a mixture of Casamino Acids and vitamins. The end products of glucose and xylose fermentation are acetate, CO_2, H_2, L-alanine, and traces of ethanol. Uses thiosulfate as an electron acceptor during glucose fermentation, and under these conditions the biomass is greater than the biomass obtained with no added electron acceptor. H_2S is produced from thiosulfate reduction. Elemental sulfur cannot be used as an electron acceptor, and growth is not inhibited by elemental sulfur. The G+C content of the DNA is 50 mol% (as determined by high-performance liquid chromatography). Isolated from an oil-producing well. The type strain is SEBR 7054 (= DSM 11164).

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