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*. We name this new species *Thermotoga hypogea* nov. The type strain of *T. hypogea* is SEBR 7054 (DSM 11164).

**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 10069 was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM).

**Culture media.** Enrichment was performed by using MB medium containing 1 g of NH\(_4\)Cl, 0.3 g of KH\(_2\)PO\(_4\), 0.3 g of KH\(_2\)PO\(_4\), 0.5 g of MgCl\(_2\), 0.1 g of CaCl\(_2\), 10 g of NaCl, 0.2 g of KC, 0.3 g of cysteine-HCl, 2 g of yeast extract (Difco Laboratories, Detroit, Mich.), 2 g of bio-Trypticase (bioMCriew, Marcy l’Etoile, France), 10 g of yeast, 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\(_2\)S \(\cdot\) 9H\(_2\)O, Na\(_2\)CO\(_3\), and thiosulfate were injected from sterile stock solutions to obtain final concentrations of 0.04%, 0.2%, and 20mM, 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 Barois (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 sulfur. The pH of the medium in Hungate tubes was adjusted by injecting NaHCO\(_3\) or Na\(_2\)CO\(_3\) from 10% sterile anaerobic stock solutions. Mineral oil baths were used to obtain incubation temperatures of \(\geq 50°C\). 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% (w/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% (w/vol) glutaraldehyde in HCl-cacodylate buffer (pH 6.0) containing 0.7 M sucrose, and postfixed overnight in 1% (w/vol) osmium tetroxide in 0.75 M cacodylate buffer (pH 7.4) at 4°C.
were used throughout the analytical studies. Growth was measured by inserting tubes directly into a model U1-160A spectrophotometer (Shimadzu Corp., Kyoto, Japan) and measuring the optical density at 580 nm. Sulfide was determined photometrically as colloidial Cs by using the method of Cord-Ruwisch (7). H₂, CO₂, sugars, alcohols, and volatile and nonvolatile fatty acids were measured as described previously (13, 14). CO₂ was measured in MB medium that lacked xylan but contained 20 mM glucose. In this medium, N₂, CO₂, and bicarbonate buffer were replaced by N₂ and phosphate buffer, respectively. L-Alanine was determined enzymatically with an L-alanine dehydrogenase kit (catalog no. A-7633, Sigma). Cell-free supernatant was deproteinized with an equal volume of 1 M sodium perchlorate, and the mixture was centrifuged and neutralized with KHCO₃. 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 xylene 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 a pH of 7.0 and 70°C.

**Determination of G+C content.** The G+C content of DNA was determined at the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturbetrieb 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 7054T 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 H₂S was detected from thioulate reduction. Microscopic examination revealed the presence of rod-shaped bacteria with an outer sheathlike structure characteristic of members of the order Thermotoga. Colonies that were 1 mm in diameter developed in Phytetag roll tubes after 7 days of growth at 70°C. Single colonies were picked, and serial dilution in Phytetag 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 Thermotogae were obtained. One strain, strain SEBR 7054T, was used for further characterization.

**Morphology.** Strain SEBR 7054T was a rod-shaped bacterium. The cells were 0.5 to 1 by 1.5 to 4 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 7054T revealed a typical toga cell wall ultrastructure with a spongy periplasm layer (Fig. 1d). The cells stained gram negative.

**Optimum growth conditions.** Strain SEBR 7054T 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 (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 7054T. Yeast extract could not be replaced by Casamino Acids (1 g/liter), vitamins (42), or a mixture of Casamino Acids and vitamins. Strain SEBR 7054T 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-xylanose, and xylan (10 g/liter). It could not utilize d-arabinose, d-ribose, l-sorbos, l-xyllose, and fatty acids (acetate, butyrate, lactate, and propionate). Acetate, l-alanine, CO₂, H₂, and traces of ethanol were produced during xylose fermentation irrespective of the presence of thioulate (Table 1); a similar profile of metabolites was produced by glucose fermentation. In all cases, about 1 mol of CO₂ 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, dl-maltose, d-mannose, d-arabinose, and l-sorbos. T. thermaurum also produced traces of ethanol during glucose fermentation.

**Effect of added electron acceptors.** Strain SEBR 7054T reduced thioulate but not sulfate or sulfur to sulfide. The presence of thioulate 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 thioulate modified the metabolic pathways of strain SEBR 7054T. The changes observed were as follows: (i) thioulate was reduced to sulfide with a concomitant decrease in H₂ to barely detectable levels; and (ii) the ratio of acetate produced to sugar consumed increased in the presence of thioulate, whereas the ratio of l-alanine produced to sugar consumed decreased (Table 1). T. thermarum also reduced thioulate to sulfide.

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

**Xylanase production in batch culture.** Strain SEBR 7054T 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 7054T was 50 mol%.

**16S rRNA sequence analysis.** Using 12 primers, we determined 1,497 bases of the 16S rRNA gene sequence of strain SEBR 7054T. A phylogenetic analysis revealed that strain SEBR 7054T was a member of the order Thermotogae and
FIG. 1. (a) Phase-contrast micrograph of strain SEBR 7054T, showing the toga (arrows), a characteristic structure of Thermotoga species. Bar = 10 μm. (b) Electron micrograph of a negatively stained culture of strain SEBR 7054T, showing laterally inserted flagella. Bar = 2 μm. (c) Electron micrograph of a thin section of strain SEBR 7054T, 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 7054T, 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.
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 *Thermoaerobacter* 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).

**TABLE 1. Fermentation of xylose and glucose in the presence or absence of thiosulfate by strain SEBR 7054<sup>T</sup>**

<table>
<thead>
<tr>
<th>Culture conditions&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Amt of substrate utilized (mM)</th>
<th>Maximum optical density at 580 nm</th>
<th>Amt of end products formed (mM)</th>
<th>Ratio of acetate produced to sugar consumed</th>
<th>Ratio of t-alanine produced to sugar consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylose</td>
<td>12.9</td>
<td>0.40</td>
<td>19.0</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Xylose + thiosulfate</td>
<td>12.0</td>
<td>0.66</td>
<td>1.8</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>7.0</td>
<td>0.35</td>
<td>9.4</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Glucose + thiosulfate</td>
<td>13.0</td>
<td>0.92</td>
<td>0.9</td>
<td>1.6</td>
<td>15.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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<sub>2</sub>S production after 1 week of incubation at 70°C in the presence or absence of thiosulfate.

<sup>b</sup> Amount of H<sub>2</sub> produced in millimolar equivalents.

Strain SEBR 7054<sup>T</sup> is an anaerobic, rod-shaped thermophile with an outer sheathlike structure (toga) similar to that described for the members of the genera *Thermosiphio*, *Petrotoga*, and *Thermotoga*. However, the outer sheathlike structure is marginally wider than the cell, and therefore strain SEBR 7054<sup>T</sup> 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<sup>T</sup> also differs from *Thermosiphio* 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<sup>T</sup> clearly differs from *Petrotoga* and *Geotoga* species (9).

The phylogenetic analysis of 16S rRNAs indicated that strain SEBR 7054<sup>T</sup> is a member of the genus *Thermotoga*. However, the sequence of the 16S rRNA gene of strain SEBR 7054<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> uses D-fructose,
TABLE 2. Characteristics that differentiate members of the genus Thermotoga

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type strain</strong></td>
<td>DSM 11164</td>
<td></td>
<td>DSM 9442</td>
<td>DSM 3109</td>
<td>DSM 4359</td>
<td>DSM 5069</td>
<td>DSM 4559</td>
<td>DSM 1016</td>
<td>DSM 9912</td>
<td>DSM 3109</td>
<td>DSM 4359</td>
<td>DSM 5069</td>
</tr>
<tr>
<td><strong>Source</strong></td>
<td>African oil well</td>
<td></td>
<td>African oil well</td>
<td>Submarine thermal vent</td>
<td>Submarine thermal vent</td>
<td>Submarine thermal vent</td>
<td>Paris oil well</td>
<td>African oil well</td>
<td>Submarine thermal vent</td>
<td>Submarine thermal vent</td>
<td>Submarine thermal vent</td>
<td>Hot spring</td>
</tr>
<tr>
<td><strong>Temp range (°C)</strong></td>
<td>50-72</td>
<td>50-75</td>
<td>55-80</td>
<td>55-90</td>
<td>55-80</td>
<td>55-90</td>
<td>55-90</td>
<td>55-90</td>
<td>55-90</td>
<td>55-90</td>
<td>55-90</td>
<td>55-90</td>
</tr>
<tr>
<td><strong>Optimum temp (°C)</strong></td>
<td>66</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td><strong>pH range</strong></td>
<td>5-6.5</td>
<td>5.5-8.5</td>
<td>5.5-8.5</td>
<td>5.5-8.5</td>
<td>5.5-8.5</td>
<td>5.5-8.5</td>
<td>5.5-8.5</td>
<td>5.5-8.5</td>
<td>5.5-8.5</td>
<td>5.5-8.5</td>
<td>5.5-8.5</td>
<td>5.5-8.5</td>
</tr>
<tr>
<td><strong>Optimum pH</strong></td>
<td>6.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td><strong>NaCl concn (%)</strong></td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
</tr>
<tr>
<td><strong>Optimum NaCl concn (%)</strong></td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
<td>0-2.4</td>
</tr>
<tr>
<td><strong>G+C content (mol%)</strong></td>
<td>50</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td><strong>Reduction of</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>So</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>S,03'</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Flagella</strong></td>
<td>Lateral</td>
<td>Peritrichous</td>
<td>N/a</td>
<td>N/a</td>
<td>N/a</td>
<td>N/a</td>
<td>Lateral</td>
<td>Peritrichous</td>
<td>N/a</td>
<td>N/a</td>
<td>N/a</td>
<td>N/a</td>
</tr>
<tr>
<td><strong>Substrates used</strong></td>
<td>bio-Trypticase, arabinose, fructose, glucose, lactose, maltose, ribose, sucrose, xylose</td>
<td>bio-Trypticase, arabinose, fructose, glucose, lactose, maltose, ribose, sucrose, xylose</td>
<td>bio-Trypticase, arabinose, fructose, glucose, lactose, maltose, ribose, sucrose, xylose</td>
<td>bio-Trypticase, arabinose, fructose, glucose, lactose, maltose, ribose, sucrose, xylose</td>
<td>bio-Trypticase, arabinose, fructose, glucose, lactose, maltose, ribose, sucrose, xylose</td>
<td>bio-Trypticase, arabinose, fructose, glucose, lactose, maltose, ribose, sucrose, xylose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Diagnostic fermentation product(s) from glucose</strong></td>
<td>Ethanol, L-alanine</td>
<td>Ethanol, L-alanine</td>
<td>Ethanol, L-alanine</td>
<td>Ethanol, L-alanine</td>
<td>Ethanol, L-alanine</td>
<td>Ethanol, L-alanine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND, not determined.

*All Thermotoga species are characterized by the presence of outer sheaths, termed togas.

Data from this study.

Data from reference 34.

Data from reference 23.

Data from reference 18.

Data from reference 44.

Data from reference 6.

Data from reference 36.

J.

Other substrates were not tested.

Data for sucrose and xylose were determined in this study.

*All species that were tested produced acetate, H2, and CO2.

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

The ability of *Thermotoga* sp. strain SEBR 7054T 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 7054T 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 7054T, 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 7054T 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 7054T causes a shift in the flow of electrons, favoring H₂S 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 *Thermoaerobacter 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 *Thermoaerobacter* 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 7054T 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 7054T, *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 7054T, and 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 7054T (50 mol%) differs substantially from the values reported for the five previously described *Thermotoga* species (40 to 46 mol%). Isolate SEBR 7054T 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. hypogeo, under; Gr. n. ge, earth; L. fem. adj. hypogeo, 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, D-l-fructose, D-galactose, D-lactose, D-maltose, D-mannose, D-sucrose, D-syrulose, and xylan but not D-arabinose, D-ribose, L-sorbose, L-xylene, 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, CO₂, H₂, 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. H₂S 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).

**Acknowledgments**

The financial assistance provided to B.K.C.P. by the Australian Research Council is gratefully acknowledged.

We thank J. L. Crolet for suggesting the species name, P. A. Roger for revising the manuscript, and J. L. Coyer for helpful discussions.

**References**


