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Thermotoga elfii sp. nov., a Novel Thermophilic Bacterium from an African Oil-Producing Well

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A thermophilic, glucose-fermenting, strictly anaerobic, rod-shaped bacterium, strain SEBR 6459^T (T = type strain), was isolated from an African oil-producing well. This organism was identified as a member of the genus *Thermotoga* on the basis of the presence of the typical outer sheath-like structure (toga) and 16S rRNA signature sequences and its ability to grow on carbohydrates (glucose, arabinose, fructose, lactose, maltose, and xylose). Major differences in its 16S rRNA gene sequence, its lower optimum temperature for growth (66°C), its sodium chloride range for growth (0 to 2.8%), its lack of lactate as an end product from glucose fermentation, and its peritrichous flagella indicate that strain SEBR 6459^T is not similar to the three previously described *Thermotoga* species. Furthermore, this organism does not belong to any of the other genera related to the order *Thermotogales* that have been described. On the basis of these findings, we propose that this strain should be described as a new species, *Thermotoga elfii*. The type strain of *T. elfii* is SEBR 6459 (= DSM 9442).

The order *Thermotogales* currently comprises three genera, *Thermotoga* (17), *Thermosipho* (18), and *Fervidobacterium* (19, 35). All of the species belonging to these genera are rod shaped and have a characteristic outer sheath-like structure which can be observed under in situ conditions (34). Members of the recently described genera *Geotoga* and *Petrotoga* (9) also possess this morphological feature and, as determined by a 16S rRNA sequence analysis, are distantly related to members of the *Thermotogales*. Collectively, the five genera mentioned above represent one of the deepest phylogenetic branches in the domain *Bacteria* (42). These taxa can be differentiated on the basis of their optimum temperatures for growth; *Thermotoga* species are extreme thermophiles that have optimum temperatures for growth of around 80°C (17, 20, 21, 41), *Thermosipho* and *Fervidobacterium* species have optimum temperatures for growth of 65 to 75°C (18, 19, 35) and are regarded as thermophiles, and *Geotoga* and *Petrotoga* species are moderate thermophiles having optimum temperatures for growth of less than 60°C (9). Until recently, members of the three genera belonging to the order *Thermotogales* (*Thermotoga*, *Thermosipho*, and *Fervidobacterium*) have been isolated only from volcanic aquatic environments. Different species have different sodium chloride requirements and optimum temperatures for growth. These differences reflect the restricted ecological habitats (hydrothermal marine environments, hydrothermal terrestrial environments) from which the organisms were isolated. However, *Thermotoga* species capable of growth at 85°C have recently been isolated from oil wells in the North Sea (39). Davey et al. (9) have also described the isolation of members of two new genera of moderate thermoanaerobes, the genera *Petrotoga* and *Geotoga*, from oil fields. These reports have extended our knowledge concerning the ecological habitats of these bacteria. In this paper, we describe the results of an investigation of a new thermophilic *Thermotoga* strain isolated from an African oil field and discuss the diversity of similar strains in such environments.

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MATERIALS AND METHODS

Sample collection and sample source. Strain SEBR 6459^T (T = type strain) was isolated from an oil-producing well in Africa. The in situ temperature was 68°C, and the concentration of sodium chloride was 12 g/liter. A 1-liter sample was collected at the wellhead as described elsewhere (5). In the laboratory, portions of this sample were inoculated onto culture media.

Enrichment, isolation, and growth conditions. Basal medium MB was used for all studies; this basal medium was modified when necessary, as discussed below. Basal medium MB contained (per liter) 1 g of NH_4Cl, 0.3 g of K_2HPO_4, 0.3 g of KH_2PO_4, 0.2 g of $MgCl_2 \cdot 6\ H_2O$, 0.1 g of $CaCl_2 \cdot 2H_2O$, 10 g of NaCl, 0.1 g of KCl, 0.5 g of cysteine-HCl, 0.5 g of sodium acetate (anhydrous), 2 g of yeast extract (Difco Laboratories, Detroit, Mich.), 2 g of bio-Trypticase (bioMérieux, Graponne, France), 10 ml of the trace mineral element solution of Balch et al. (3), 1 mg of resazurin, and 1,000 ml of distilled water. The pH was adjusted to 8.0 with 10 M KOH. The medium was boiled under a stream of O_2-free N_2 gas and cooled to room temperature. Then 5-ml portions of the medium were distributed into Hungate tubes under a stream of N_2-CO_2 (80:20), and the tubes were autoclaved for 45 min at 110°C. Just prior to inoculation, 0.1 ml of $Na_2S \cdot 9H_2O$ (from a 2% sterile anaerobic stock solution) and 0.1 ml of Na_2CO_3 (from a sterile 10% anaerobic stock solution) were injected into each tube.

For enrichment cultures and isolation, basal medium MB was modified as follows. The medium was dispensed into 20-ml serum vials under a stream of H_2-CO_2 (80:20; 2×10^5 Pa), and thiosulfate (final concentration, 20 mM) was added as an electron acceptor. An enrichment culture was initiated by injecting 2 ml of a sample, and the preparation was incubated without shaking at 60°C. An isolate was purified by repeatedly performing the agar shake dilution technique in the same medium containing 2% Noble agar (Difco), as described previously (33). The purity of each culture was checked by microscopic examination.

pH, temperature, and sodium chloride ranges for growth. For growth studies, the yeast extract and bio-Trypticase concentrations in the basal medium were changed to 1 and 5 g/liter, respectively. For pH studies, the pH values of media in prereduced anaerobic tubes were adjusted with $NaHCO_3$ or Na_2CO_3 (10% sterile anaerobic stock solutions). For temperature studies, mineral oil baths were used when incubation temperatures were greater than 50°C and water baths were used when the incubation temperatures were less than 50°C. For growth

studies in which different salt concentrations were used, sodium chloride was weighed directly in the tubes to give the desired concentrations when concentrations higher than 1% were used, and the medium was dispensed into the tubes as described above. When sodium chloride concentrations less than 1% were used, different amounts from a 10% NaCl stock solution were injected into the prereduced medium to give the desired concentrations. The strain was subcultured at least once under the same experimental conditions prior to inoculation.

Substrate utilization tests. Tests to determine utilization of substrates were performed in the medium described above containing thiosulfate (20 mM), 1 g of yeast extract per liter, and 1 g of bio-Trypticase per liter. Substrates were added from sterile anaerobic stock solutions. The electron acceptors thiosulfate, sulfate, and elemental sulfur were added to the medium when they were required at concentrations of 20 mM, 20 mM, and 2%, respectively.

Light and electron microscopy. Cells were observed with a Nikon microscope to determine purity, morphology, and the Gram reaction. Phase-contrast microscopy was used when cells were observed unstained. Cells were negatively stained with uranyl acetate (4% [wt/vol] in distilled water). For thin-section electron microscopy, exponentially growing cells were fixed for 1 h in 0.07 M sodium cacodylate buffer (pH 7.3) containing 1.2% glutaraldehyde and 0.05% ruthenium red and then in 0.07 M cacodylate buffer (pH 7.3) containing 1% (wt/vol) osmium tetraoxide. The samples were then embedded in Epon, dehydrated in an ethanol series, and sectioned, and the sections were stained with 2% uranyl acetate in 50% ethanol and then with 2% lead citrate. The sections were observed with a JEOL model 1200CX electron microscope at an accelerating voltage of 80 kV.

Analytical techniques. Unless indicated otherwise, duplicate culture tubes were used throughout this study. 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 contents were determined photometrically as collodial CuS by using the method of Cord-Ruwisch (7). Contents of H_2 and fermentation products (alcohols and volatile and nonvolatile fatty acids) were measured as described previously (14).

Determination of G+C content. The G+C content of DNA was determined by workers at DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. The DNA was isolated and purified by chromatography on hydroxyapatite. The G+C content was determined by using high-performance liquid chromatography (HPLC) as described by Mesbah et al. (31). Nonmethylated lambda DNA (Sigma) was used as the standard.

16S rRNA sequence studies. Semipurified DNA was extracted for amplification of the 16S rRNA gene by 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 pipetted into a microcentrifuge tube. The suspension was microwaved on high for four cycles, each consisting of 15 s with the heat on and 5 s with the heat off with an open lid. 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 (27, 37). 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 (37).

The 16S ribosomal DNA sequence obtained from the sequencing data was aligned by using sequence editor ae2 with the sequences of various members of the bacterial phylum; the latter 16S rRNA sequences were obtained from the Ribosomal Database Project (version 4.0) (26). Positions of sequence and alignment uncertainty were omitted from the analysis, and pairwise evolutionary distances based on 1,170 unambiguous nucleotides were computed by using the method of Jukes and Cantor (24). 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 (25), were also used in the analysis. All of the programs except MEGA were run on a Sun Sparc workstation; MEGA was run on a 386 Toshiba model T3100SX laptop IBM-compatible computer.

Nucleotide sequence accession number. The 16S rRNA sequence of strain SEBR 6459^T determined in this study has been deposited in the EMBL database under accession number X80790.

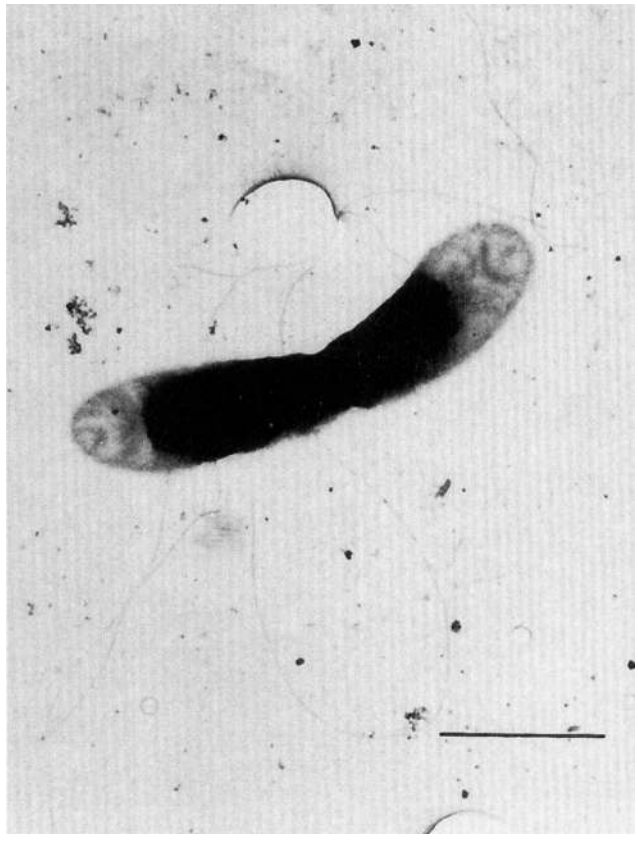

FIG. 1. Electron micrograph of a negatively stained cell of SEBR 6459^T showing the typical outer sheath-like structure of the genus *Thermotoga*. Bar = 1 μm.

RESULTS

Enrichment and isolation. Enrichment cultures were positive after incubation at 60°C for 3 days. H_2S was also produced from thiosulfate reduction. Microscopic examination revealed the presence of rod-shaped bacteria having an outer sheath-like structure characteristic of members of the *Thermotogales* (Fig. 1). Enrichment cultures were serially diluted and inoculated into agar shake tubes. Colonies that were 1 mm in diameter were observed after 7 days of growth at 60°C. Single colonies were picked, and serial dilution in agar shake tubes was repeated at least twice before the culture was considered pure. We obtained several axenic cultures that had the typical outer polar sheath-like structures found in members of the *Thermotogales*. A strain designated SEBR 6459^T was used for further characterization.

Morphology. Strain SEBR 6459^T was a strictly anaerobic, rod-shaped organism. The cells were 0.5 to 1 by 2 to 3 μm and occurred singly or in pairs. Strain SEBR 6459^T was gram negative and had a typical gram-negative cell wall ultrastructure, as determined by observing thin sections with an electron microscope (Fig. 2). Each cell was surrounded by a characteristic toga, a sheath-like structure ballooning over the ends (Fig. 1), and had peritrichous flagella.

Optimum growth conditions. Strain SEBR 6459^T grew at temperatures ranging from 50 to 72°C; optimum growth occurred at 66°C. No growth was observed at 45 and 75°C. This

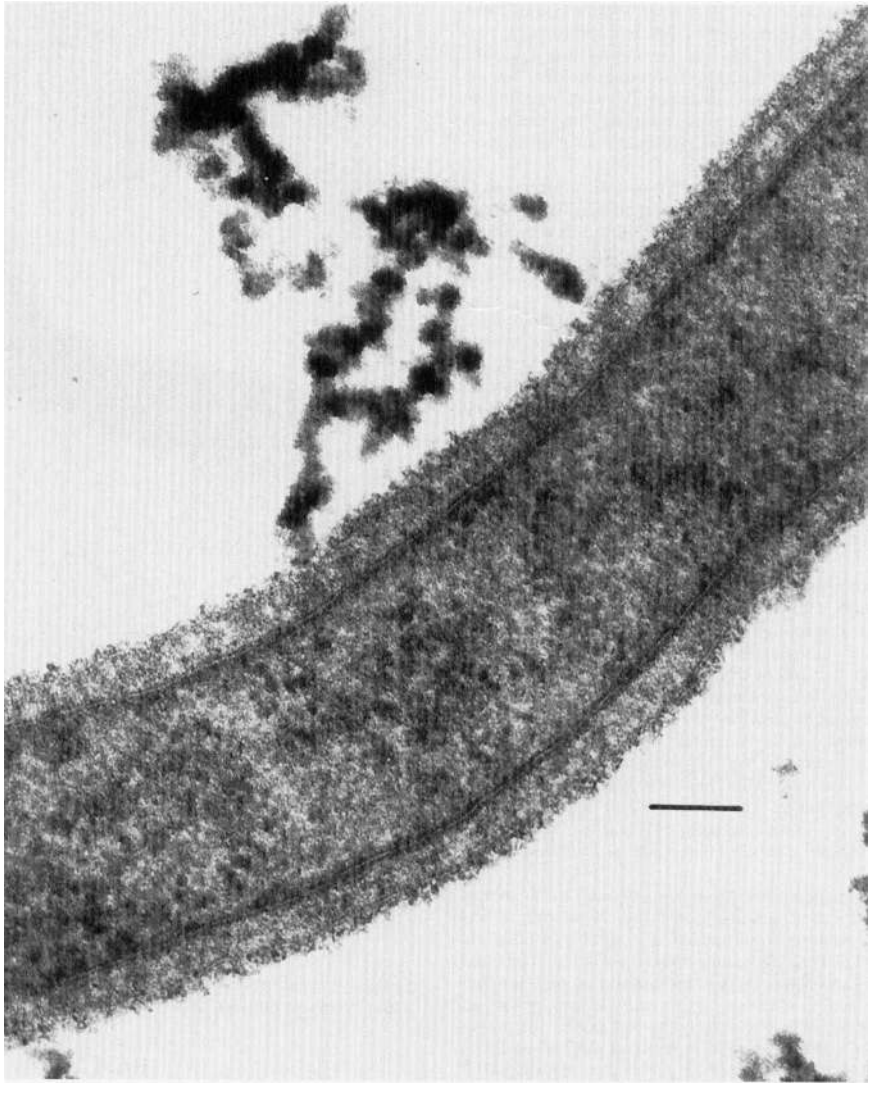

FIG. 2. Thin section of isolate SEBR 6459^T showing a typical gram-negative cell wall ultrastructure. Bar = 0.1 μm.

organism grew in the presence of sodium chloride concentrations ranging from 0 to 2.8%, and optimum growth occurred in the presence of approximately 1% NaCl. The optimum pH for growth was 7.5, and growth occurred at pH 5.5 but not at pH 9.1. When the temperature, pH, and sodium chloride concentration were optimal, the generation time was 2.8 h.

Substrates used for growth. In the presence of thiosulfate, strain SEBR 6459^T was nutritionally versatile and was able to oxidize D-glucose, D-arabinose, D-fructose, lactose, maltose, D-mannose, D-ribose, sucrose, and D-xylose but not L-arabitol, D-mannitol, L-rhamnose, L-sorbose, L-xylose, or fatty acids (acetate, butyrate, lactate, and propionate). Yeast extract (0.1%) and bio-Trypticase (0.1%) were required for growth. Yeast extract could not be replaced by Casamino Acids, vitamins (40), or a mixture of Casamino Acids and vitamins. During the fermentation of glucose in the absence of thiosulfate, acetate, CO_2, and H_2 were produced. The isolate was also able to ferment bio-Trypticase in the presence of yeast extract.

Effects of added electron acceptors. The ability of strain SEBR 6459^T to use electron acceptors, including sulfate, thiosulfate, and elemental sulfur, was determined by using a medium containing 1 g of yeast extract per liter and 5 g of bio-Trypticase per liter. Strain SEBR 6459^T used only thiosulfate as an electron acceptor (Fig. 3). Furthermore, elemental sulfur completely inhibited growth of this organism (Fig. 3). When thiosulfate was added to a medium containing glucose, growth of the isolate increased twofold (Fig. 4). Under these conditions, utilization of glucose was significantly higher than utilization of glucose in the absence of thiosulfate (Table 1). In the presence or absence of thiosulfate, the only fatty acid produced during glucose fermentation was acetate (Table 1).

G+C content. The G+C content of isolate SEBR 6459^T was 39.6 mol%.

16S rRNA sequence analysis. Using eight primers, we determined an almost complete sequence consisting of 1,519 bases for the 16S rRNA gene of strain SEBR 6459^T. This sequence (positions 36 to 1,540; *Escherichia coli* numbering of Winker and Woese [42]) 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 6459^T was a member of the order *Thermotogales*. Additional sequence alignments and phylogenetic analyses performed with members of this order indicated that the closest relatives of strain SEBR 6459^T were *Thermo-*

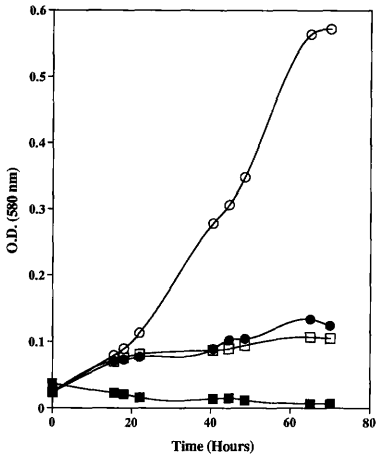

FIG. 3. Effects of different electron acceptors on the growth of strain SEBR 6459^T. Symbols: ●, control; □, sulfate (20 mM); ○, thiosulfate (20 mM); ■, elemental sulfur (2%). The medium contained 1 g of yeast extract per liter and 5 g of bio-Trypticase per liter. O.D. (580 nm), optical density at 580 nm.

toga maritima and *Thermotoga thermarum* (average level of similarity, 92%) and *Thermosipho africanus* (level of similarity, 90%). Strain SEBR 6459^T was only distantly related to other members of the *Thermotogales* and related bacteria, including *Fervidobacterium* sp. (average level of similarity, 86%), *Petrotoga miotherma*, *Geotoga subterranea*, and *Geotoga petraea* (average level of similarity, 79%) (Table 2). Figure 5 is a dendrogram generated by the neighbor-joining method (15) from a Jukes-Cantor evolutionary similarity matrix (24) (Table 2) and shows this relationship.

Strain SEBR 6459^T is related to *Thermotoga thermarum* and *Thermotoga maritima* as shown by identical structural idiosyncrasies (represented by a relatively long helix at positions 184 to 193, 450 to 480, and 1438 to 1463) but is distinctly different from *Thermosipho*, *Fervidobacterium*, *Geotoga*, and *Petrotoga* species. Although strain SEBR 6459^T, *Thermotoga thermarum*, and *Thermotoga maritima* all have identical structural idiosyncrasies in these regions, their sequences are different.

In addition, our parsimony analysis produced the same tree

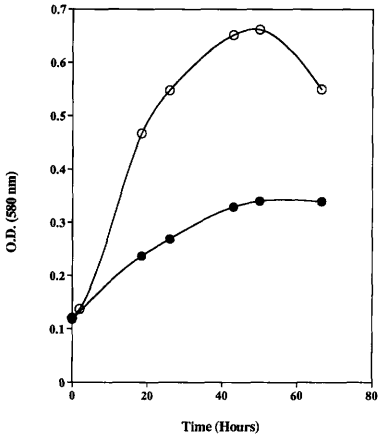

FIG. 4. Effect of thiosulfate on the growth of strain SEBR 6459^T cultivated in basal medium MB containing 20 mM glucose, 1 g of yeast extract per liter, and 1 g of bio-Trypticase per liter. Symbols: ●, no thiosulfate present; ○, 20 mM thiosulfate present. O.D. (580 nm), optical density at 580 nm.

TABLE 1. End products formed during glucose fermentation by *Thermotoga elfii* in the absence and presence of thiosulfate[a]

Growth conditions	Amt of substrate used (mM)	Amt of acetate produced (mM)	Amt of H_2 produced (mM)	Amt of H_2S produced (mM)
Glucose	3.1	4.0	8.8	0
Glucose + thiosulfate	10.4	17.9	2.0	23.0

[a] Basal medium MB contained 1 g of yeast extract per liter, 1 g of bio-Trypticase per liter, and 20 mM glucose. The amounts of volatile fatty acids, H_2, and H_2S produced in the same medium in the absence of glucose were subtracted when we calculated the amounts of products formed during glucose fermentation.

topology, but our bootstrap analysis revealed a low level of relatedness (60%). The level of relatedness might be increased by isolating and sequencing the 16S rRNA genes of more *Thermotoga* species, a task which is currently being undertaken in our laboratories.

DISCUSSION

Oil fields represent a novel environment with respect to their physicochemical conditions (halophilic, thermophilic, mesophilic) and therefore provide an exciting source from which taxonomically, physiologically, and phylogenetically unusual microbes can be isolated. Some of these isolates may be useful in microbe-enhanced oil recovery processes (6, 30). The new thermophilic, anaerobic, heterotrophic microbe isolate SEBR 6459^T from an African oil field extends the physiological and taxonomic diversity of thermophiles that have been obtained from deep subsurface environments. Previous microbiological studies of such environments have led to the identification of several thermophilic microbes, including anaerobic fermenters (14, 36), homoacetogens (11), sulfate-reducing bacteria (1), methanogens (4, 10, 32), and sulfur-reducing anaerobes (39), but not any isolate similar to the organism described in this paper.

It is thought that oil pipeline biofouling and corrosion are microbially mediated, and sulfate-reducing bacteria have been implicated as the major causative agents (12, 13, 16). Stetter et al. (39) have demonstrated that numerous sulfur-reducing hyperthermophilic bacteria which do not use sulfate as an electron acceptor are present in oil fields. The isolation from an oil field of strain SEBR 6459^T, an organism that can utilize thio-

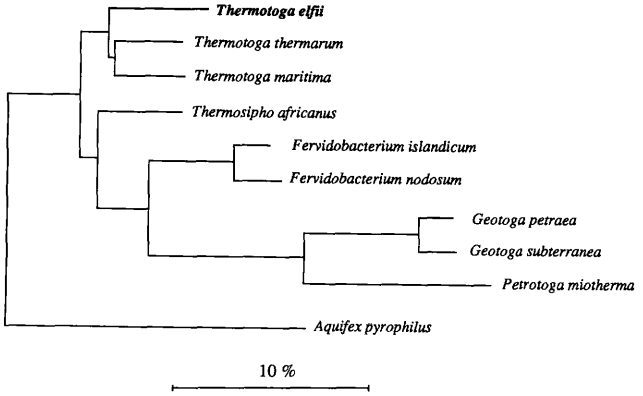

FIG. 5. Phylogenetic position of *Thermotoga elfii* within the *Thermotogales*. Bar = evolutionary distance of 10%.

TABLE 2. Evolutionary similarity matrix for members of the *Thermotogales* determined by using the method of Jukes and Cantor[a]

Species	% Similarity								
	Thermotoga elfii	*Thermotoga thermarum*	*Thermotoga maritima*	*Thermosipho africanus*	*Fervidobacterium nodosum*	*Fervidobacterium islandicum*	*Petrotoga miotherma*	*Petrotoga subterranea*	*Geotoga petraea*
Thermotoga thermarum	92.6								
Thermotoga maritima	91.9	93.8							
Thermosipho africanus	90.3	91.5	90.9						
Fervidobacterium nodosum	85.6	86.7	87.0	88.3					
Fervidobacterium islandicum	86.5	87.4	87.3	89.5	96.1				
Petrotoga miotherma	78.5	77.8	78.8	79.6	80.8	80.5			
Petrotoga subterranea	79.7	79.9	80.8	81.1	81.9	81.4	85.0		
Geotoga petraea	79.8	79.8	80.7	80.9	82.0	81.5	85.5	96.6	
Aquifex pyrophilus	78.7	80.3	81.4	77.8	77.3	76.7	71.9	73.2	73.0

[a] See Materials and Methods. The sequences used in this analysis were obtained from the Ribosomal Database Project, version 4.0 (26). Only 1,170 unambiguous nucleotide positions were used in this analysis.

sulfate but not sulfate or elemental sulfur as an electron acceptor and produces H_2S, raises some interesting questions about the involvement of such thermophilic sulfide producers in the process of biocorrosion in oil fields. Indeed, it has been recently hypothesized that thiosulfate, which results from chemical oxidation of sulfide (22, 23, 29), could increase the risk of corrosion (8, 28).

Isolate SEBR 6459^T is an anaerobic, rod-shaped thermophile with an outer sheath-like structure that is characteristic of genera belonging to the order *Thermotogales*. This sheath-like flattened structure is marginally wider than the diameter of the cell and is referred to as a toga. The toga is a common feature of members of the genera *Thermosipho*, *Geotoga*, *Petrotoga*, and *Thermotoga* and is distinctly different from the spheroids of *Fervidobacterium* species (19, 35); the latter are spherical and at least six to eight times the diameter of the cell. On the basis of morphological characteristics, isolate SEBR 6459^T is not a member of the genus *Fervidobacterium* (19, 35). Strain SEBR 6459^T oxidizes carbohydrates and is therefore physiologically different from *Thermosipho* species (18). Our results indicate that the presence of thiosulfate not only improves glucose oxidation, but also increases the ratio of amount of acetate produced to amount of glucose consumed. On the basis of its optimum temperature for growth and sodium chloride requirements, isolate SEBR 6459^T is clearly different from *Petrotoga* and *Geotoga* species (9). The major evidence that strain SEBR 6459^T is different from all other members of the order *Thermotogales* except *Thermotoga neapolitana*, *Thermotoga maritima*, and *Thermotoga thermarum* comes from a phylogenetic analysis of 16S rRNAs. In contrast to *Thermotoga neapolitana* and *Thermotoga maritima*, both of which grow at hyperthermophilic temperatures and have an optimum temperature for growth of around 80°C (17, 21), and *Thermotoga thermarum*, which also grows at 80°C but has a lower optimum temperature for growth (70°C) (41), strain SEBR 6459^T does not grow at 80°C and has an optimum temperature for growth of 66°C.

Thermotoga neapolitana and *Thermotoga maritima* grow in the presence of 0.25 to 6% NaCl, whereas *Thermotoga thermarum* grows in the presence of 0.2 to 0.55% NaCl; these values reflect the marine and continental habitats of these organisms, respectively. Strain SEBR 6459^T has an NaCl range for growth of 0 to 2.8% and an optimum NaCl concentration for growth of around 1%, values which fall between the values obtained for *Thermotoga neapolitana* and *Thermotoga maritima* and the values obtained for *Thermotoga thermarum*. These concentrations correspond to the in situ concentrations found in the oil field. In addition, the optimum temperature for growth is also close to the temperature found in situ.

Clearly, isolate SEBR 6459^T is phenotypically distinct from other *Thermotoga* species (Table 3). The sequence of the 16S rRNA gene of SEBR 6459^T also differs significantly (8%) from the sequences of the previously described members of the genus *Thermotoga*. On the basis of the data described above, a new species is warranted (2, 38). Thus, we propose that strain SEBR 6459^T should be placed in a new species of the genus *Thermotoga*, *Thermotoga elfii*.

Description of *Thermotoga elfii* sp. nov. *Thermotoga elfii* (el.fi'i. L. gen. n. *elfii*, named after Elf-Aquitaine). Round colonies (diameter, 1 mm) are present after 7 days of incubation at 60°C. Cells are rods (0.5 to 1 by 2 to 3 μm), and each cell has an outer sheath-like structure (toga). The cells occur singly or in pairs and have peritrichous flagella. The cell wall is gram negative, as determined by electron microscopy or Gram staining. Chemoorganotrophic and obligately anaerobic members of the domain *Bacteria*. The optimum temperature for growth is 66°C. No growth occurs at 45 or 75°C. The optimum pH is 7.5; growth occurs at pH 5.5 but not at pH 9.1. The optimum sodium chloride concentration for growth is around 1%; only marginal growth occurs in the absence of sodium chloride, and no growth occurs in the presence of 2.8% NaCl. Oxidizes D-glucose, D-arabinose, D-fructose, lactose, maltose, D-mannose, D-ribose, sucrose, and D-xylose in the presence of thiosulfate but not L-arabitol, D-mannitol, L-rhamnose, L-sorbose, L-xylose, acetate, butyrate, lactate, or propionate. Requires yeast extract and bio-Trypticase for growth. Yeast extract cannot be replaced by Casamino Acids, a vitamin solution, or a mixture of Casamino Acids and vitamins. Bio-Trypticase can be fermented in the presence of yeast extract. The end products of glucose fermentation in the presence of yeast extract and bio-Trypticase are acetate, CO_2, and hydrogen. Uses thiosulfate as an electron acceptor during glucose fermentation, and under these conditions the cell density is greater than the cell density when the organism is grown with no added electron acceptor. H_2S is produced from thiosulfate reduction. Elemental sulfur cannot be used as an electron acceptor, and growth is inhibited by elemental sulfur. The G+C content of the DNA is 39.6 mol% (as determined by

TABLE 3. Characteristics that differentiate members of the *Thermotogales*

Species	Type strain	Reference	Outer sheath	Source	Temp range (°C)	Optimum temp (°C)	pH range	Optimum pH	NaCl concn range (%)	Optimum NaCl concn (%)	Generation time (h)	G+C content (mol%)	Reduction of S^0	Flagella	Substrates	Metabolites from glucose fermentation
Thermotoga elfii	DSM 9442	This study	Toga	Oil well	50–72	66	5.5–8.7	7.5	0–2.4	1.2	2.8	39.6	−	Peritrichous	Arabinose, bio-Trypticase, fructose, glucose, lactose, maltose, ribose, sucrose, xylose	Acetate, CO_2, H_2
Thermotoga maritima	DSM 3109	17	Toga	Geothermal heated sea floor	55–90	80	5.5–9	6.5	0.25–3.75	2.7	1.25	46	+	One, subpolar	Galactose, glucose, glycogen, maltose, ribose, starch, sucrose, xylose, yeast extract	L-Lactate, acetate, CO_2, H_2[a]
Thermotoga neapolitana	DSM 4359	21	Toga	Submarine thermal vent	55–90	80	5.5–9	7	ND[b]	ND	0.75	41	+	−	Galactose, glucose, glycogen, lactose, maltose, ribose, starch, sucrose, xylose	ND
Thermotoga thermarum	DSM 5069	41	Toga	Solfataric spring	55–84	70	5.5–9	7	0.2–0.55	0.35	1.25	40	−	Lateral	Glucose, maltose, starch, yeast extract	ND
Petrotoga miotherma	ATCC 51224	9	Toga	Oil well	35–65	55	5.5–9	6.5	0.5–10	3	7	40	+	−	Galactose, glucose, lactose, maltodextrins, maltose, mannose, starch, sucrose, xylose	Acetate, ethanol, CO_2, H_2
Geotoga subterranea	ATCC 51225	9	Toga	Oil well	30–60	45	5.5–9	6.5	0.5–10	4	14	30	+	ND	Galactose, glucose, lactose, maltodextrins, maltose, mannose, starch, sucrose	Acetate, ethanol, CO_2, H_2
Fervidobacterium nodosum	ATCC 35602	35	Spheroid	New Zealand hot spring	41–79	70	6–8	7	Low	0.1	1.75	33.7	ND	ND	Arabinose, fructose, galactose, glucose, glycerol, lactose, maltose, mannose, pectin, raffinose, sorbitol, sucrose	Lactate, acetate, ethanol, CO_2, H_2
Fervidobacterium islandicum	DSM 5733	19	Spheroid	Icelandic hot spring	50–80	65	6–8	7.2	Low	0.2	2.5	41	+	ND	Cellulose, glucose, maltose, pyruvate, raffinose, ribose, starch	L-Lactate, acetate, ethanol, CO_2, H_2
Thermosipho africanus	DSM 5309	18	Toga	Marine hydrothermal area	35–77	75	6–8	7.2	0.11–3.6	ND	0.5	29	+	ND	Peptone, yeast extract	ND

[a] Two unidentified compounds were also produced.
[b] ND, not determined.

HPLC). Isolated from an oil-producing well. The type strain is SEBR 6459 (= DSM 9442).

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