Kosmotoga olearia gen. nov., sp. nov., a thermophilic, anaerobic heterotroph isolated from an oil production fluid

Jonathan L. DiPippo,1 Camilla L. Nesbø,2† Håkon Dahle,3 W. Ford Doolittle,2 Nils-Kåre Birkland3 and Kenneth M. Noll1

Correspondence
Kenneth M. Noll
kenneth.noll@uconn.edu

1Department of Molecular and Cell Biology, University of Connecticut, Unit 3125, 91 N. Eagleville Road, Storrs, CT 06269-3125, USA
2Department of Biochemistry and Molecular Biology, Dalhousie University, 5850 College Street, Halifax, NS B3H 1X5, Canada
3Department of Biology and Centre for Geobiology, University of Bergen, PO Box 7800, N-5020 Bergen, Norway

A novel thermophilic, heterotrophic bacterium, strain TBF 19.5.1T, was isolated from oil production fluid at the Troll B oil platform in the North Sea. Cells of strain TBF 19.5.1T were non-motile rods with a sheath-like structure, or toga. The strain was Gram-negative and grew at 20–80 °C (optimum 65 °C), pH 5.5–8.0 (optimum pH 6.8) and NaCl concentrations of 10–60 g l⁻¹ (optimum 25–30 g l⁻¹). For a member of the order Thermotogales, the novel isolate is capable of unprecedented growth at low temperatures, with an optimal doubling time of 175 min (specific growth rate 0.24 h⁻¹) and a final optical density of >1.4 when grown on pyruvate at 37 °C. Various carbohydrates, proteinaceous compounds and pyruvate served as growth substrates. Thiosulfate, but not elemental sulfur, enhanced growth of the isolate. Sulfate also enhanced growth, but sulfide was not produced. The strain grew in the presence of up to approximately 15 % oxygen, but only if cysteine was included in the medium. Growth of the isolate was inhibited by acetate, lactate and propionate, while butanol and malate prevented growth. The major fermentation products formed on maltose were hydrogen, carbon dioxide and acetic acid, with traces of ethanol and propionic acid. The G+C content of the genomic DNA was 42.5 mol%.

Phylogenetic analyses of the 16S and 23S rRNA gene sequences as well as 29 protein-coding ORFs placed the strain within the bacterial order Thermotogales. Based on the phylogenetic analyses and the possession of a variety of physiological characteristics not previously found in any species of this order, it is proposed that the strain represents a novel species of a new genus within the family Thermotogaceae, order Thermotogales. The name Kosmotoga olearia gen. nov., sp. nov. is proposed. The type strain of Kosmotoga olearia is TBF 19.5.1T (=DSM 21960T =ATCC BAA-1733T).

Bacteria of the order Thermotogales possess a sheath-like ‘toga’ and grow optimally at high temperatures. The first described species were isolated from heated sea floors, marine hydrothermal vents and terrestrial hot springs (Huber et al., 1986, 1989, 1990). One species, Thermotoga lettingae, and many 16S rRNA gene sequences from members of the Thermotogales have been detected in both high- and low-temperature anaerobic waste digesters and contaminated sediments (Balk et al., 2002; Briones et al., 2007; Chouari et al., 2005; Nesbo et al., 2006; and references therein). Additionally, several genera and the majority of described species of the Thermotogales have been detected in oil reservoirs and oil production fluids.

Abbreviations: ME, minimum-evolution; ML, maximum-likelihood; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the sequence of fosmid tbf19.5.1.d05, including the 16S rRNA gene, from strain TBF 19.5.1T is EU980631.

Annotation of the fosmid clone and an ME tree constructed from ML distances estimated from the KO_3 ORF encoding chaperonin GroEL are available as supplementary material with the online version of this paper.
throughout the world (Davey et al., 1993; Fardeau et al., 1997; L’Haridon et al., 2001, 2002; Lien et al., 1998; Magot et al., 2000; Miranda-Tello et al., 2004; Orphan et al., 2000; Ravot et al., 1995; Takahata et al., 2001). The quantity and diversity of sequences of members of the Thermotogales that have been detected in oil reservoirs suggest that these organisms may play important roles in these environments (Grassia et al., 1996; Li et al., 2006, 2007; Magot et al., 2000). While numerous species of the Thermotogales have been shown to grow very slowly at relatively low temperatures (30–40 °C) (Davey et al., 1993; Huber et al., 1989; Miranda-Tello et al., 2004; Nunoura et al., 2007; Postec et al., 2005), robust growth at these temperatures has not been observed with isolated strains. There is evidence from metagenomic studies that species of the Thermotogales with low optimal growth temperatures exist in nature (Nesbø et al., 2006). None of these organisms, belonging to the informally described ‘mesotoga’ clade, have yet been isolated. Here, we report the isolation and characterization of a strain that belongs to a new genus of the Thermotogales that is characterized by unprecedented growth at low temperatures.

Strain TBF 19.5.1T was isolated from oil production fluids of the Troll B oil platform (60° 46′ 27.8″ N 03° 30′ 11.5″ E). The reservoir is located 1560 m below the sea floor, with an in situ temperature of 68 °C and a predicted in situ pH of 6. The pH of the formation water at 15 °C was 7.2 and it contained the following ions (approximate concentrations in g l⁻¹): sodium, 17; potassium, 0.4; calcium, 10; magnesium, 0.45; barium, 0.2; strontium, 0.25; chloride, 29; bicarbonate, 0.6. No sulfate and only trace amounts of total iron were detected.

Sterile Pyrex bottles filled with argon gas were used to collect samples at the upper riser on the platform. Sampling lines were flushed for more than 20 min prior to sampling. The bottles were filled completely with a mixture of oil and water and transported to the laboratory at ambient temperature. One millilitre of production fluid was injected into 20 ml minimal medium containing (l⁻¹ NanoPure water): 20 g NaCl, 0.9 g MgCl₂.6H₂O, 1.4 g MgSO₄.7H₂O, 0.33 g KCl, 0.25 g NH₄Cl, 0.14 g CaCl₂.2H₂O, 0.45 g KH₂PO₄, 10 ml trace mineral solution (Balch et al., 1979) adjusted to pH 6.5, 10 ml trace vitamin solution (Balch et al., 1979), 2 g yeast extract, 0.5 g cysteine hydrochloride and 1 mg resazurin. The pH was adjusted to 6.8 with 1 M NaOH. Two solutions of the basal medium were prepared, containing either no NaCl or 50 g NaCl l⁻¹, and intermediate concentrations of NaCl were achieved by combining appropriate volumes of the two solutions. The medium, in foil-topped Wheaton bottles, was tyndallized for 20 min, sealed with rubber stoppers and flushed with O₂-free N₂ gas before being dispensed in an anaerobic chamber. Bellco tubes (28 ml) containing 10 ml medium were sealed in the anaerobic chamber, flushed with O₂-free N₂ gas for 15 min and autoclaved at 121 °C for 20 min. For NaCl studies, the strain was grown in the basal medium containing 20 g NaCl l⁻¹ and this culture was used to inoculate test tubes containing 0–5 % (w/v) NaCl.

For temperature and pH studies, the basal medium was modified to contain (l⁻¹ NanoPure water) 5 g yeast extract and 20 g NaCl (temperature studies) or 30 g NaCl (pH studies). Water baths were used for incubation of tubes in all studies except for studies of growth at 37 °C, which were done using a gravity convection incubator. The pH range for growth was determined at 65 °C. For pH studies, the medium contained 20 mM of the following buffers: for pH 4 and 10, no buffer; pH 5–6, MES; pH 6.5–7, PIPES; pH 7.5 and 8, HEPES; pH 9, AMPSO. The pH of the medium was adjusted by addition of 1 M HCl or NaOH before autoclaving.

The basal medium for all tests, with the exception of substrate utilization studies, was supplemented with 0.5 % (w/v) maltose. Carbon sources and electron acceptors were tested at 65 °C using a basal medium modified as follows: (l⁻¹ NanoPure water): 30 g NaCl, 6.7 g PIPES, 1 g yeast extract and either 0.9 g MgCl₂.6H₂O and 1.4 g MgSO₄.7H₂O (for carbon source tests) or 2.3 g MgCl₂.6H₂O and no MgSO₄.7H₂O (for electron acceptor tests). Carbon sources were tested at a final concentration of 0.5 % (w/v) and growth of the strain was compared with controls lacking added carbon source. Elemental sulfur (approx. 1% w/v), sulfate (20 mM), sulfite (5 mM), thiosulfate (20 mM), nitrate (20 mM) and nitrite (5 mM) were tested as electron acceptors. When sulfate was tested, the basal medium was modified by replacing magnesium sulfate with magnesium chloride. The stock slurry of white, round colonies were picked from the 10⁻⁵ dilution after incubation at 70 °C for 3 days, and their 16S rRNA gene sequences were determined after amplification by PCR using primers 16S.1406R (5'AGAGTTGTATCCTGCTCAG-3') and 16S.1406R (5'-ACGGCCGATCCTGTRC-3'). The three sequences were identical and all subsequent work was done on isolate TBF 19.5.1T.
elemental sulfur had been tyndallized by steaming for 20 min per day for four consecutive days. Growth of the strain with added electron acceptor was compared with controls lacking the electron acceptor. For carbon source and electron acceptor studies, positive cultures were transferred at least once into the test medium (2% inoculum) to confirm growth. The production of H2S was measured qualitatively by suspending lead acetate strips in the headspace of stationary-phase cultures. Sensitivity to ampicillin, carbenicillin, chloramphenicol, kanamycin, rifampicin, streptomycin and vancomycin was tested at 10, 25, 50 and 100 µg ml−1 in an optimized medium [basal medium modified to contain (1−1 NanoPure water) 30 g NaCl, 6.7 g PIPES and 5 g yeast extract], hereafter referred to as TBFXP medium. Antibiotics were tested at 65 °C. An ethanol control (chloramphenicol solvent) and a DMSO control (rifampicin solvent) were used. Growth was monitored in culture tubes using a Bausch & Lomb Spectronic 20 spectrometer at 600 nm. All experiments were performed in triplicate.

The morphology and Gram reaction of strain TBF 19.5.1T were evaluated using an Olympus BH-2 phase-contrast microscope with cells grown in TBFXP medium at 65 °C. The Gram reaction of strain TBF 19.5.1T was determined using Gram stain Set-S (Difco). The presence of a murein cell wall was inferred using a lysozyme assay (Huber et al., 1989) with cells grown in TBFXP medium. The effect of lysozyme was determined at 37 °C. Motility was investigated on glass microscope slides and coverslips, which were preheated to about 70 °C. A drop of mid-exponential-phase cell culture grown in TBFXP at 65 °C was placed on the slides and observed immediately using the phase-contrast microscope. A comparison of optimal growth rates was conducted at 65 °C using cells grown in TBFXP medium supplemented with 0.5% (w/v) maltose or 0.5% (w/v) sodium pyruvate. The strain was subcultured once in the presence of each substrate before optimal growth rates were determined. For scanning electron microscopy, mid-exponential-phase cells grown in TBFXP at 65 °C were prepared as described previously (Lie et al., 1999) with the modification that cells were fixed in a solution containing 3% (w/v) glutaraldehyde, 0.3 M NaCl and 3 mM CaCl2 in 0.15 M sodium cacodylate buffer (pH 6.8).

Fermentation products were determined by gas chromatography and enzymic assay. An Agilent 6890N gas chromatograph was used to measure gaseous products, ethanol, acetic acid, propionic acid and butyric acid, using flame-ionization detection. Alanine dehydrogenase was used to measure alanine in spent medium. The detection limit for the assay was 2 mM.

The heat resistance of cells and the presence of spores were determined in TBFXP medium. Mid-exponential-phase cultures grown at 65 °C were heated to 80 °C (4 h), 90 °C (1–23 h) or 100 °C (1 h). Heated cultures were used subsequently to inoculate new culture tubes, which were incubated at 65 °C. Morphology was determined by phase-contrast microscopy. The effect of oxygen on growth was determined in two sets of Bellco tubes containing anaerobic TBFXP medium lacking resazurin. One set of tubes contained 0.5 g cysteine hydrochloride, while another lacked cysteine as a reducing agent. Two or twenty millilitres of the 20 ml headspace was removed and the same volume of filter-sterilized air was added before inoculation to provide approximately 2 and 15% oxygen, respectively. All cultures were incubated without shaking at 65 °C and growth rates were measured. To confirm the results of tests with 20 ml air, cells grown in the presence of 20 ml air were used to inoculate a second set of air-exposed tubes. The G+C content of genomic DNA was determined using HPLC at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Mesbah et al., 1989).

DNA was isolated using the protocol of Charbonnier & Forterre (1995). A fosmid library was constructed using the CopyControl Fosmid Library Production kit (Epicentre) following the protocol of the manufacturer. Three clones containing the 23S rRNA gene were identified, among 192 screened, using primers originally used in screening for the 23S rRNA intron found in some Thermotoga species (Nesbø & Doolittle, 2003). One of the clones was subcloned using the TOPO Shotgun Subcloning kit (Invitrogen) and sequenced to 15-fold final coverage (after low-quality regions and gaps were corrected by PCR). The sequence was assembled using phdPhap and consed (http://www.phap.org/phedphapconsed.html) (Ewing & Green, 1998; Ewing et al., 1998; Gordon et al., 1998). ORFs were identified using the run-glimmer2 script using the standard settings provided in this script (Delcher et al., 1999), and ORFs shorter than 100 bp were eliminated. If two overlapping ORFs were identified, we selected the one that had significant homologues in GenBank. tRNAs were identified with tRNAscan-SE (Lowe & Eddy, 1997). The ORFs where annotated using BLASTP searches (Altschul et al., 1997) of GenBank (http://www.ncbi.nlm.nih.gov/BLAST/) and Pfam searches (Bateman et al., 2004) (http://www.sanger.ac.uk/Software/Pfam/search.shtml). The ORFs were designated KO_1 to KO_29.

Phylogenetic analysis of the sequences of the 23S rRNA gene fragments and 16S rRNA genes were done in PAUP* (Swofford, 2002). Minimum-evolution (ME) trees were constructed using LogDet distances and maximum-likehood (ML) trees were constructed using a general time-reversible model with gamma-distributed rates with four categories and invariable sites (GTR+Γ+I). Ten random addition cycles of the sequences and tree bisection and reconnection (TBR) branch swapping were used in both cases.

ML trees [WAG (Whelan & Goldman, 2001) + Γ+I model] were constructed from protein-coding ORFs using the PhyloGenie package (Frickey & Lupas, 2004). In addition to these ‘automated’ trees, we also constructed trees manually for some ORFs. Homologues of the ORF were then identified and retrieved from GenBank using BLASTP searches (http://www.ncbi.nlm.nih.gov/BLAST/). In
addition, we retrieved ORFs from the genome of *Thermosipho africanus* TCES52B that was sequenced by Genome Atlantic (Nesbo et al., 2009). Clusters of very similar sequences from the same or sister taxa were trimmed down to one representative sequence. We also removed sequences that were considerably shorter than the rest of the alignment, as well as sequences that were difficult to align. The alignments were edited by deleting regions with many or large gaps, and were used to make both simple neighbour-joining (NJ) trees with bootstrap analysis and ME trees (with bootstrap analysis; 100 replicates with global rearrangements) estimated from ML distances (the distance chosen by TREE-PUZZLE 5.2 (Schmidt et al., 2002) + Γ, global rearrangements and 10 random addition replicates).

Anaerobic, sheath-bearing thermophilic bacteria (strain TBF 19.5.1^T^) were isolated from oil production water. Typical mid-exponential-phase cells were short rods, approximately 0.4–0.7 μm wide and 0.8–1.2 μm long, with one cell per sheath (Fig. 1a). Dividing cells, with a constriction at the midline, were 2–3 μm long. Cells were surrounded by a sheath-like structure or ‘toga’ that is characteristic of strains of the *Thermotogales*. An outer sheath was usually seen at each pole of growing cells and was about 0.4–1 μm long. In mid-exponential phase, spherical forms and chains of up to five cells were seen rarely. In early to mid-exponential phase, cellular aggregations of up to approximately 40 cells were seen occasionally. In stationary phase, the majority of rods became spherical, with a diameter of approximately 0.6 μm and, in some cells, the outer sheath was enlarged up to 3 μm in diameter, as has been reported previously (Huber et al., 1989). Occasionally, cells with diminished cytoplasmic content and an enlarged outer sheath were seen (Fig. 1b). The addition of lysozyme (1 mg ml^-1^) caused mid-exponential-phase rods to become spherical within 3 min, and cells remained spherical for at least 6 h. Motility was not detected using either room temperature or heated microscope slides, and flagella were not observed in any scanning electron micrographs. Cells stained Gram-negative.

Phylogenetic analysis of the 16S rRNA and 23S rRNA gene sequences of strain TBF 19.5.1^T^ revealed that it represents a novel species from a new genus within the *Thermotogales* (Fig. 2), most closely related to several mesophilic members of the *Thermotogales* informally called mesotoga (Nesbo et al., 2006). We constructed a fosmid library from genomic DNA from the isolate to obtain more sequence data. One rRNA operon-containing fosmid clone was fully sequenced; the insert was 38 287 bp long and had a G+C content of 42.65 mol%. The clone contained one rRNA operon and two tRNA genes (Supplementary Table S1, available in IJSEM Online). We identified 29 ORFs; however, two of these, ORF20 and ORF21 (predicted exporters of the RND superfamily), are probably pseudogenes. Phylogenetic analyses of both the rRNA genes (Fig. 2) and the predicted ORFs (not shown) demonstrated that strain TBF 19.5.1^T^ represents a novel species within the *Thermotogales* distinct from all previously described genera (Fig. 2 and Supplementary Fig. S1). In the rRNA trees, strain TBF 19.5.1^T^ clusters as a sister to the *Marinitoga*–*Geotoga*–*Petrogro* clade together with uncultured members of the *Thermotogales* found in low-temperature environments (Nesbo et al., 2006). Analysis of the protein-coding ORFs also place strain TBF 19.5.1^T^ in a new genus within the *Thermotogales*. This analysis included genome sequences from strains of *Petrogro mobilis*, *Fervidobacterium nodosum*, *Thermosipho melanesiensis*, *Thermotoga maritima* and *Thermotoga petrophila*.

Twenty-five of the ORFs (ORF20 and 21 were combined in one alignment) could be used in phylogenetic analyses and, in 21 (84%) of these trees, strain TBF 19.5.1^T^ clustered with other members of the *Thermotogales*. Only two of the trees constructed from the ORFs showed a topology identical to those of the rRNA trees (ORF13 and ORF15), and strain TBF 19.5.1^T^ was the sister of *P. mobilis* in five trees only (data not shown). In most cases, the discrepancy from the rRNA trees was due to the placement of the root and, in 13 of the trees, strain TBF 19.5.1^T^ and *P. mobilis* branched outside the other members of the *Thermotogales*, in agreement with the rRNA trees (see e.g. Supplementary Fig. S1). However, in six of the trees, strain TBF 19.5.1^T^ branched at the base of the *Thermotogales* clade and, in four trees, *P. mobilis* branched at the root of the *Thermotogales*. In comparison, *Thermotoga* was the deepest branching lineage in three trees, while *Thermosipho* and *Fervidobacterium* appeared at the root in one tree each.
This raises the possibility that strain TBF 19.5\textsuperscript{T}, and its closest relative mesotogas, could be early-branching lineages of the \textit{Thermotogales}. We therefore recalculated ML trees from the predicted ORFs in the mesotoga fosmid clones analysed by Nesbø \textit{et al.} (2006), and here ‘mesotoga’ was the root in only four of 19 trees that could be polarized and that also contained \textit{P. mobilis}. In these trees, \textit{P. mobilis} branched at the base of the \textit{Thermotogales} in five trees, while seven trees had the same topology as the rRNA trees. The large number of conflicting topologies suggests that high levels of lateral gene transfer may have occurred among different lineages of the \textit{Thermotogales}.

Strain TBF 19.5\textsuperscript{T} is thermophilic, and grew at 20–80 °C, with optimum growth at 65 °C. The isolate grew in the presence of 10–60 g NaCl l\textsuperscript{-1}, with an optimum at 25–30 g l\textsuperscript{-1}. Growth was observed at pH 5.5–8.0, with optimal growth at pH 6.8. In an optimized medium (TBFXP) at 65 °C, the doubling time was 124 ± 12 min with maltose as the substrate and 103 ± 8 min with pyruvate as the substrate (means ± SD of six determinations). At 37 °C, the minimal doubling time was 644 ± 56 min with maltose and 175 ± 59 min with pyruvate (means ± SD of three determinations). Pyruvate is clearly preferred over maltose, but the discrepancy in relative growth rates at these two temperatures is surprising. Perhaps the growth rate-limiting factor for maltose is either its transporter or an \( \alpha \)-glucosidase. The activity of one or both of these could be unusually cold-sensitive relative to the other catabolic enzymes. It will be interesting to determine whether the genes encoding these were inherited relatively recently from a more thermophilic organism and so are maladapted to low-temperature growth.

Spores were not observed in any growth phase or in any medium. Incubation at 80 °C for 4 h caused cells to become enlarged and spherical, and these cells grew when transferred to new medium. Cells incubated at 90 °C for up to 1 h grew when transferred to fresh medium; however, cells could not grow in new medium after 2 h at 90 °C. Incubation at 100 °C for 3 h caused typical rod-shaped cells with togas to form tiny cocci without visible togas. These cells were unable to grow in new medium. Strain TBF 19.5\textsuperscript{T} grew in an anaerobic, unreduced medium (lacking cysteine) with growth rates and optical densities not significantly different from those of cells grown in the same medium supplemented with cysteine (reduced medium). With 2 % oxygen in the headspace over a cysteine-reduced medium, the strain grew nearly as well as control cells grown under a 100 % nitrogen atmosphere. Growth was also observed in a reduced medium when 15 % oxygen was added to the headspace over cysteine-reduced medium. No growth was observed in a medium lacking cysteine that was shaken in air.

Maltose, ribose, sucrose, starch, Casamino acids, tryptone and pyruvate allowed good growth on basal medium containing 0.1 % (w/v) yeast extract. Hydrogen, carbon dioxide and acetic acid were the major products of growth.
on maltose. Traces of ethanol and propionic acid were detected. Butyric acid and alanine (detection limit 2 mM) could not be detected. Fructose, galactose, mannose, raffinose, xylan, casein and peptone were less preferred growth substrates. Arabinose, CM-cellulose, cellobiose, glucose, lactose, xylose, methanol, propanol, chitin, myo-inositol, putrescine and glycerol did not serve as carbon sources. Acetate, lactate and propionate inhibited growth slightly compared with controls (grown on 0.1 % yeast extract alone). Two substrates were toxic to the strain: with malate as the substrate, the OD did not rise above that of the starting inoculum, while butanol produced a sharp decline in OD. Cells incubated in the presence of butanol were unable to grow when inoculated into butanol-free medium. The strain could grow in basal medium containing 0.01 % (w/v) yeast extract as the sole carbon source. The strain could grow in the presence of 100 μg rifampicin ml−1 (grew less than control) and in the presence of 5 days) and 10 μg vancomycin ml−1 (total inhibition up to 5 days) and 10 μg chloramphenicol ml−1 (total inhibition up to 6 days, at which time the cultures were discarded). The G+C content of genomic DNA from strain TBF 19.5.1T was 42.5 mol%.

The strain grew in the presence of 100 μg kanamycin (grew as well as control), ampicillin, carbenicillin and streptomycin ml−1 (grew less than control) and in the presence of 25 μg rifampicin ml−1 (grew less than DMSO control). No growth was observed with 50 μg rifampicin ml−1 (for up to 8 h), 10 μg vancomycin ml−1 (total inhibition up to 5 days) and 10 μg chloramphenicol ml−1 (total inhibition up to 6 days, at which time the cultures were discarded). The G+C content of genomic DNA from strain TBF 19.5.1T was 42.5 mol%.

The genotypic and phenotypic characters of strain TBF 19.5.1T show that it represents a novel bacterial species within the family Thermotogaceae and is distinct from existing genera. The strain possesses the classical sheath-like structure, or toga, found in all characterized species of the Thermotogales. The most closely related genera, based on sequence analysis using the 16S rRNA gene, are Marinitoga, Petrotoga and Departotoga; however, the 16S rRNA gene sequence of strain TBF 19.5.1T is most similar to sequences from uncultured members of the Thermotogales found in low-temperature contaminated marine sediments and anaerobic waste reactors (Nesbo et al., 2006). Members of several genera of the Thermotogales have been found in oil environments; Petrotoga and Geotoga species have only been found in oil reservoirs (Davey et al., 1993; L’Haridon et al., 2002; Lien et al., 1998; Miranda-Tello et al., 2004, 2007), while Thermosiphon and Thermotoga contain species found in oil reservoirs and other high-temperature environments such as shallow marine hydrothermal vents and an anaerobic waste digester (Antoine et al., 1997; Balk et al., 2002; Fardeau et al., 1997; Huber et al., 1986, 1989; Jannasch et al., 1988; Jannasch et al., 1995; L’Haridon et al., 2001; Ravot et al., 1995; Takai & Horikoshi, 2000; Urios et al., 2004; Windberger et al., 1989). Geotoga, Petrotoga and Marinitoga contain characterized species most closely related to the new isolate. However, the difference in the 16S rRNA gene sequence between members of these three genera and strain TBF 19.5.1T is significant (18–20 %). Additionally, several physiological characteristics of strain TBF 19.5.1T have not been reported for any existing genus of the Thermotogales. The novel isolate is capable of unprecedented growth at low temperature, with a minimal doubling time of 175 min (specific growth rate 0.24 h−1) and final OD>1.4 when grown on pyruvate at 37 °C. Such significant low-temperature growth has not been reported for any other species of the Thermotogales. Strain TBF 19.5.1T differs from members of existing genera of the Thermotogales in that it is capable of growth under a 15 % oxygen atmosphere and also by the fact that its growth is inhibited by nitrite and sulfite. In terms of substrate utilization, the novel strain shows strong growth on pyruvate and Casamino acids and reduced growth on acetate, lactate and propionate. Butanol and malate were toxic to the strain. This substrate utilization profile distinguishes the strain from all described species of the Thermotogales. The DNA G+C content of the strain is higher than values reported for all other genera of the Thermotogales except Thermotoga.

These phylogenetic data and phenotypic characteristics indicate that the strain TBF 19.5.1 represents a novel species within a previously unknown genus, which we propose to name Kosmotoga olearia gen. nov., sp. nov. Table 1 summarizes the differences between Kosmotoga and the six established genera of the Thermotogales.

**Description of Kosmotoga gen. nov.**

*Kosmotoga* (Kos.mo.to’ga. Gr. masc. n. kosmos universe or world; L. fem. n. toga toga, a Roman outer garment; N.L. fem. n. Kosmotoga a worldly toga, referring to the
Table 1. Characteristics that distinguish strain TBF 19.5.1\textsuperscript{T} from species of the six recognized genera of the Thermotogales

Data were taken from this study (strain TBF 19.5.1\textsuperscript{T}), Alain et al. (2002), Nunoura et al. (2007), Postec et al. (2005) and Wery et al. (2001) (Marinitoga), Davey et al. (1993) (Geotoga), Davey et al. (1993), L’Haridon et al. (2002), Lien et al. (1998) and Miranda-Tello et al. (2004, 2007) (Petrotoga), Antoine et al. (1997), Huber et al. (1989), L’Haridon et al. (2001), Takai & Horikoshi (2000) and Urios et al. (2004) (Thermosiphon), Balk et al. (2002), Fardeau et al. (1997), Huber et al. (1986), Jannasch et al. (1988), Jeanthon et al. (1995), Ravot et al. (1995), Takahata et al. (2001) and Windberger et al. (1989) (Thermotoga) and Andrews & Patel (1996), Friedrich & Antranikian (1996), Huber et al. (1990) and Patel et al. (1985) (Fervidobacterium). ND, No data available; --, does not enhance growth; —, negative effect on growth; ——, strong negative effect on growth; +, enhanced growth; ±, enhanced growth for some, but not all, species; \(t_d\), doubling time.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain TBF 19.5.1\textsuperscript{T}</th>
<th>Marinitoga</th>
<th>Geotoga</th>
<th>Petrotoga</th>
<th>Thermosiphon</th>
<th>Thermotoga</th>
<th>Fervidobacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source(s)</td>
<td>Oil reservoir</td>
<td>Hydrothermal vents</td>
<td>Continental oil reservoir</td>
<td>Oil reservoirs</td>
<td>Hydrothermal vents and oil reservoir</td>
<td>Hydrothermal vents, oil reservoirs and bioreactor</td>
<td>Terrestrial hot springs</td>
</tr>
<tr>
<td>Morphology</td>
<td>Rods, single or pairs, chains rarely observed; not motile</td>
<td>Motile rods, may form chains; polar flagella</td>
<td>Motile rods, 1–5 cells per sheath</td>
<td>Rods, chains observed, 1–6 cells per sheath, most motile</td>
<td>Rods, 1–12 cells per sheath, not motile, or ND</td>
<td>Rods, 1–5 cells per sheath, most motile with flagella</td>
<td>Rods, 1–2 cells per sheath or chains; motile or ND</td>
</tr>
<tr>
<td>Stationary-phase morphology</td>
<td>Spheres form, rarely enlarged, chains up to 5 cells rare</td>
<td>Spherical, chains or ND</td>
<td>Become spherical</td>
<td>Most species form enlarged spheres</td>
<td>Cells become spherical or form chains</td>
<td>Cells become spherical or ND</td>
<td>Enlarged spheres form or ND</td>
</tr>
<tr>
<td>Temperature optimum (°C)</td>
<td>65</td>
<td>55–65</td>
<td>45–50</td>
<td>55–60</td>
<td>65–75</td>
<td>65–80</td>
<td>65–70</td>
</tr>
<tr>
<td>Growth at low temperatures</td>
<td>Excellent growth at 37 °C ((t_d) 175 min), growth to 20 °C</td>
<td>No growth below 30–40 °C</td>
<td>No growth below 30 °C</td>
<td>Poor growth at 37 °C, no growth at 30 °C</td>
<td>Poor or no growth at 40 °C</td>
<td>No growth below 45–55 °C</td>
<td>No growth below 40–45 °C</td>
</tr>
<tr>
<td>Oxygen tolerance</td>
<td>Growth at 15% O(_2)</td>
<td>Strict anaerobes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electron acceptors</td>
<td>Elemental sulfur</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Thiosulfate</td>
<td>+</td>
<td>±</td>
<td>ND</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Nitrate</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td>—</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Nitrite</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td>—</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Sulfite</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>±</td>
<td>—</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Cystine</td>
<td>—</td>
<td>+</td>
<td>ND</td>
<td>—</td>
<td>±</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Sulfate</td>
<td>+*</td>
<td>—</td>
<td>ND</td>
<td>±</td>
<td>—</td>
<td>±</td>
</tr>
<tr>
<td>Substrate utilization</td>
<td>Arabinose</td>
<td>—</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Casamino acids</td>
<td>+</td>
<td>±</td>
<td>ND</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Casein</td>
<td>+</td>
<td>±</td>
<td>ND</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>+</td>
<td>±</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>—</td>
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<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Pyruvate</td>
<td>—</td>
<td>±</td>
<td>ND</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Ribose</td>
<td>+</td>
<td>±</td>
<td>ND</td>
<td>—</td>
<td>—</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Xylose</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>42.5</td>
<td>28–29</td>
<td>29.5–29.9</td>
<td>31–39.8</td>
<td>29–33</td>
<td>39.2–50</td>
<td>33.7–40</td>
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</tbody>
</table>

*Slight growth enhancement, but no sulfide produced.
placement of the genus within a clade of the Thermotogales whose members appear to inhabit diverse environments such as oil reservoirs, marine sediments and low-temperature bioreactors).

Cells are Gram-negative, non-motile, short rods that possess a sheath-like outer structure. Non-spore-forming. Thermophilic, with optimal growth at 65 °C, pH 6.8 and 2.5–3 % NaCl. Anaerobic chemo-organotrophs, able to ferment carbohydrates, peptides and pyruvate. The DNA G + C content of the only known strain is 42.5 mol%. The 16S rRNA gene sequence places the genus within the family Thermotogaceae. The type species is Kosmotoga olearia.

Description of Kosmotoga olearia sp. nov.

Kosmotoga olearia (o.le.a’ri.a. L. fem. adj. olearia of or belonging to oil, describing the environment from which the type strain was isolated).

Displays the following properties in addition to those given for the genus. Cells are approximately 0.4–0.7 μm wide and 0.8–1.2 μm long, with one to three cells per sheath (toga). Rarely found in chains or aggregations of up to 50 cells. Spherical forms appear in stationary phase. Motile forms are not observed. Colonies grown on TBFXP are Spherical forms (but not spores) are detected up to 90 °C. Growth occurs in the presence of or without 2.5–3 % NaCl. Anaerobic chemo-organotrophs, able to ferment carbohydrates, peptides and pyruvate. The DNA G + C content of the only known strain is 42.5 mol%. The 16S rRNA gene sequence places the genus within the family Thermotogaceae. The type species is Kosmotoga olearia.

Growth characteristics and Morten Andre Teigland at StatoilHydro for assistance in measuring fermentation products and determining growth substrates. Fructose, galactose, mannose, raffinose, xylan, casein and glycerol do not serve as carbon sources. Thiosulfate enhances growth, while elemental sulfur, cinine and glycerol do not serve as carbon sources. Fructose, galactose, mannose, raffinose, xylan, casein and peptone allow relatively weaker growth. starch, Casamino acids, tryptone and pyruvate can serve as growth substrates. Fructose, galactose, mannose, raffinose, xylan, casein and peptone allow relatively weaker growth. Requires yeast extract for growth. Acetate, lactate and propionate inhibit growth slightly, while malate and butanol prevent growth. Under anaerobic conditions, malate is fermented primarily into hydrogen, carbon dioxide and acetic acid. Traces of ethanol and propionic acid are detected, but not butyric acid or alanine. Arabinose, CM-cellulose, cellulbiose, glucose, lactose, xylose, methanol, propanol, chitin, myo-inositol, putrescine and glycerol do not serve as carbon sources. Thiosulfate enhances growth, while elemental sulfur, nitrate and cystine do not. Sulfate also enhances growth, but sulfide is not produced. Sulfite inhibits growth slightly and nitrite prevents growth. Growth is inhibited by vancomycin and chloramphenicol (each at 10 μg ml⁻¹) and 50 μg rifampicin ml⁻¹. Growth occurs in the presence of ampicillin, carbenicillin, kanamycin and streptomycin (each at 100 μg ml⁻¹). The G + C content of genomic DNA of the type strain is 42.5 mol%.

The type strain, TBF 19.5.1T (=DSM 21960T =ATCC BAA-1733T), was isolated from oil production fluid from the Troll B platform in the North Sea.

Note added in proof

An upcoming report by Feng et al. [Feng, Y., Cheng, L., Zhang, X., Li, X., Deng, Y. & Zhang, H. (2010). Thermococcoides shengliensis gen. nov., sp. nov., from oil-production fluid, representing a novel genus of the order Thermotogales. Int J Syst Evol Microbiol (in press). doi:10.1099/ijs.0.013912-0] describes a new Thermotogales isolate, Thermococcoides shengliensis strain DSM-21960T, which we found to share 99.9% 16S rRNA gene sequence similarity with that of Kosmotoga olearia sp. nov. TBF 19.5.1 if 21 nt are removed from the 5′ end and 16 nt are removed from the 3′ end of the sequence (see the complete genome sequence of K. olearia strain TBF 19.5.1T in GenBank, NCBI reference sequence NC_012785.1).

Acknowledgements

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References


