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Thermotoga lettingae sp. nov., a novel thermophilic, methanol-degrading bacterium isolated from a thermophilic anaerobic reactor

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A novel, anaerobic, non-spore-forming, mobile, Gram-negative, thermophilic bacterium, strain TMO^T, was isolated from a thermophilic sulfate-reducing bioreactor operated at 65 °C with methanol as the sole substrate. The G+C content of the DNA of strain TMO^T was 39·2 mol%. The optimum pH, NaCl concentration, and temperature for growth were 7·0, 1·0%, and 65 °C, respectively. Strain TMO^T was able to degrade methanol to CO_2 and H_2 in syntrophic culture with *Methanothermobacter thermautotrophicus* ΔH or *Thermodesulfovibrio yellowstonii*. Thiosulfate, elemental sulfur, Fe(III) and anthraquinone-2,6-disulfonate were able to serve as electron acceptors during methanol degradation. In the presence of thiosulfate or elemental sulfur, methanol was converted to CO_2 and partly to alanine. In pure culture, strain TMO^T was also able to ferment methanol to acetate, CO_2 and H_2. However, this degradation occurred slower than in syntrophic cultures or in the presence of electron acceptors. Yeast extract was required for growth. Besides growing on methanol, strain TMO^T grew by fermentation on a variety of carbohydrates including monomeric and oligomeric sugars, starch and xylan. Acetate, alanine, CO_2, H_2, and traces of ethanol, lactate and α-aminobutyrate were produced during glucose fermentation. Comparison of 16S rDNA genes revealed that strain TMO^T is related to *Thermotoga subterranea* (98%) and *Thermotoga elfii* (98%). The type strain is TMO^T (= DSM 14385^T = ATCC BAA-301^T). On the basis of the fact that these organisms differ physiologically from strain TMO^T, it is proposed that strain TMO^T be classified as a new species, within the genus *Thermotoga*, as *Thermotoga lettingae*.

Keywords: *Thermotoga lettingae* sp. nov., thermophilic, syntrophic, methanol oxidation, thiosulfate reduction

INTRODUCTION

Methanol is formed during microbial pectin degradation and is released as a fungal biodegradation product of natural methoxylated aromatics, which are the building blocks of lignin (Donnelly & Dagley, 1980; Schink & Zeikus, 1980). Methanol is also known to be the main pollutant in evaporator condensates from the kraft pulping industry (Minami *et al*., 1986).

In anaerobic environments, methanol can be utilized by several groups of micro-organisms. In the presence of CO_2, homoacetogens are able to produce acetate and butyrate from methanol (Lettinga *et al*., 1979; Zeikus *et al*., 1980; van der Meijden *et al*., 1984). Methanol can be converted to CO_2 either by sulfate-reducing bacteria, which produce H_2S from sulfate (Braun & Stolp, 1985; Nanninga & Gottschal, 1986; Hattori *et al*., 2000), or by nitrate-reducing bacteria, which reduce nitrate to N_2 (Bamforth & Quayle, 1978; Urakami *et al*., 1995). Methanogens degrade methanol to methane and carbon dioxide (Schnellen, 1947; Sowers & Ferry, 1983; Ollivier *et al*., 1984; Ni & Boone, 1991). Methanol can also be degraded by syntrophic cultures of anaerobic micro-organisms (Heijthuijsen & Hansen, 1986; Cord-Ruwisch & Ollivier, 1986; Davidova & Stams, 1996; Daniel *et al*.,

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain TMO^T is AF355615.

1999). However, none of these methanol-degrading micro-organisms is restricted to the use of methanol as a substrate.

Recently, the conversion of methanol in a thermophilic sulfate-reducing bioreactor was investigated by Weijma (2000). The bioreactor experiments indicated that a syntrophic type of conversion of methanol might play an important role. Therefore, we aimed to isolate the bacterium originating from the highest dilution series of the reactor sludge in methanol-containing media. Here, we describe the isolation and characterization of a methanol-oxidizing bacterium, strain TMO^T, from the thermophilic methanol-oxidizing consortium. Its morphological, physiological and phylogenetic characteristics are presented, and its taxonomic position is discussed.

METHODS

Sample source. Strain TMO^T was isolated from a thermophilic (65 °C) sulfate-reducing and slightly saline (0·7% NaCl) bioreactor that was fed with methanol as the sole carbon and energy source, as previously described by Weijma (2000). From the reactor biomass a methanogenic enrichment culture was obtained with *Methanothermobacter thermautotrophicus* ΔH. *M. thermautotrophicus* ΔH (= DSM 1053) was kindly provided by J. T. Keltjens (University of Nijmegen, The Netherlands). *Thermodesulfovibrio yellowstonii* (= DSM 11347), *Thermotoga maritima* (= DSM 3109), *Thermotoga elfii* (= DSM 9442), *Thermotoga subterranea* (= DSM 9912), *Thermotoga thermarum* (= DSM 5069), *Thermotoga neapolitana* (= DSM 4359) and *Thermotoga hypogea* (= DSM 11164) were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany).

Culture media. The composition of the bicarbonate-buffered medium (MB) used for enrichment and routine growth experiments was based on medium 664 of the DSMZ (http://www.dsmz.de), with the following modifications: biotrypticase and sodium acetate were omitted and the amount of yeast extract was lowered to 0·5 g l^{-1}. The pH was adjusted to 7·0 with 10 M KOH and the medium was boiled and cooled to room temperature under a stream of O_2-free N_2 gas. The medium was anaerobically dispensed into serum bottles under a N_2/CO_2 (80:20, v/v) gas atmosphere. The bottles were closed with butyl rubber stoppers sealed with crimp seals. The medium was autoclaved for 20 min at 121 °C. Prior to inoculation, the medium was reduced with sterile stock solutions of $Na_2S.7–9H_2O$ and $NaHCO_3$ to obtain final concentrations of 0·04 and 0·2%, respectively. The pure culture of strain TMO^T and the syntrophic culture with *M. thermautotrophicus* ΔH were maintained by weekly transfer of a 10% (v/v) inoculum to fresh medium. Bottles were incubated in the dark without shaking.

For isolation, the enriched syntrophic co-culture with *M. thermautotrophicus* ΔH was incubated in the presence of methanol at 62 °C by using the soft-agar dilution method. Colonies which were visible after 3 weeks of incubation were picked with a sterile needle and subcultured in liquid medium containing 20 mM of pyruvate (as a substrate) and bromoethanesulfonate (to inhibit methanogenesis). Serial agar and liquid dilutions in pyruvate-containing media were repeated until a pure culture was obtained.

Substrate-utilization tests. The ability of strain TMO^T to metabolize soluble substrates was tested in MB medium. Substrates were added from sterile, anoxic, concentrated stock solutions to final concentrations of 20 mM, unless otherwise indicated. To test for electron acceptors, sodium thiosulfate (20 mM), sodium sulfate (20 mM), sodium nitrate (20 mM), sodium sulfite (5 mM), elemental sulfur (2%, w/v), Fe(III) (10 mM), anthraquinone-2,6-disulfonate (20 mM) and cystine (10 mM) were added to the medium at the concentrations indicated.

For syntrophic growth on substrates, strain TMO^T was inoculated in hydrogen-pregrown cultures of *M. thermautotrophicus* ΔH. Prior to inoculation, the gas phase of the bottles was changed to N_2/CO_2 (80:20, v/v), and substrates from sterile, concentrated stock solutions were added. Syntrophic growth of strain TMO^T on methanol was also tested in co-culture with *Thermodesulfovibrio yellowstonii* in MB medium to which 20 mM sulfate had been added. Yeast extract was not added to MB medium when syntrophic growth with *M. thermautotrophicus* ΔH was being tested on methanol.

pH, temperature, and sodium chloride concentration ranges for growth. The pH, temperature, and NaCl concentration ranges for growth were determined in MB medium containing 1 g yeast extract l^{-1} and 5 g peptone l^{-1}. The pH of the medium was adjusted by injecting calculated amounts of sterile Na_2CO_3 or HCl from the sterile, anaerobic stock solutions. To study the NaCl requirement, NaCl was weighed directly in the bottles before the medium was dispensed. Strain TMO^T was subcultured at least once under the same experimental conditions before the growth rates were determined.

Light- and electron microscopy. Cell morphology and purity were examined with a phase-contrast microscope. Gram staining and motility were studied according to the standard procedure (Doetsch, 1981). Transmission electron microscopy was performed as described by Plugge *et al.* (2000).

Antibiotic susceptibility. Chloramphenicol, vancomycin, streptomycin, rifampicin or penicillin G from filter-sterilized stock solutions was added to a final concentration of 100 μg ml^{-1} to sterile, prereduced MB medium.

Analytical techniques. Most substrates were measured by HPLC, as described previously by Stams *et al.* (1993). Methanol was analysed by GC using a Chrompack gas chromatograph (model CP9000) equipped with a Sil5 CB column (25 m × 0·32 mm) and a flame-ionization detector at 300 °C. The column temperature was 50 °C and the injection port temperature was 250 °C. The carrier gas was N_2 saturated with formic acid. Amino acids were analysed by HPLC, as described by Kengen & Stams (1994). Sulfide was determined as described by Trüper & Schlegel (1964). Hydrogen and methane were determined quantitatively by GC (Stams *et al.*, 1993). Thiosulfate was analysed by HPLC (Scholten & Stams, 1995).

G+C content. DNA was isolated and purified by the method of Marmur (1961). The G+C content of the DNA was determined by thermal denaturation, as described by Owen *et al.* (1969).

16S rDNA sequence analysis. DNA was extracted as described by Zoetendal *et al.* (1998). PCR was performed with the bacterial primers 7f and 1510r by using the *Taq* DNA polymerase kit from Life Technologies to amplify the

bacterial 16S rDNA. The PCR products were purified with the Qiaquick PCR purification kit (Qiagen) according to the manufacturer's instructions. Primers 1100r, 519r, 530f, 342r (Lane, 1991), 803f (Stackebrandt & Charfreitag, 1990) and 968f (Nübel *et al*., 1996), labelled with Infrared Dye 41 (MWG-Biotech), were used as sequencing primers. The sequences were automatically analysed on a LI-COR DNA 4000L sequencer and corrected manually. The sequences were checked for reading errors with the alignment programs of the ARB package (Strunk & Ludwig, 1991). Homology searches of the ARB, EMBL and GenBank DNA databases for these partial sequences were performed with FASTA and the homologies were checked with the ARB programs.

DNA–DNA hybridization. DNA–DNA hybridizations were performed with strain TMO^T, *Thermotoga elfii* (= DSM 9442) and *Thermotoga subterranea* (= DSM 9912) at the Institute of Microbiology of the Russian Academy of Sciences in Moscow. DNA–DNA hybridization was performed by using the optical reassociation method as described by DeLey *et al*. (1970).

RESULTS AND DISCUSSION

Enrichment and isolation

Strain TMO^T was enriched from a thermophilic anaerobic reactor described previously by Weijma (2000). The original co-culture was enriched without yeast extract. The strain was obtained first in co-culture with *M. thermautotrophicus* ΔH, using soft-agar media with methanol as the carbon and energy source. After 3 weeks incubation at 62 °C, colonies were about 1 mm in diameter, circular and opaque/whitish. A single colony was picked and serially diluted in agar and liquid media containing 20 mM pyruvate and 10 mM bromoethanesulfonate. A pure culture of strain TMO^T was used for further studies.

Morphology

Strain TMO^T is a rod-shaped bacterium. The cells stained Gram-negative and were 0·5–1 × 2–3 μm, occurring singly or in pairs. Each cell was surrounded by a sheath-like structure that ballooned over the cell ends, and each had peritrichous flagella. The envelope was easily visible in all growth phases by phase-contrast microscopy. In the late stationary phase, the rods became spheres and were still surrounded by the sheath-like structures (Fig. 1). No endospores were observed in old cultures.

Growth conditions

Strain TMO^T grew at temperatures ranging from 50 to 75 °C; optimum growth occurred at 65 °C, whereas no growth was observed at 45 or 80 °C. Growth was

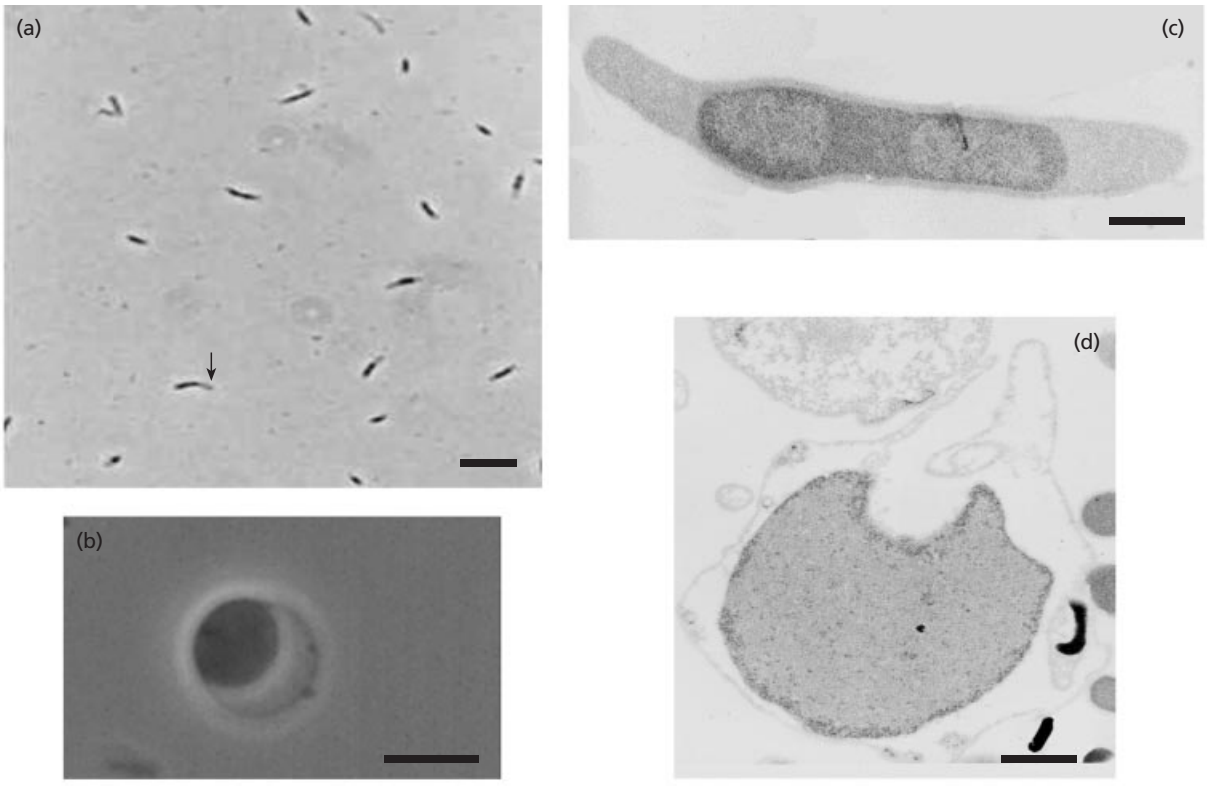

Fig. 1. (a, b) Phase-contrast micrograph of strain TMO^T, showing the toga (a, arrow) and a ballooning cell (b). Bars, 2·5 μm. (c, d) Electron micrographs of a thin section of strain TMO^T, showing the typical outer sheath-like structure (c) of the genus *Thermotoga* and a spherical cell (d) in stationary growth phase. Bars, 0·5 μm.

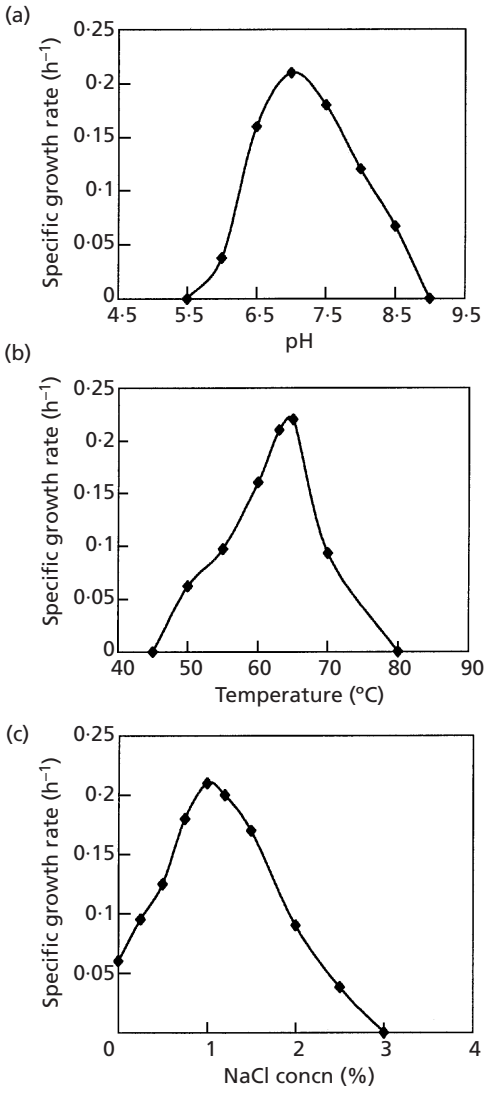

Fig. 2. Effect of pH (a), temperature (b) and NaCl concentration (c) on the growth of strain TMO^T in MB medium. The specific growth rates were calculated from the slopes of the growth curves (not shown).

obtained between pH 6·0 and pH 8·5, optimum growth being at pH 7·0. The isolate grew in the presence of sodium chloride concentrations ranging from 0 to 28 g l^{-1}, the optimum being at 10 g l^{-1} (Fig. 2). At the optimum temperature (65 °C), pH (7·0) and salinity (10 g NaCl l^{-1}) for growth, the doubling time in the medium containing yeast extract and peptone was about 4 h.

Phylogeny, DNA base composition and DNA reassociation

Using six primers, an almost complete sequence consisting of 1513 bases for the 16S rDNA gene of strain TMO^T was obtained. On the basis of the

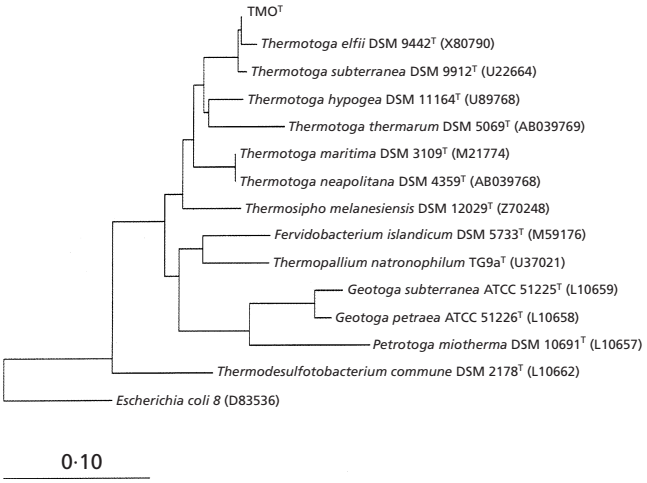

Fig. 3. Dendogram showing the position of strain TMO^T among the members of the order *Thermotogales* and related bacteria. The bar represents an evolutionary distance of 0·10.

phylogenetic analysis (Fig. 3), strain TMO^T appeared to be a member of the order *Thermotogales*, the closest relatives being *Thermotoga subterranea* (Jeanthon *et al.*, 1995) and *Thermotoga elfii* (Ravot *et al.*, 1995) (both 98 % similarity).

Until now, representatives of the genus *Thermotoga* have not been isolated from a thermophilic anaerobic bioreactor (Huber *et al.*, 1986; Jannasch *et al.*, 1988; Windberger *et al.*, 1989; Jeanthon *et al.*, 1995; Ravot *et al.*, 1995; Fardeau *et al.*, 1997; Takahata *et al.*, 2001). Furthermore, *Thermotoga* species were described as carbohydrate-fermenters that are able to utilize thiosulfate as an electron acceptor (Ravot *et al.*, 1995).

Physiologically, strain TMO^T differs from the species *Thermotoga hypogea* (Fardeau *et al.*, 1997), *Thermotoga maritima* (Huber *et al.*, 1986), *Thermotoga neapolitana* (Jannasch *et al.*, 1988), *Thermotoga thermarum* (Windberger *et al.*, 1989), *Thermotoga petrophila* and *Thermotoga naphtophila* (Takahata *et al.*, 2001) by its lower optimal temperature, from the species *Thermotoga maritima* and *Thermotoga neapolitana* by its lower salinity range, but from the species *Thermotoga hypogea* and *Thermotoga thermarum* by its higher salinity range. Its physiological properties (temperature, pH optima and salinity requirement) are more similar to those of *Thermotoga elfii* and *Thermotoga subterranea*.

The DNA base composition (G+C) of strain TMO^T was 39·2 mol %, which is within the range reported for other *Thermotoga* species (Huber & Stetter, 1992).

DNA–DNA hybridizations of strain TMO^T with *Thermotoga subterranea* and *Thermotoga elfii* revealed 35 and 30 % similarity, respectively. Although the results showed their low DNA homology, in the same

Table 1. Methanol utilization of strain TMO^T under different conditions

Duplicate cultures were incubated at 65 °C until complete methanol degradation was measured.

Growth conditions for strain TMO^T on methanol*	Methanol degraded (mM)	Acetate formed (mM)	Alanine formed (mM)	Sulfide formed (mM)	CH_4 formed (mmol l^{-1})	Carbon balance (%)†	Electron balance (%)‡
–	19·7	13·7	–	–	–	92	105
In co-culture with methanogen	20·7	–	–	–	18·2	88	85
In co-culture with sulfate-reducer	19·2	–	–	10·2	–	98	93
With thiosulfate	18·7	–	5·8	11·2	–	93	120
With elemental sulfur	10·6	–	3·1	7·3	–	87	120

* In a pure culture of strain TMO^T, the yeast extract concentration was 0·5 g l^{-1} in MB medium. Incubations were stopped when methanol was completely degraded.

† Calculated to reduction of CO_2 according to the energetics.

‡ Calculated as available hydrogen balance.

genus phylogenetically closely related bacteria (99% similarity; *Thermotoga neapolitana* and *Thermotoga maritima*) also showed only 25% homology (Jannasch *et al.*, 1988).

Growth of strain TMO^T in MB medium containing glucose was inhibited by chloramphenicol and streptomycin but was not inhibited by rifampicin and penicillin G (each at a final concentration of 100 μg ml^{-1}). However, slight growth was observed with the same concentration of vancomycin.

Growth on methanol

Methanol utilization in a pure culture of strain TMO^T was slow, and concomitantly low optical densities were obtained. Complete degradation of methanol occurred in around 30 days when 0·5 g yeast extract l^{-1} was present (Table 1). Methanol degradation by a pure culture of strain TMO^T did not occur when yeast extract was completely omitted.

Strain TMO^T was able to convert methanol within 12 days in the presence of thiosulfate (Table 1). Similarly, in the presence of elemental sulfur, methanol was converted to alanine, CO_2 and sulfide as end-products.

A remarkable characteristic of strain TMO^T is its capacity to form alanine from methanol when thiosulfate or elemental sulfur is present. Although alanine formation from glucose metabolism has been reported for *Pyrococcus furiosus* (Kengen & Stams, 1994), for a moderately thermophilic *Clostridium* species (Örlygsson *et al.*, 1995) and for the order *Thermotogales* (Ravot *et al.*, 1996), alanine formation from a C_1 substrate has not been reported until now.

Growth of strain TMO^T was also possible on methanol after inoculation into a hydrogen-pregrown culture of *M. thermautotrophicus* ΔH. Methanol was completely oxidized to CO_2 in syntrophy with the methanogen

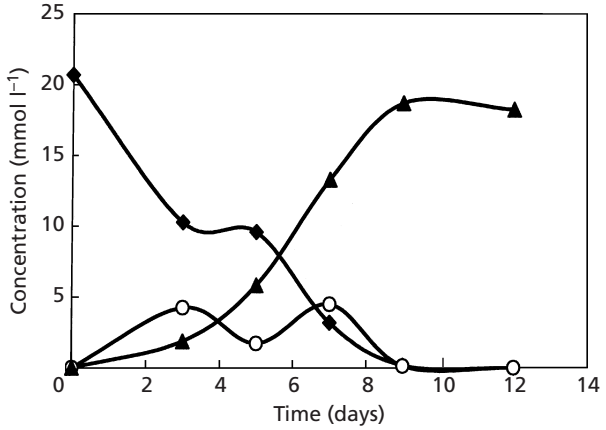

Fig. 4. Utilization of methanol (◆) and production of acetate (○) and methane (▲) by strain TMO^T in co-culture with *Methanothermobacter thermautotrophicus* ΔH in MB medium. The pH value, NaCl concentration, and temperature for growth were 7·0, 1·0%, and 65 °C, respectively.

within 9 days (Fig. 4). Methanol oxidation also occurred when *Thermodesulfovibrio yellowstonii* was used in co-culture, where the products are CO_2 and sulfide.

All of the *Thermotoga* species described to date require yeast extract for growth on carbohydrates. This suggests a requirement for certain growth factors. The fact that strain TMO^T does not require yeast extract during syntrophic growth on methanol indicates that the methanogens provide these growth factors to strain TMO^T.

To our knowledge, the methanol-utilization capabilities of the *Thermotoga* species described to date have not been studied. We tested some *Thermotoga* species for their methanol-degradation abilities, and preliminary results indicated that *Thermotoga subterranea*,

Table 2. Characteristics that differentiate strain TMO^T from two close relatives, *T. elfii* DSM 9442^T and *T. subterranea* DSM 9912^T

All *Thermotoga* species are characterized by the presence of outer sheaths, termed togas. Strain TMO^T (= DSM 14385^T), *T. elfii* DSM 9442^T and *T. subterranea* DSM 9912^T are able to utilize glucose and reduce thiosulfate. Their salinity range is around 1 %. NR, Not reported.

Characteristic	Strain TMO^T	*T. subterranea*	*T. elfii*
Source	Anaerobic bioreactor	Oil well, Paris	Oil well, Africa
Temp. range (°C)	50–70	50–75	50–72
Optimum temp. (°C)	65	70	66
pH range	6·0–8·5	6·0–8·5	5·5–8·7
Optimum pH	7·0	7·0	7·5
NaCl range (%)	0–2·8	0–2·4	0–2·4
Optimum NaCl conc. (%)	1·0	1·2	1·2
G+C content (mol %)	39·2	40	39·6
Reduction of S^0	+	−	−
Reduction of cystine	−	+	NR
Substrates used			
Methanol	+	NR	NR
Pyruvate	+	NR	NR
Pectin	+	NR	NR
Cellobiose	+	NR	NR
Xylose	+	NR	+
Xylan	+	NR	NR
Starch	+	NR	NR
Methylamines	+	NR	NR
Acetate + thiosulfate	+	NR	−
Acetate in co-culture with the methanogen	+	NR	NR

Thermotoga elfii, *Thermotoga thermarum* and *Thermotoga maritima* are also able to grow on methanol. However, methanol oxidation by *Thermotoga hypogea* and *Thermotoga neapolitana* was hardly detectable.

Growth on acetate

Strain TMO^T was able to grow on acetate in the presence of the methanogen or thiosulfate: 20 mM acetate was converted to CO_2 by strain TMO^T in co-culture with *M. thermautotrophicus* ΔH in about 28 days when 0·5 g yeast extract l^{-1} was present. As is the case for methanol conversion, strain TMO^T converted 19 mM acetate to 6 mM alanine and 10 mM sulfide when thiosulfate was present. In this case, acetate was converted within 36 days. Although slow growth of strain TMO^T on acetate was observed, these results are in agreement with those for previously described acetate-degrading bacteria that are either thermophilic or mesophilic and coupled to a methanogenic partner or an electron acceptor (Zinder & Koch, 1984; Schnürer *et al.*, 1996; Hattori *et al.*, 2000).

Utilization of other substrates

Growth was observed on complex substrates such as yeast extract, peptone, biotrypticase, gelatin or Casamino acids, and these complex substrates were fermented by strain TMO^T. Single substrates were tested in MB medium containing 0·5 g yeast extract l^{-1}. The following substrates were utilized in the absence or presence of the methanogen or thiosulfate: methanol, lactate, pyruvate, glucose, fructose, galactose (10 mM), mannose (10 mM), xylose, lactose, maltose, sucrose (10 mM), arabinose (10 mM), ribose (10 mM), cellobiose, rhamnose, glycerol, starch, xylan, pectin, methylamine, dimethylamine, trimethylamine, 2-oxoglutarate and serine. Acetate, betaine, leucine, isoleucine and valine were utilized in the presence of the methanogen or thiosulfate. In the presence of thiosulfate, formate (10 mM) and H_2/CO_2 (80:20, v/v) were also utilized for growth. The following substrates were tested, but not utilized, for growth in the absence or presence of the methanogen or thiosulfate: choline (10 mM), benzoate (10 mM), mannitol (10 mM), butanol (10 mM), isobutanol (10 mM), 2,3-butandiol (10 mM), propanol (10 mM), butyrate (10 mM), succinate (10 mM). Thiosulfate, elemental sulfur, Fe(III) and anthraquinone-2,6-disulfonate were able to serve as electron acceptors, unlike sulfate (20 mM), sulfite (5 mM), nitrate (20 mM) and cystine (10 mM) (Table 2). The presence of thiosulfate or elemental sulfur affected the pattern of fermentation products, as has been reported for *Thermotoga hypogea* (Fardeau *et al.*, 1997).

Strain TMO^T was nutritionally versatile in its ability to use different sugars. During the sugar fermentation, acetate, alanine, CO_2, H_2, traces of ethanol, lactate and α-aminobutyrate were produced. It is probable that α-aminobutyrate is the unknown product that was found during glucose fermentation by *Thermotoga maritima* (Huber *et al*., 1986). The production of alanine and α-aminobutyrate is a way of relieving hydrogen inhibition, as described previously for *P. furiosus* (Kengen & Stams, 1994). In co-culture with the methanogen, strain TMO^T degraded glucose to acetate and CO_2. The other products were not detected.

The presence of thiosulfate had an effect on the alanine/acetate ratio produced during glucose fermentation. In the absence of thiosulfate, strain TMO^T shifted its metabolism to alanine production; consequently, less acetate was produced. Depending on the growth conditions and the substrate used, the alanine concentration could be up to 13·4 mM.

Strain TMO^T is also able to use elemental sulfur as an electron acceptor. In this respect, strain TMO^T differs from the most closely related species, i.e. *Thermotoga elfii* and *Thermotoga subterranea*. Elemental sulfur can also be utilized as an electron acceptor by *Thermotoga maritima* and *Thermotoga neapolitana* during glucose fermentation. Moreover, in contrast to *Thermotoga subterranea*, strain TMO^T is unable to utilize cystine as an electron acceptor. The other electron acceptors for methanol utilization by strain TMO^T are Fe(III) and anthraquinone-2,6-disulfonate. The ability of hyperthermophilic micro-organisms, including *Thermotoga maritima*, to reduce humic substances and Fe(III) was reported by Lovley *et al*. (2000).

On the basis of genomic DNA–DNA hybridization and particular physiological differences (i.e. the ability to grow on C_1 substrates and acetate and the ability to use elemental sulfur), we consider isolate TMO^T to be a new species of the genus *Thermotoga*. We propose to name it *Thermotoga lettingae*.

Description of *Thermotoga lettingae* sp. nov.

Thermotoga lettingae (let′tin.gae. N.L. gen. n. *lettingae* of Lettinga, named after Gatze Lettinga, a Dutch biotechnologist, in recognition of his pioneering work on anaerobic methanol conversion in methanogenic bioreactors).

The cells are Gram-negative rods 2–3 μm long × 0·5–1 μm wide. Each rod is surrounded by a sheath-like structure, ballooning over the ends. Cells occur singly, in pairs or, rarely, in chains comprising a maximum of four rods. The rods tend to become large spheres in the stationary phase. Motile, strictly anaerobic, thermophilic. Growth occurs between 50 and 75 °C, the optimum being at 65 °C; between pH 6·0 and pH 8·5, the optimum being at pH 7·0; and at NaCl concentrations between 0 and 28 g l^{-1}, the optimum being at 10 g l^{-1}. The doubling time at optimum temperature and salinity at pH 7·0 is 4 h. Growth is possible on complex substrates such as yeast extract, peptone, gelatin, biotrypticase or Casamino acids. Uses thiosulfate, elemental sulfur, Fe(III) and anthraquinone-2,6-disulfonate, but not sulfate, sulfite, nitrate or cystine, as electron acceptors. Grows on methanol, lactate, pyruvate, glucose, fructose, galactose, mannose, xylose, lactose, maltose, sucrose, arabinose, ribose, cellobiose, rhamnose, glycerol, pectin, methylamines, starch, xylan, 2-oxoglutarate and serine. In the presence of thiosulfate or a methanogen, it can grow on acetate, betaine, leucine, isoleucine and valine. In the presence of thiosulfate, it grows on formate and H_2/CO_2 but not on choline, benzoate, mannitol, butanol, isobutanol, 2,3-butandiol, propanol, butyrate or succinate. Methanol degradation occurs in syntrophic culture with *Methanothermobacter thermautotrophicus* ΔH or *Thermodesulfovibrio yellowstonii*. Thiosulfate enhances the growth rate and substrate conversion. Isolated from an anaerobic bioreactor. The type strain is TMO^T, which has been deposited in the DSMZ as DSM 14385^T and in the ATCC culture collection as BAA-301^T.

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