

***Thermoanaerobacter tengcongensis* sp. nov., a novel anaerobic, saccharolytic, thermophilic bacterium isolated from a hot spring in Tengcong, China**

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A new, extremely thermophilic bacterium, designated strain MB4^T, was isolated from a Chinese hot spring. The new isolate was an obligately anaerobic, rod-shaped, Gram-negative, saccharolytic bacterium. Spore formation was not observed. Growth occurred at temperatures between 50 and 80 °C, with an optimum of around 75 °C; at pH values between 5.5 and 9.0, with an optimum of 7.0–7.5; and at salinities between 0 and 2.5% NaCl, with an optimum of around 0.2% NaCl. The organism utilized glucose, galactose, maltose, cellobiose, mannose, fructose, lactose, mannitol and starch. Acetate was the main end product from glucose fermentation. Thiosulfate and sulfur were reduced to hydrogen sulfide. Sulfate, sulfite and nitrate were not reduced. Growth was inhibited by hydrogen. The G+C content of the DNA was 33 mol%. Phylogenetic analyses based on the 16S rDNA sequence indicated that the isolate was a new member of the genus *Thermoanaerobacter* and formed a monophyletic unit within the *Thermoanaerobacter* cluster. Based on its phenotypic and phylogenetic characteristics, the isolate was proposed as a new species, *Thermoanaerobacter tengcongensis*. The type strain is MB4^T (= Chinese Collection of Microorganisms AS 1.2430^T = JCM 11007^T).

Keywords: *Thermoanaerobacter*, thermophiles, saccharolytic bacterium

INTRODUCTION

The biotechnological potential and evolutionary significance of thermophiles has led to intensive studies on the biology of anaerobic, saccharolytic thermophiles, which are found in all types of thermal habitats (Brock, 1986; Wiegel *et al.*, 1985; Kristjansson & Stetter, 1992). Most of these isolates have been characterized and described as members of the genera *Thermoanaerobacter*, *Thermoanaerobacterium* and *Clostridium* (Wiegel & Ljungdahl, 1981; Lee *et al.*, 1993).

Anaerobic thermophiles have been isolated from widely distributed hot springs, for example *Thermoanaerobacter brockii* (Zeikus *et al.*, 1979) and *Thermoanaerobacterium xylanolyticum* (Lee *et al.*, 1993) from the hot springs of the Yellowstone National Park, *Fervidobacterium nodosum* from a thermal spring in

New Zealand (Patel *et al.*, 1985) and *Thermoanaerobacter italicus* from a thermal spa in Italy (Kozianowski *et al.*, 1997). To date, there are few reports on anaerobic thermophiles from Chinese hot springs. During investigations on the microbial diversity of Chinese hot springs, we isolated a new thermophilic bacterium designated strain MB4^T, which is classified as a new member of the genus *Thermoanaerobacter* on the basis of phenotypic and phylogenetic analyses. The name *Thermoanaerobacter tengcongensis* is proposed.

METHODS

Sample source. Mixed sediment and water samples were taken from a hot spring in Tengcong, located in Yunnan Province, China. At the site of sampling, the temperature was 86 °C, the pH value was 7.0 and the NaCl concentration was 0.25% (w/v).

Enrichment, isolation and cultivation. The modified MB medium (Fardeau *et al.*, 1997) used for the experimental studies contained (per litre distilled water): NH₄Cl, 1.0 g;

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The GenBank accession number for the 16S rRNA gene sequence of strain MB4^T is AF209708.

$K_2HPO_4 \cdot 3H_2O$, 0.3 g; KH_2PO_4 , 0.3 g; $MgCl_2 \cdot 6H_2O$, 0.5 g; NaCl, 2.0 g; KCl, 0.2 g; $CaCl_2$, 0.05 g; trace element solution (Balch *et al.*, 1979), 9 ml; cysteine·HCl, 0.5 g; yeast extract, 1.0 g; tryptone, 2.0 g; starch, 10 g. The medium was boiled under a stream of oxygen-free N_2 gas and cooled to about 80 °C. The pH of the medium was adjusted to 7.5 with 2 M NaOH, then dispensed into Hungate tubes under oxygen-free N_2 gas and autoclaved. Prior to inoculation, thiosulfate was injected from sterile stock solutions to obtain a final concentration of 20 mM. All incubations were at 75 °C unless otherwise noted. The pH value of the medium did not change more than 0.3 pH units at the different temperatures used.

Enrichment was performed anaerobically in 30 ml glass bottles containing 20 ml modified MB medium. Media were inoculated with 2 ml samples and incubated at 80 °C without agitation for 3 d. Purification was repeated at 65 °C in modified MB agar deeps (2%, w/v, agar) and Hungate roll tubes (Hungate, 1969) with 3% agar.

Characterization. Gram reaction was determined by staining (Doetsch, 1981) and by the KOH lysis method (Buck, 1982). Cell morphology and motility were examined by phase-contrast microscopy and electron microscopy during exponential-growth phase in modified MB medium. Amino acid composition of the cell wall was determined by the method of Schleifer (1985).

pH, temperature and NaCl concentration ranges for growth were determined in modified MB medium. For the pH range, the medium was adjusted to pH values between 5 and 10 with HCl or NaOH and 10 ml CO_2 was injected into the headspace of the tube by syringe prior to autoclaving. The pH value of the medium was adjusted by injecting $NaHCO_3$ or Na_2CO_3 from 10% (w/v) sterile anaerobic stock solutions and measured at 75 °C with a model $\phi 71$ pH meter (Beckman) equipped with a temperature probe and calibrated at 75 °C prior to inoculation. For NaCl requirements, different amounts of NaCl were weighed directly into the tubes prior to dispensing the medium.

Substrate utilization was tested in modified MB medium containing 1 g tryptone l^{-1} and 1 g yeast extract l^{-1} and without starch. Substrates in sterile stock solutions were added to the medium at a final concentration of 20 mM except for yeast extract (0.2%, w/v), tryptone (0.2%, w/v) and Casamino acids (0.2%, w/v). The controls were grown without any substrate addition.

To test for electron acceptors, compounds prepared as sterile stock solution were added to the modified MB medium at a final concentration of 20 mM except for oxygen (atmospheric concentration) and elemental sulfur (1 g l^{-1}). The medium was prerduced with cysteine in the experiments with sulfate, sulfite, thiosulfate and elemental sulfur. No reducing agents were present in medium with oxygen. Both reduced and reducing-agent-free media were used in the experiment with nitrate. The utilization of the electron acceptors was monitored by measuring growth and sulfide production. The effect of a hydrogen atmosphere on growth of strain MB4^T was tested using modified MB medium containing 0.5% glucose with and without thiosulfate or sulfur.

To test antibiotic susceptibility, ampicillin, chloramphenicol, penicillin, polymyxin B, streptomycin sulfate or tetracycline·HCl from filter-sterilized stock solutions were each added at a final concentration of 100 $\mu g ml^{-1}$ to sterile medium.

Analytical techniques. All experiments were performed in duplicate. The strain was subcultured at least once under the same experimental conditions prior to determination of growth rates. Bacterial growth was monitored by optical density at 600 nm.

For negative staining, cells were suspended in 0.9% NaCl solutions and allowed to attach to a grid, followed by washing with 2% (w/v) ammonium acetate and negatively staining with 2% (w/v) ammonium molybdate. For platinum shadowing, cells were applied to a carbon-coated grid, washed once with distilled water and shadowed with platinum-carbon at an angle of 18°. For thin sectioning, cells were fixed with 0.5% (w/v) glutaraldehyde and 1% (w/v) OsO_4 at 4 °C, embedded in Epon epoxy resin and stained with 1% (w/v) uranyl acetate and lead citrate. Photomicrographs were taken with a Hitachi transmission electron microscope.

Short-chain fatty acids and alcohols were analysed by GC on a Shimadzu GC-9A gas chromatograph with 5% FAPP on a Chromosorb-G glass column and an FID detector. H_2 and CO_2 were analysed by GC on a Shimadzu GC-7AG gas chromatograph with a Porapak Q glass column and a TCD detector. Glucose was determined by HPLC (Waters) with a Sugar-pak-1 column (300 × 6.5 mm; Waters) and a Waters 410 RI detector. Hydrogen sulfide formation was detected by the addition of $FeSO_4$ to the culture, a black precipitate indicating the presence of sulfide.

Sporulation test. Cultures grown in media with or without glucose or starch were examined for the presence of spores at different growth phases. The heat resistance of cells was determined in modified MB medium. After 2 d incubation at 75 °C, duplicate cultures were heated at 100 °C for 20 or 30 min and subcultured into fresh medium (20%, v/v, inoculum). The resulting cultures were incubated at 75 °C for 3 d.

DNA and 16S rDNA studies. Genomic DNA was prepared by the method of Marmur (1961), and the purity was checked spectrophotometrically. The G + C content of the DNA was determined by the thermal denaturation method according to Marmur & Doty (1962) using *Escherichia coli* DNA (51 mol% G + C) as standard.

16S rDNA was amplified from strain MB4^T genomic DNA by PCR with *Taq* DNA polymerase (Promega) and primers 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-AAGGAGGTGATCCAGCCGCA-3', which correspond to positions 8–27 and 1541–1525 in the 16S rDNA (*E. coli* numbering; Brosius *et al.*, 1978). The PCR products were cloned into vector pGEM-T using the pGEM-T vector system I kit according to the manufacturer's instructions (Promega). The sequences were determined using the dideoxy sequencing method (Sanger *et al.*, 1977) supplied in kit form on an ABI 373S DNA sequencer (Applied Biosystems).

Phylogenetic analysis of 16S rDNA sequences. The 16S rDNA sequence from strain MB4^T was compared against all deposited nucleotide sequences in the GenBank database by using the Basic BLAST 2.0 option (Altschul *et al.*, 1997), implemented as a sequence similarity search tool at the home page of the National Center of Biotechnology Information (<http://www.ncbi.nlm.nih.gov>). Close relatives were retrieved and aligned with each other using CLUSTAL W version 1.8 (Thompson *et al.*, 1994). The phylogenetic tree was drawn by using the neighbour-joining method (Saitou & Nei, 1987) with the Kimura two-parameter calculation model in TREECON for Windows version 1.2 (Van de Peer &

De Wachter, 1994). Tree topologies were evaluated by bootstrap analysis based on 100 resamplings of the neighbour-joining dataset.

The accession numbers of the reference strains used in the sequence comparison were as follows: *Thermoanaerobacter acetoethylicus* ATCC 33265^T, L09163; *Thermoanaerobacter brockii* subsp. *brockii* ATCC 33075^T, L09165; *Thermoanaerobacter ethanolicus* ATCC 31550^T, L09162; *Thermoanaerobacter kivui* DSM 2030^T, L09160; *Thermoanaerobacter brockii* subsp. *finii* DSM 3389^T, L09166; *Thermoanaerobacter brockii* subsp. *lactiethylicus* DSM 9801^T, U14330; *Thermoanaerobacter thermocopriae* IAM 13577^T, L09167; *Thermoanaerobacter thermohydrosulfuricus* DSM 567^T, L09161; *Thermoanaerobacter wiegelii* DSM 10319^T, X92513; *Thermoanaerobacter siderophilus* DSM 12299^T, AF120479; *Thermoanaerobacter italicus* DSM 9252^T, AJ250846; *Thermoanaerobacter mathranii* DSM 11426^T, Y11279; *Thermoanaerobacterium saccharolyticum* DSM 7060^T, L09169; *Thermoanaerobacterium xylanolyticum* DSM 7097^T, L09172; *Thermoanaerobacterium thermosulfurigenes* ATCC 33743^T, L09171.

RESULTS

Enrichment and isolation

For enrichment and isolation of anaerobic, saccharolytic thermophiles, modified MB medium was inoculated with 10% (w/v) of samples. Prior to inoculation,

Na₂S·9H₂O and NaHCO₃ in sterile stock solution were injected to obtain final concentrations of 0.05% (w/v) and 0.2% (w/v), respectively. Turbidity caused by cell growth was observed after incubation at 80 °C for 3 d. Microscopic examination revealed bacterial populations composed of nonsporulating rods. After several successive transfers, the positive enrichment cultures were serially diluted and purified in agar deeps and roll tubes. Single colonies developed in the agar deeps and roll tubes after incubation at 65 °C for 2 d, and were then picked and subcultured in liquid medium. The process was repeated five times, after which the culture was considered to be pure. A pure culture was designated strain MB4^T.

Colony and cellular characteristics

Colonies of strain MB4^T in agar deeps or roll tubes were round, creamy white and had diameters of about 1–2 mm after 2 d. The cells of strain MB4^T were rods, 1–10 µm in length with a diameter of 0.5–0.6 µm, and occurred singly or in pairs or chains. The cells were Gram-negative by staining and by the KOH lysis test. Electron microscopy of ultrathin sections revealed a cell wall with a thin, intensely stained inner layer and a less densely stained outer layer (Fig. 1). No endospores were observed by phase-contrast microscopy under the conditions used and no cells survived in the heat

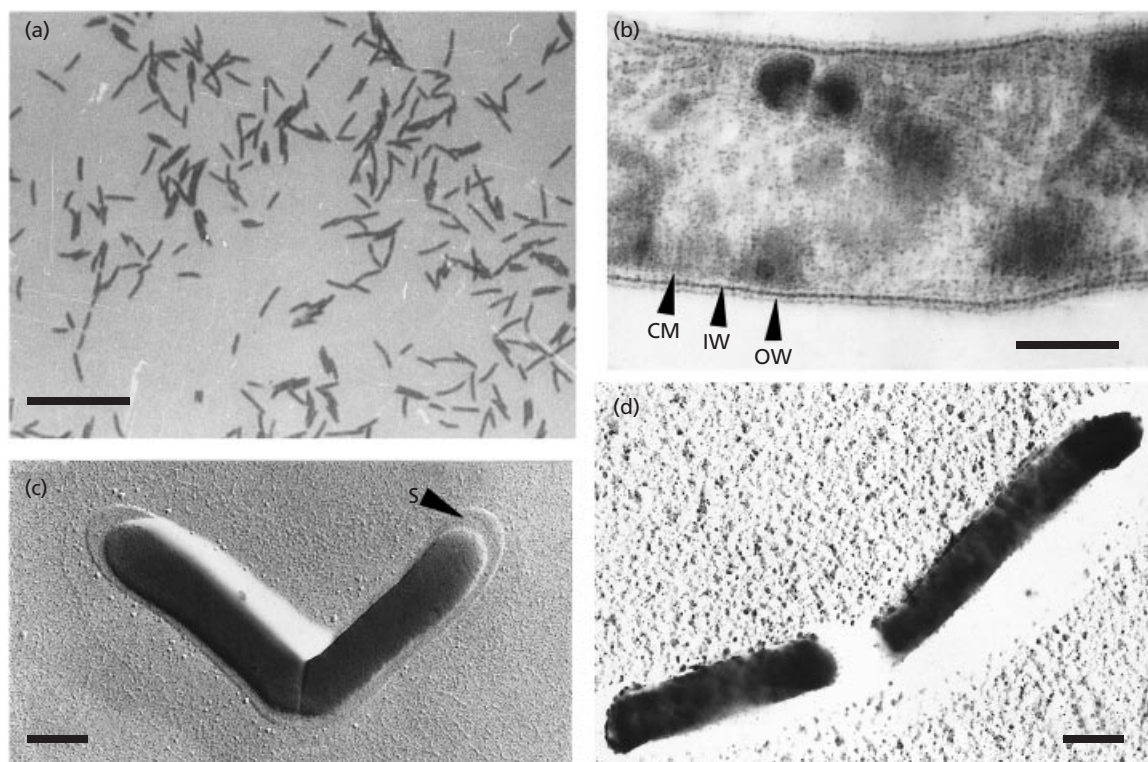


Fig. 1. Photomicrographs of strain MB4^T. (a) Phase-contrast micrograph. Bar, 10 µm. (b) Electron micrograph of ultrathin section showing the cell wall structure. OW, less densely stained outer layer; IW, intensely stained inner layer; CM, cytoplasmic membrane. Bar, 0.2 µm. (c) Electron micrograph of platinum-shadowed cells grown on glucose-MB medium showing the outer sheathlike structure (S). Bar, 0.5 µm. (d) Electron micrograph of platinum-shadowed cells grown on starch-MB medium. Bar, 0.5 µm.

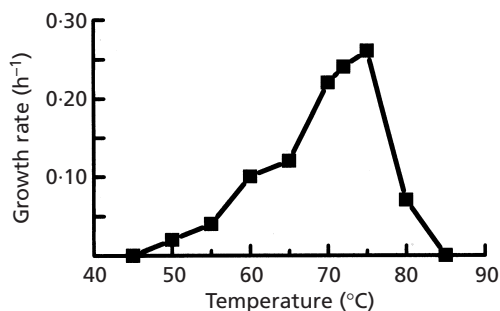


Fig. 2. Effect of temperature on the growth rate of strain MB4^T.

resistance tests, indicating the absence of heat-resistant forms. Motility was not observed by phase-contrast microscopy and flagella were not observed in negatively stained electron microscopic preparations. The cell walls contained *meso*-diaminopimelic acid. A marginally wider outer sheathlike structure was present in cells grown on glucose. No such material was present in cells grown on starch.

Physiological characteristics

Strain MB4^T required yeast extract for growth and carbohydrate metabolism. Yeast extract could not be replaced with Casamino acids or Tryptone. In the absence of glucose but in the presence of yeast extract, little growth was obtained, indicating that yeast extract did not serve as a sole carbon and energy source. Strain MB4^T grew well on starch, but could not degrade cellulose or xylan. Growth occurred on the following substrates: glucose, galactose, maltose, cellobiose, mannose, lactose, fructose and mannitol. No growth occurred on the following substrates: arabinose, sucrose, ribose, xylose, acetate, pyruvate, butyrate, lactate, citric acid, malic acid or glycerol. During glucose fermentation, 1.0 mmol acetate, 0.7 mmol ethanol, 1.5 mmol CO₂ and 0.3 mmol H₂ were produced per mmol glucose consumed. Propionate, isobutyrate and isovalerate were detected in trace amounts (< 0.1 mM). No lactate was detected.

Strain MB4^T grew in reduced MB medium. In the presence of oxygen (indicated by the pink colour of the resazurin), strain MB4^T did not grow, suggesting that it was obligately anaerobic. When thiosulfate or sulfur was included in the medium, the growth of strain MB4^T increased, with an increase in sulfide production. Sulfate or nitrate were not used as electron acceptors and did not affect the growth of strain MB4^T. Sulfite and H₂ inhibited growth of strain MB4^T, and H₂ inhibition could not be eliminated by addition of sulfur or thiosulfate to the culture.

Growth of strain MB4^T occurred at initial pH values ranging from 5.5 to 9.0, with an optimum of 7.0–7.5 at 75 °C. Growth occurred in the medium containing 0–2.5% NaCl, with an optimum of 0.2% at pH 7.5 and

75 °C. The temperature range for growth was 50–80 °C, with an optimum of around 75 °C at pH 7.5 (Fig. 2). Under optimal conditions, the shortest doubling time was approximately 65 min. At the end of optimal growth, the pH had decreased by 1.5–2.0 units.

Growth of strain MB4^T was completely inhibited by 100 µg ml⁻¹ chloromycetin, polymyxin B, streptomycin sulfate or tetracycline.HCl. Ampicillin or penicillin did not inhibit growth at the same concentration.

DNA base composition

The G+C content of the DNA of strain MB4^T determined by the thermal denaturation temperature was 33 ± 0.2 mol %.

16S rDNA sequence analysis

A 1512 bp sequence of 16S rDNA from strain MB4^T was determined and compared with the members of the genus *Thermoanaerobacter* and related organisms. Positions of sequence alignment uncertainty were omitted from the analysis, and 1420 unambiguous nucleotides were used for computing evolutionary distance. The resulting phylogenetic tree (Fig. 3) showed strain MB4^T to be in the cluster comprising members of the genus *Thermoanaerobacter* and to constitute a monophyletic unit in the genus *Thermoanaerobacter*. The 16S rDNA sequence of strain MB4^T exhibited levels of similarity ranging from 92.4 to 93.7% with the sequences of the bacteria currently assigned to the genus *Thermoanaerobacter* (Table 1). The highest level of sequence similarity (93.7%) was with *Thermoanaerobacter brockii* subsp. *brockii* (Zeikus *et al.*, 1979).

DISCUSSION

The genus *Thermoanaerobacter* was first introduced by Wiegel & Ljungdahl (1981) and 14 species are currently recognized as valid: *Thermoanaerobacter acetethylicus* (Ben-Bassat & Zeikus, 1981), *Thermoanaerobacter brockii* subsp. *brockii* (Zeikus *et al.*, 1979), *Thermoanaerobacter brockii* subsp. *finnii* (Schmid *et al.*, 1986), *Thermoanaerobacter brockii* subsp. *lactiethylicus* (Cayol *et al.*, 1995), *Thermoanaerobacter ethanolicus* (Wiegel & Ljungdahl, 1981), *Thermoanaerobacter kivui* (Leigh & Wolfe, 1983), *Thermoanaerobacter mathranii* (Larsen *et al.*, 1997), *Thermoanaerobacter thermohydrosulfuricus* (Lee *et al.*, 1993), *Thermoanaerobacter thermocopriae* (Jin *et al.*, 1988), *Thermoanaerobacter wiegelii* (Cook *et al.*, 1996), *Thermoanaerobacter italicus* (Kozianowski *et al.*, 1997), *Thermoanaerobacter siderophilus* (Slobodkin *et al.*, 1999), *Thermoanaerobacter subterraneus* (Fardeau *et al.*, 2000) and *Thermoanaerobacter sulfurophilus* (Bonch-Osmolovskaya *et al.*, 1997).

The new isolate MB4^T shares some phenotypic characteristics with members of the genus *Thermo-*

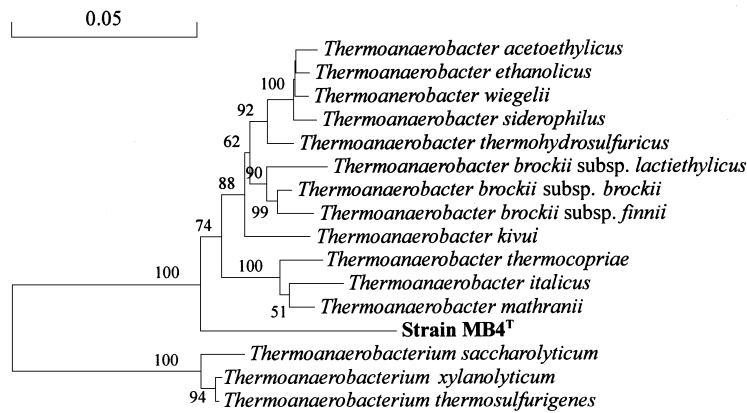


Fig. 3. Phylogenetic tree showing the position of strain MB4^T within the genus *Thermoanaerobacter* and related taxa based on 16S rDNA sequence similarity values. The numbers at the nodes of branches are the confidence values (expressed as percentages) obtained from 100 replications. Only values above 50 % are indicated. Bar represents 5 base substitutions per 100 nucleotides.

Table 1. Distinguishing properties among strain MB4^T and other members of the genus *Thermoanaerobacter*

1, Strain MB4^T; 2, *Thermoanaerobacter brockii* subsp. *brockii*; 3, *Thermoanaerobacter ethanolicus*; 4, *Thermoanaerobacter acetoethylicus*; 5, *Thermoanaerobacter kivui*; 6, *Thermoanaerobacter thermohydrosulfuricus*; 7, *Thermoanaerobacter wiegelii*; 8, *Thermoanaerobacter italicus*; 9, *Thermoanaerobacter thermocopriae*; 10, *Thermoanaerobacter siderophilus*; 11, *Thermoanaerobacter mathranii*.

Feature	1	2	3	4	5	6	7	8	9	10	11
Gram reaction	-	+	v	-	-	v	-	-	-	+	v
Spores	-	+	-	-	-	+	+	+	+	+	+
Cell wall peptidoglycan diamino acid	<i>m</i> -DAP	NR	<i>m</i> -DAP	NR	NR	<i>m</i> -DAP	NR	<i>m</i> -DAP	NR	NR	NR
Motility	-	+	+	+	-	+	+	-	+	+	+
Optimum temperature (°C)	75	68	69	65	66	68	66	70	60	70	70
DNA G + C content (mol%)	33	31	32	31	38	37	35	34	37	32	37
H ₂ inhibition	+	NR	NR	-	-	+	NR	NR	NR	-	+
Starch hydrolysis	+	+	+	+	-	+	+	+	+W	+	NR
Xylan degradation	-	+	+	-	-	NR	NR	+	+W	-	+
Growth substrates:											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Sucrose	-	+	+	+	-	+	+	+	-	+	+
Ribose	-	-	+	NR	-	+	-	NR	-	NR	+
Xylose	-	-	+	-	NR	+	+	+	-	+	+
Arabiose	-	+	-	NR	NR	+W	-	+	NR	-	+
Mannitol	+	-	-	NR	-	v	+	NR	+	NR	+
Glycerol	-	NR	-	NR	-	-	+	NR	-	+	-
Pyruvate	-	+	+	-	+	+	-	NR	NR	+	NR
Fermentation products:											
Acetate	+	+	+	+	+	+	+	+	+	-	+
Lactate	-	+	+	-	-	+	+	+	+	+	+
Ethanol	+W	+	+	+	-	+	+	+	+	+	+
Antibiotic inhibition:											
Chloromycetin	+	+	+	-	NR	NR	-	NR	NR	+	+
Penicillin	-	+	-	+	NR	+	-	NR	NR	-	+
Streptomycin	+	+	-	+	NR	NR	-	NR	NR	-	NR
Tetracycline	+	+	-	+	NR	NR	+	NR	NR	NR	+
16S rDNA sequence similarity with MB4 ^T (%)		93.7	92.7	92.9	92.6	93.2	93.1	92.4	92.5	93.0	92.6

* NR, Not reported; v, variable; +, positive; -, negative; +w, weak.

anaerobacter, i.e. obligately anaerobic rods, growth above 70 °C, reduction of thiosulfate and sulfur to hydrogen sulfide, fermentation of glucose to acetate

and ethanol and a DNA G + C content of 33 mol %. In these respects, strain MB4^T may be considered to be related to the genus *Thermoanaerobacter*.

However, strain MB4^T is unlike the currently accepted species of *Thermoanaerobacter* in several important phenotypic properties, such as absence of spore production, Gram-staining reaction, motility, lactate production, xylan utilization, growth on some substrates and antibiotic susceptibility (Table 1). Furthermore, the optimum temperature for growth of strain MB4^T (75 °C) is the highest among species of the genus *Thermoanaerobacter*. These data suggest that strain MB4^T can be clearly differentiated from the validly described *Thermoanaerobacter* species.

The proposed relationship between strain MB4^T and the other species of the genus *Thermoanaerobacter* is also supported by the phylogenetic analysis. As shown previously, the genus *Thermoanaerobacter* forms a distinct phylogenetic cluster (Rainey *et al.*, 1993; Collins *et al.*, 1994). The results of our 16S rDNA sequence analysis indicated that strain MB4^T is clearly related to *Thermoanaerobacter brockii* subsp. *brockii* (level of similarity, 93.7%) and falls into the *Thermoanaerobacter* cluster (Fig. 3). Moreover strain MB4^T represents a distinct branch within the *Thermoanaerobacter* cluster. The levels of 16S rDNA similarity between strain MB4^T and other *Thermoanaerobacter* spp. range from 92.4 to 93.7%, which is lower than that between species previously assigned to the genus *Thermoanaerobacter* on the basis of DNA–DNA homology values (Lee *et al.*, 1993; Cayol *et al.*, 1995). Stackebrandt & Goebel (1994) demonstrated that strains belonging to the same genus that exhibit less than 97% 16S rDNA sequence similarity should be considered members of different species. The level of 16S rDNA sequence similarity of 93.7% is low enough to designate strain MB4^T as a new species.

On the basis of the results described above, we propose strain MB4^T as a new species of the genus *Thermoanaerobacter*, namely *Thermoanaerobacter tengcongensis*. The type strain is MB4^T, which has been deposited in the Chinese Collection of Microorganisms as strain AS 1.2430^T.

Description of *Thermoanaerobacter tengcongensis* sp. nov

Thermoanaerobacter tengcongensis (teng.con.gen'sis. N.L. masc. adj. *tengcongensis* pertaining to Tengcong, China).

Cells are Gram-negative rods, 0.5–0.6 µm in diameter and 1–10 µm in length, occurring singly, in pairs or in chains. No spores are observed. Strictly anaerobic and thermophilic. Growth occurs at temperatures between 50 and 80 °C, with an optimum of around 75 °C; at pH values between 5.5 and 9.0, with an optimum of 7.0–7.5; and at salinities between 0 and 2.5% NaCl, with an optimum of around 0.2% NaCl. Optimal doubling time is about 65 min. Heterotrophic growth requires yeast extract, which cannot be replaced by Tryptone or Casamino acids. Utilizes glucose, galactose, maltose, cellobiose, mannose, fructose, lactose, mannitol and starch, but not arabinose, sucrose,

ribose, xylose, cellulose, alginate, xylan, acetate, pyruvate, butyrate, lactate, citrate, malate or glycerol. The major fermentation end product on glucose is acetate. Hydrogen and sulfite inhibit growth. Thio-sulfate and sulfur stimulate growth. Chloromycetin, polymyxin B, streptomycin sulfate and tetracycline. HCl inhibit growth. The genomic DNA has a G+C content of 33 mol%. The isolate was obtained from a hot spring in Tengcong, China. The type strain is MB4^T (= Chinese Collection of Microorganisms AS 1.2430^T = JCM 11007^T).

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