***Thermoanaerobacter subterraneus* sp. nov., a novel thermophile isolated from oilfield water**

Marie-Laure Fardeau,¹ Michel Magot,² Bharat K. C. Patel,³
Pierre Thomas,¹ Jean-Louis Garcia¹ and Bernard Ollivier^{1,3}

Author for correspondence: Bernard Ollivier. Tel: +33 4 91 82 85 76. Fax: +33 4 91 82 85 70.
e-mail: ollivier@esil.univ-mrs.fr

¹ Laboratoire de Microbiologie IRD, Universités de Provence et de la Méditerranée, ESIL case 925, 163 Avenue de Luminy, 13288 Marseille cedex 9, France

² Sanofi Recherche, Unité de Microbiologie, 31676 Labège, France

³ School of Biomolecular and Biomedical Sciences, Faculty of Science, Griffith University, Brisbane, Queensland 4111, Australia

A new thermophilic, anaerobic glucose-fermenting, Gram-positive, rod-shaped bacterium, designated strain SEBR 7858^T, was isolated from an oilfield water sample. Under optimal conditions on a glucose-containing medium (3% NaCl, 65 °C and pH 7.5), the generation time was 2.5 h. No growth occurred at 35 or 80 °C, nor at pH 5.5 or 9.0. Strain SEBR 7858^T possessed lateral flagella. Spores were undetected but heat-resistant forms were present. Strain SEBR 7858^T fermented a range of carbohydrates to acetate, L-alanine, lactate, H₂ and CO₂. The isolate reduced thiosulfate and elemental sulfur, but not sulfate or sulfite to sulfide. In the presence of thiosulfate, the ratio of acetate produced per mole of glucose consumed increased, suggesting a shift in the use of electron acceptors during carbohydrate metabolism. The DNA G+C content was 41 mol%. Based on 16S rRNA gene sequence analysis, the strain was almost equidistantly related to all members of the genus *Thermoanaerobacter* (mean similarity 92%). Based on phenotypic, genomic and phylogenetic characteristics, strain SEBR 7858^T was clearly different from all members of the genus *Thermoanaerobacter* and was therefore designated as a new species, *Thermoanaerobacter subterraneus* sp. nov. The type strain is SEBR 7858^T (= CNCM I-2383^T, DSM 13054^T).

Keywords: thiosulfate reduction, *Thermoanaerobacter subterraneus* sp. nov., thermophile, phylogeny, oil well

INTRODUCTION

In the last two decades, intensive research on anaerobic, thermophilic, carbohydrate-fermenting micro-organisms from marine and terrestrial volcanic hot springs has led to the isolation of several new genera and species in the domains *Bacteria* and *Archaea*. The major rationale for this research stems from the biotechnological potential and the basic evolutionary traits of these microbes. In *Bacteria*, particular attention has been paid to members of the order *Thermotogales* and the family *Thermoanaerobiaceae*. The latter includes the genera *Thermoanaerobacter* and *Thermoanaerobacterium*, whose members reduce thiosulfate or elemental sulfur, respectively, to sulfide (Lee *et al.*, 1993; Wiegel & Ljungdahl, 1981). *Thermoanaerobacter* species are thermophilic, heterotrophic, saccharolytic anaerobes found in soil, faeces,

sugar beet and sugar cane extraction juices, thermal spas, volcanic hot springs, non-volcanic geothermally heated subsurface aquifers, hydrothermal vents and oil-producing wells (Cayol *et al.*, 1995; Cook *et al.*, 1996; Jin *et al.*, 1988; Kozianowski *et al.*, 1997; Larsen *et al.*, 1997; Lee *et al.*, 1993; Leigh *et al.*, 1981; Slobodkin *et al.*, 1999; Wiegel, 1986; Wynter *et al.*, 1996). *Thermoanaerobacter* species originating from oil-producing wells have only recently been studied and have been found to be similar to surface-inhabiting *Thermoanaerobacter* species (Cayol *et al.*, 1995; Magot *et al.*, 2000; Wynter *et al.*, 1996). We report here on the characterization of a new member of this genus, *Thermoanaerobacter subterraneus* sp. nov., which was isolated from a French oilfield.

METHODS

Collection site. Strain SEBR 7858^T was isolated from the 'Lacq Supérieur' oilfield located in south-west France. The *in situ* temperature of the geological formation, at a depth of 645 m, was 52 °C. A water sample was aseptically collected

The GenBank accession number for the 16S rRNA gene sequence of strain SEBR 7858^T is AF195797.

at the wellhead, as described previously (Bernard *et al.*, 1992), transported to the laboratory and stored at 4 °C until required.

Medium, enrichment and isolation. Enrichment and routine growth were performed using a culture medium containing (l^{-1}): NH_4Cl , 1.0 g; K_2HPO_4 , 0.3 g; KH_2PO_4 , 0.3 g; $MgCl_2 \cdot 6H_2O$, 1.0 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $NaCl$, 10.0 g; KCl , 0.2 g; $Na_2S_2O_3$, 3.16 g; glucose, 20 mM; sodium thiosulfate, 20 mM; cysteine/HCl, 0.5 g; yeast extract (Difco), 2.0 g; Bio-trypticase (BioMérieux), 2.0 g; trace mineral element solution (Balch *et al.*, 1979), 10 ml; resazurin, 1.0 mg. The pH was adjusted to 7.0 with 10 M KOH and the medium was boiled under a stream of O_2 -free N_2 gas and cooled to room temperature. Five or 20 ml aliquots were dispensed into Hungate tubes or serum bottles, respectively, under a stream of N_2/CO_2 (80:20, v/v) gas and the vessels were autoclaved for 45 min at 110 °C. Prior to inoculation, $Na_2S \cdot 9H_2O$ and $NaHCO_3$ were injected from sterile stock solutions to final concentrations of 0.04 and 0.2 %, respectively. An aliquot of the water sample was inoculated into 20 ml medium and incubated at 70 °C without agitation to initiate an enrichment culture. Several pure cultures were obtained by picking well isolated colonies that developed in the culture medium solidified with 4 % (w/v) Phytigel (Sigma; Deming & Baross, 1986) by the repeated use of the Hungate roll-tube method (Hungate, 1969). One of these was used for subsequent studies.

Characterization studies. pH, temperature and NaCl ranges for growth were determined using the culture medium. The medium in Hungate tubes was adjusted to the desired pH, measured at ambient temperature, by injecting 10 % (w/v) sterile anaerobic stock solutions of $NaHCO_3$ or Na_2CO_3 . NaCl was weighed directly in the tubes prior to dispensing the culture medium. The strain was subcultured at least once under the same experimental conditions prior to determination of growth rates. Substrates were tested in culture medium lacking glucose at a final concentration of 20 mM. To test for electron acceptors, sodium thiosulfate, sodium sulfate, sodium sulfite and elemental sulfur were added to the culture medium at final concentrations of 20 mM, 20 mM, 2 mM and 2 % (w/v), respectively.

Light and electron microscopy. Light microscopy was performed as described by Cayol *et al.* (1994). Electron microscopy was performed as described by Fardeau *et al.* (1997a).

Analytical techniques. Unless otherwise indicated, duplicate culture tubes were used throughout this study. Growth was measured by inserting tubes directly into a model UV-160A spectrophotometer (Shimadzu) and measuring changes in OD_{580} . Sulfide was determined photometrically as colloidal CuS by using the method of Cord-Ruwisch (1985). H_2 , CO_2 , sugars, alcohols, volatile and non-volatile fatty acids were measured as described previously (Fardeau *et al.*, 1996). L-Alanine was measured by HPLC as described by Moore *et al.* (1958).

Determination of DNA G+C content. The DNA G+C content was determined by the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany). DNA was isolated and purified by chromatography on hydroxyapatite and its G+C content was determined by using HPLC as described by Mesbah *et al.* (1989). Non-methylated λ DNA (Sigma) was used as the standard.

16S rDNA sequence analysis. The methods for the purification and extraction of DNA and the amplification and

sequencing of the 16S rRNA gene have been described previously (Andrews & Patel, 1996). The 16S rRNA gene sequence was manually aligned with reference sequences of various members of *Bacteria* using the editor ae2 (Maidak *et al.*, 1999). Reference sequences were obtained from the Ribosomal Database Project (Maidak *et al.*, 1999), EMBL and GenBank databases. Positions of sequence and alignment uncertainty were omitted from the analysis. The pairwise evolutionary distances based on 1224 unambiguous nucleotides were computed by using the method of Jukes & Cantor (1969) and dendrograms were constructed from these distances by using the neighbour-joining method, both of which were from part of the PHYLIP suite of programs (Felsenstein, 1993). A maximum-likelihood approach with FastDNAm1 was used as an alternative method for tree construction (Olsen *et al.*, 1994).

RESULTS

Enrichment and isolation

Enrichment cultures were positive after incubation at 70 °C for 3 d and H_2S was detected, presumably derived from thiosulfate reduction. Microscopic examination revealed the presence of rod-shaped bacteria. Single, well isolated colonies (3 mm diam.) that developed in Phytigel roll tubes after 3 d incubation at 70 °C were picked and serially diluted in Phytigel roll tubes at least twice before the culture was considered pure. Several axenic cultures were obtained and the culture designated strain SEBR 7858^T was used for further studies.

Morphology

Strain SEBR 7858^T was a rod-shaped bacterium ($0.5\text{--}0.7 \times 2\text{--}8 \mu m$), which occurred singly (Fig. 1a). No motility was observed but the cells possessed laterally inserted peritrichous flagella (Fig. 1b). No spores were observed under microscopic examination (electron and light microscopy), but cultures exposed at 120 °C for 45 min could be subcultured, suggesting the presence of heat-resistant forms. Electron microscopy of ultrathin sections revealed a Gram-positive cell wall composed of a thin dense surface layer separated from a thicker inner layer (Fig. 1c).

Optimum growth conditions

Strain SEBR 7858^T was strictly anaerobic and did not grow in culture medium containing traces of oxygen, as indicated by the anaerobiosis indicator, resazurin. The strain grew optimally at 65 °C (temperature range between 40 and 75 °C, but not at 80 °C; Fig. 2a) and a pH of 7.5 (pH range between 6.0 and 8.5; Fig. 2b). The isolate grew in the presence of 0–3 % NaCl with an optimum of 0 % at pH 7.0 and 70 °C (Fig. 2c).

Substrates for growth

Yeast extract or Bio-trypticase was required for growth on carbohydrates. Yeast extract could not be replaced by a vitamin mixture (Balch *et al.*, 1979).

Fig. 1. (a) Phase-contrast micrograph of strain SEBR 7858^T. Bar, 10 μ m. (b) Electron micrograph of a negatively stained culture of strain SEBR 7858^T showing laterally inserted flagella. Bar, 2 μ m. (c) Electron micrograph of an ultrathin section of strain SEBR 7858^T showing the cytoplasmic membrane (cm), the inner layer (il) and the outer layer (ol). Bar, 0.5 μ m.

SEBR 7858^T grew on the following substrates (at a concentration of 20 mM unless otherwise indicated) in the presence of thiosulfate as the electron acceptor: D-

Fig. 2. Effect of (a) temperature (pH 7.5, no NaCl), (b) NaCl (65 °C, pH 7.5) and (c) pH (65 °C, no NaCl) on the growth of strain SEBR 7858^T cultivated in basal medium.

fructose, D-galactose, D-glucose, DL-lactose, DL-maltose, D-mannose, D-xylose, D-ribose, mannitol, cellobiose, pyruvate, melibiose, starch and xylan, but not L-xylose, L-arabinose, L-rhamnose, sorbose or sucrose. Glycerol was used poorly. Acetate, L-alanine, lactate, H₂ and CO₂ were produced from glucose fermentation. From 0.6 to 0.8 mol L-alanine was produced per mole glucose consumed (Table 1).

Effect of electron acceptors

Strain SEBR 7858^T reduced thiosulfate, elemental sulfur and sulfite, but not sulfate to sulfide. The presence of thiosulfate altered the concentration of metabolites during glucose oxidation, indicating that thiosulfate modified the metabolic pathway of strain

Table 1. End products from glucose metabolism by *Thermoanaerobacter subterraneus*, in the presence or absence of thiosulfate (20 mM)

Growth conditions	Amount of glucose consumed (mmol)	Amount of product (mmol)*				
		Acetate	Lactate	L-Alanine	H ₂	H ₂ S
Glucose	9.4	10.6	1.2	8.0	5.7	0
Glucose + thiosulfate	8.6	20.3	0	0	0.2	21.9

* Data were corrected for non-utilizable glucose present in the medium.

Fig. 3. Dendrogram indicating the position of strain SEBR 7858^T amongst members of the genus *Thermoanaerobacter*. Sequences of the following type strains were obtained from the Ribosomal Database Project, version 7.0 (Maidak *et al.*, 1999): *Thermoanaerobacter acetoethylicus* HTB2/W^T (= ATCC 33265^T, DSM 2359^T), *Thermoanaerobacter Brockii* subsp. *Brockii* HTD4^T (= ATCC 33075^T, DSM 1457^T), *Thermoanaerobacter Brockii* subsp. *finni* AKO-1^T (= DSM 3389^T), *Thermoanaerobacter Brockii* subsp. *lactiethylicus* SEBR 5268^T (= DSM 9801^T), *Thermoanaerobacter ethanolicus* JW 200^T (= ATCC 31550^T, DSM 2246^T), *Thermoanaerobacter kivui* ATCC 33488^T (= DSM 2030^T), *Thermoanaerobacter mathranii* A3^T (= DSM 11426^T), *Thermoanaerobacter siderophilus* SR4^T (= DSM 12299^T), *Thermoanaerobacter sulfurophilus* L-64^T (= DSM 11584^T), *Thermoanaerobacter thermocopriae* (= IAM 13577^T), *Thermoanaerobacter thermohydrosulfuricus* E100-69^T (= DSM 567^T), *Thermoanaerobacter wiegelii* Rt8.B1^T (= DSM 10319^T). Bootstrap values, expressed as a percentage of 100 replications, are shown at branching points. Only values above 95% were considered significant and reported. Scale bar indicates 5 substitutions per 100 nt.

SEBR 7858^T. In the presence of thiosulfate, L-alanine was not detected, hydrogen production decreased, whereas sulfide production increased and acetate was the only acid detected (Table 1).

16S rDNA sequence analysis

A total of 1372 nt of the 16S rDNA of strain SEBR 7858^T, corresponding to position 20–1470 of the *Escherichia coli* 16S rDNA, was sequenced. 16S rDNA sequence analysis and comparison with species representative of *Bacteria* indicated that strain SEBR 7858^T was a member of the genus *Thermoanaerobacter* and formed a deep line of descent almost equidistant to the 12 taxonomically validated species and subspecies with available sequences, with a mean similarity of 93% (Fig. 3).

DNA G + C content

The G + C content of isolate SEBR 7858^T was 41 mol% (as determined by HPLC).

DISCUSSION

Strains of various physiological groups of thermophilic bacteria have been previously isolated from deep subsurface environments such as oilfields and aquifers. In addition to methanogens and sulfate-reducing bacteria, heterotrophic micro-organisms, including members of the order *Thermotogales* and the family *Thermoanaerobiaceae* (e.g. the genus *Thermoanaerobacter*), have been isolated and described (Andrews & Patel, 1996; Cayol *et al.*, 1995; Fardeau *et al.*, 1997a; Jeanthon *et al.*, 1995; Magot *et al.*, 2000; Ravot *et al.*, 1995; Slobodkin *et al.*, 1999; Stetter *et al.*, 1993;

Wynter *et al.*, 1996). Strain SEBR 7858^T, a Gram-positive thermophilic anaerobic bacterium, isolated from a French oilfield, is phylogenetically related to *Thermoanaerobacter* species. The presence of a *Thermoanaerobacter* species in oilfields was first reported in 1995. The isolate was found to be a phylogenetic relative of *Thermoanaerobacter brockii* and *Thermoanaerobacter finnii* (Cayol *et al.*, 1995). It was recognized as a novel subspecies of the terrestrial micro-organism *Thermoanaerobacter brockii* and named *Thermoanaerobacter brockii* subsp. *lactiethylicus*. Because of the large number of new isolates belonging to *Thermoanaerobacter*, the taxonomy of this genus was revised on the basis of 16S rRNA sequence and DNA–DNA hybridization and now comprises 11 validated species, 3 subspecies and 3 non-validated species (Table 2).

The isolation of strain SEBR 7858^T extends our knowledge on the diversity of microbes from sub-terrestrial ecosystems and confirms that *Thermoanaerobacter* species, similar to members of the order *Thermotogales*, may be important components of the heterotrophic microflora of oilfield reservoirs (Grassia *et al.*, 1996; Magot *et al.*, 2000). Studies on a methanogenic archaeon, *Methanocalculus halotolerans* isolated from an African oilfield (Ollivier *et al.*, 1998), and the results reported by L'Haridon *et al.* (1995) suggest that indigenous micro-organisms might inhabit oilfield reservoirs. However, the presence of *Thermoanaerobacter* species in oilfield waters as a result of anthropogenic contamination rather than as an indigenous microflora cannot be ruled out entirely and further investigations are needed to understand their distribution. Nevertheless, *Thermoanaerobacter* species have a wide nutritional spectrum for growth, which includes carbohydrates and amino acids (Fardeau *et al.*, 1997b; Faudon *et al.*, 1995; Magot *et al.*, 2000); oilfields may contain such nutrients, provided by other members of the bacterial community, that will possibly support their growth and/or viability.

Strain SEBR 7858^T oxidizes hydrogen in the presence of sulfur compounds. This may lead to corrosion of oilfield installations when these latter compounds are available in oilfield waters. The presence of a hydrogen-oxidizing *Thermoanaerobacter* species was previously reported from oilfields (Fardeau *et al.*, 1993a, b). In addition, thiosulfate reduction by *Thermoanaerobacter* species, including strain SEBR 7858^T, may be of significance in mineralization processes in thermal terrestrial or subterrestrial environments, since this reduction facilitates oxidation of organic matter as reported previously (Fardeau *et al.*, 1996).

Strain SEBR 7858^T has many features typical of some members of the genus *Thermoanaerobacter*. It is Gram-positive, thermophilic (optimal temperature for growth is 65 °C), anaerobic and contains heat-resistant cells. The isolate reduces thiosulfate, sulfite and elemental sulfur, but not sulfate to sulfide. In addition, it ferments carbohydrates to L-alanine, acetate, lactate,

H₂ and CO₂. This constitutes the first experimental evidence of L-alanine production by a *Thermoanaerobacter* species. We have detected the presence of L-alanine from glucose fermentation in *Thermoanaerobacter brockii* and *Thermoanaerobacter ethanolicus*, but it is a minor product compared to strain SEBR 7858^T (unpublished data). As for some members of *Archaea* (Kengen & Stams, 1994; Kobayashi *et al.*, 1995), L-alanine production from glucose metabolism has been reported in hyperthermophilic and thermophilic members of *Bacteria* (e.g. *Thermotoga*, *Thermosiphon* and *Fervidobacterium*, order *Thermotogales*) and has been suggested to be a remnant of an ancestral metabolism (Ravot *et al.*, 1996). L-Alanine is also produced by a Gram-positive anaerobe belonging to the genus *Clostridium* (Örlygsson *et al.*, 1995). It is possible that L-alanine production from carbohydrate fermentation is more widespread amongst members of *Bacteria* than previously believed.

Strain SEBR 7858^T differs from its closest phylogenetic relatives, such as *Thermoanaerobacter brockii* and *Thermoanaerobacter ethanolicus*, by fermenting melibiose and by using sucrose and xylose poorly. In contrast to *Thermoanaerobacter brockii* (Zeikus *et al.*, 1979), strain SEBR 7858^T has a much higher DNA G+C content (41% for SEBR 7858^T compared to 30–31% for *Thermoanaerobacter brockii*). The isolate also differs from *Thermoanaerobacter ethanolicus* (Wiegel & Ljungdahl, 1981) as L-alanine is the major end product. Phylogenetic, genomic and phenotypic characteristics of strain SEBR 7858^T confirm that it represents a new species of the genus *Thermoanaerobacter* for which we propose the name *Thermoanaerobacter subterraneus* sp. nov., reflecting its sub-terrestrial origin.

Description of *Thermoanaerobacter subterraneus* sp. nov.

Thermoanaerobacter subterraneus (sub.ter.ra'ne.us. L. pref. *sub* less than; L. n. *terra* earth; L. adj. *subterraneus* under the earth, describing its site of isolation).

Cells are rods (0.5–0.7 × 2–8 µm), occur singly or in pairs and possess laterally inserted flagella. Spores are not observed under microscopic examination but cultures exposed to 120 °C for 45 min could be subcultured, suggesting the presence of heat-resistant forms. Electron microscopic examinations reveal a Gram-positive cell wall. Round colonies (3 mm diam.) develop in Phytigel roll tubes after 3 d incubation at 70 °C. Chemo-organotrophic and obligately anaerobic member of the domain *Bacteria*. Optimum temperature for growth is 65 °C at pH 7.5; temperature range for growth is 40–75 °C. Optimum pH is 7.5; growth occurs between pH 6.0 and 8.5. Halotolerant, growing in the presence of up to 3% NaCl. Yeast extract or Bio-trypticase is required for growth on carbohydrates. Growth on sugars is highly enhanced by the presence of both yeast extract and Bio-trypticase. Yeast extract cannot be replaced by vitamins. Fer-

Table 2. Characteristics that differentiate members of the genus *Thermoanaerobacter*

Data were taken from the following sources for the following species: 1, *Thermoanaerobacter subterraneus*, this study; 2, '*Thermoanaerobacter acetigenus*', Nielsen *et al.* (1993); 3, *Thermoanaerobacter acetoethylicus*, Ben-Bassat & Zeikus (1981); 4, *Thermoanaerobacter brockii* subsp. *brockii*, Zeikus *et al.* (1979); 5, *Thermoanaerobacter brockii* subsp. *finnii*, Schmid *et al.* (1986); 6, *Thermoanaerobacter brockii* subsp. *lactiethylicus*, Cayol *et al.* (1995); 7, '*Thermoanaerobacter cellulolyticus*', Taya *et al.* (1988); 8, *Thermoanaerobacter ethanolicus*, Wiegel & Lungdahl (1981); 9, *Thermoanaerobacter italicus*, Kozianowski *et al.* (1997); 10,

Character	1	2	3	4	5	6	7	8
Type strain	DSM 13054 [†]	DSM 7040	DSM 2359 [†]	DSM 1457 [†]	DSM 3389 [†]	DSM 9801 [†]	DSM 8991	DSM 2246 [†]
Source	Oil well	Hot spring	Hot spring	Hot spring	Lake Kivu	Oil well	Hot spring	Hot spring
Temperature range (°C)	40–75	50–78	40–80	35–85	40–75	40–75	50–85	37–78
Optimum temperature (°C)	65	65–68	65	65–70	64–66	55–60	75	70
pH range	6–8.5	5.2–8.6	5.5–8.5	5.5–9.5	ND	5.6–8.8	6–9.5	4.4–9.9
Optimum pH	7.5	7	ND	7.5	6.5–6.8	ND	8–8.3	6–8
G + C content (mol %)	41	34.9–36.5	30–32	29–32.4	32	35	37.7	37–39
Reduction of S ⁰	+	ND	—	—	ND	+	ND	ND
Reduction of S ₂ O ₃ ²⁻	+	—	+	+	+	+	ND	ND
Flagella	Peritrichous	—	Peritrichous	—	Peritrichous	Peritrichous	Peritrichous	Peritrichous
Substrates used:								
Arabinose	—	+	—	—	ND	—	—	ND
Cellobiose	+	+	+	+	+	+	+	+
Fructose	+	+	ND	ND	+	+	+	+
Galactose	+	+	ND	ND	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Inositol	ND	ND	ND	ND	ND	ND	—	—
Lactose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Mannitol	+	—	ND	ND	+	+	—	—
Mannose	+	+	+	—	+	+	+	+
Melezitose	ND	ND	ND	ND	ND	ND	—	—
Melibiose	+	ND	ND	ND	ND	—	—	—
Raffinose	ND	+	ND	ND	ND	ND	+	—
Rhamnose	—	—	ND	ND	ND	—	—	—
Ribose	+	—	ND	ND	+	+	—	+
Starch	+	+	+	+	ND	+	+	+
Sucrose	—	+	+	+	+	+	+	+
Trehalose	ND	+	ND	ND	ND	ND	—	—
Xylose	+	+	—	—	+	+	+	+
Xylan	+	+	ND	ND	ND	ND	+	ND
Formate	ND	ND	ND	ND	ND	ND	ND	ND
Lactate	ND	—	—	—	ND	ND	ND	ND
Pyruvate	+	—	—	+	+	+	ND	+
Beef extract	ND	ND	ND	ND	ND	ND	ND	ND
Tryptone	ND	ND	+	—	ND	ND	ND	ND
Yeast extract	ND	—	+	ND	ND	+	ND	ND
Amygdalin	ND	ND	ND	ND	ND	ND	—	—
Cellulose	ND	—	ND	—	ND	—	+	—
Chitin	ND	ND	ND	ND	ND	ND	ND	ND
Aesculin	ND	ND	ND	ND	ND	ND	—	+
Glucosamine	ND	ND	ND	ND	ND	ND	ND	ND
Glycerol	+	ND	ND	ND	ND	—	—	—
Glycogen	ND	ND	ND	ND	ND	ND	—	ND
Hemicellulose	ND	ND	ND	ND	ND	ND	ND	ND
Inulin	ND	ND	ND	ND	ND	ND	—	ND
Pectin	ND	ND	—	—	ND	ND	—	ND
Diagnostic fermentation products from glucose*	Ethanol, lactate, L-alanine	Ethanol, isobutyrate	Ethanol, isobutyrate, butyrate, isovalerate, valerate	Ethanol, lactate	Ethanol, lactate	Ethanol	Lactate	Ethanol, lactate

* All species tested produced acetate, H₂ and CO₂ with the exception of *Thermoanaerobacter siderophilus* which did not produce acetate.

† Homoacetogenic species.

Table 2 (cont.)

Thermoanaerobacter kivui, Zeikus *et al.* (1979); 11, '*Thermoanaerobacter lactoethylicus*', Kondratieva *et al.* (1989); 12, *Thermoanaerobacter mathranii*, Larsen *et al.* (1997); 13, *Thermoanaerobacter siderophilus*, Slobodkin *et al.* (1999); 14, *Thermoanaerobacter sulfurophilus*, Bonch-Osmolovskaya *et al.* (1997); 15, *Thermoanaerobacter thermocopriae*, Jin *et al.* (1988); 16, *Thermoanaerobacter thermohydrosulfuricus*, Wiegel *et al.* (1979); 17, *Thermoanaerobacter wiegelii*, Cook *et al.* (1996). ND, Not determined.

9	10	11	12	13	14	15	16	17
DSM 9252 ^T Hot spring	DSM 2030 ^T Lake Kivu	DSM 9003 Hot spring	DSM 11426 ^T Hot spring	DSM 12299 ^T Hydrothermal vent	DSM 11584 ^T Cyanobacterial mats	IAM 13577 ^T Hot spring	DSM 567 ^T Hot spring	DSM 10319 ^T Geothermal water
45–78	50–72	42–75	47–78	39–78	44–75	47–74	40–78	38–78
70	66	65	70–75	69–71	55–60	60	68	65–68
ND	5.3–7.3	5–8.5	4.7–8.8	4.8–8.2	4.5–8	6–8	5–9	5.5–7.2
7	6.4	7	6.8–7.8	6.3–6.5	6.8–7.2	6.5–7.3	6.9–7.5	6.8
34.4	38	34.6	37	32	29.8–30.8	6.7–37.8	37.6	34.4–36.8
–	ND	+	+	+	+	ND	–	–
+	ND	ND	+	+	+	ND	+	+
–	–	–	Peritrichous	Peritrichous	Peritrichous	ND	Lateral	Peritrichous
+	ND	+	+	–	+	+	+	–
+	–	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+
+	–	+	–	ND	ND	–	+	+
+	+	+	+	+	+	+	+	+
ND	–	ND	ND	ND	+	ND	–	–
+	–	+	+	ND	+	+	ND	+
+	–	+	+	+	+	+	+	+
ND	–	+	+	ND	+	–	ND	+
+	+	ND	+	ND	ND	+	+	+
+	ND	ND	+	ND	ND	–	ND	–
+	ND	ND	+	ND	ND	–	ND	–
+	–	ND	+	ND	ND	–	+	+
ND	ND	+	–	ND	+	–	+	–
ND	–	ND	+	ND	ND	–	+	–
+	ND	ND	–	+	+	–	–	+
+	–	+	ND	+	+	–	+	+
+	–	+	+	+	+	–	+	+
+	–	ND	+	ND	ND	+	ND	+
+	ND	+	+	+	+	ND	+	+
ND	ND	ND	+	–	ND	–	ND	–
ND	+	ND	ND	–	–	ND	ND	ND
ND	–	ND	ND	–	+	ND	ND	–
ND	+	ND	ND	+	+	ND	+	–
ND	ND	ND	ND	+	ND	ND	ND	ND
ND	ND	ND	ND	+	ND	ND	ND	ND
+	ND	ND	–	+	ND	ND	ND	ND
–	ND	ND	+	ND	ND	+	ND	ND
ND	–	ND	–	–	–	+	ND	ND
+	ND	ND	ND	ND	ND	ND	ND	+
+	ND	ND	ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND	ND	–	+
+	–	ND	–	+	ND	ND	–	+
–	ND	ND	+	ND	ND	+	ND	ND
+	ND	ND	ND	ND	ND	+	ND	ND
+	ND	ND	–	ND	ND	–	ND	ND
Ethanol, lactate, succinate	Acetate†	Ethanol, lactate, propionate, butyrate, isovalerate	Ethanol, lactate	Ethanol, lactate	Ethanol, lactate	Ethanol, butyrate, lactate	Ethanol, lactate, formate, propanol, 2-propanol, butyrate, isovalerate, isocaproate	Ethanol, lactate, propionate

ments cellobiose, D-fructose, D-galactose, D-glucose, DL-lactose, DL-maltose, D-mannose, melibiose, D-ribose, starch, D-xylose, mannitol, pyruvate and xylan. Glycerol is poorly utilized and L-arabinose, L-rhamnose, sorbose, sucrose and L-xylose are not used. Acetate, L-alanine, lactate, H₂ and CO₂ are produced during glucose fermentation. Elemental sulfur, thiosulfate and sulfite, but not sulfate, are used as electron acceptor. The DNA G+C content is 41 mol% (as determined by HPLC). Isolated from oilfield water. Type strain is SEBR 7858^T (= CNCM I-2383^T, DSM 13054^T).

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