NOTE

***Thermanaeromonas toyohensis* gen. nov., sp. nov., a novel thermophilic anaerobe isolated from a subterranean vein in the Toyoha Mines**Koji Mori,¹ Satoshi Hanada,¹ Akihiko Maruyama¹ and Katsumi Marumo²

^{1,2} Research Institute of Biological Resources¹ and Marine Resources and Environment Institute², National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan

Author for correspondence: Satoshi Hanada. Tel: +81 298 61 6591. Fax: +81 298 61 6587. e-mail: s-hanada@aist.go.jp

A novel thermophilic, strictly anaerobic, thiosulfate-reducing bacterium, designated strain ToBE^T, was isolated from a geothermal aquifer at a depth of 550 m in the Toyoha Mines (Hokkaido, Japan). The cells of this bacterium were rod-shaped (0.6 × 2–6 µm), non-motile and sporulating. Strain ToBE^T was able to grow on formate, lactate, pyruvate or various sugars in the presence of thiosulfate as an electron acceptor. The strain could grow at 55–73 °C and pH 5.5–8.5. The optimum temperature and pH for the growth were 70 °C and pH 6.5. The G+C content of the DNA was 49.6 mol%. The major quinone and cellular fatty acids were respectively menaquinone-7 and iso-C_{15:0} and iso-C_{17:0}. Analysis of the 16S rDNA revealed that the isolate was a member of the Gram-positive bacteria and was related to the genus *Thermoanaerobacter*. However, the phylogenetic tree showed that the strain was distant from any other known bacteria, with sequence similarities of less than 90%. On the basis of phenotypic features and phylogenetic analysis, the name *Thermanaeromonas toyohensis* gen. nov., sp. nov., is proposed for the isolate, with strain ToBE^T (= DSM 14490^T = JCM 11376^T) as the type strain.

Keywords: *Thermanaeromonas toyohensis* gen. nov., sp. nov., thermophile, anaerobe, subterranean

The deep subsurface is one of the major habitats for micro-organisms (Gold, 1992; Whitman *et al.*, 1998). Over the past decade, a great deal of attention has been paid to the subsurface biosphere, since a large number of micro-organisms have been discovered in such environments. It was inferred by direct enumeration that 3.8–6.0 × 10³⁰ micro-organisms inhabit the ground-water, unconsolidated sediments and pore spaces of the subsurface. This value is equivalent to approximately 95% of the total micro-organisms living on Earth (Whitman *et al.*, 1998).

It has been demonstrated that methanogens (Kotelnikova *et al.*, 1998; Nilsen & Torsvik, 1996; Ollivier *et al.*, 1998), sulfate-reducing bacteria (Liu *et al.*, 1997; Motamedi & Pedersen, 1998) and acetogens (Davydovacharakhchyan *et al.*, 1992) are present in the subterranean biosphere. These chemoautotrophic microbes probably play an important role as primary

producers in this environment. Various heterotrophic bacteria also grow together with the autotrophs in this environment, and many researchers have reported their observations on and isolations of subterranean heterotrophic bacteria such as *Thermoanaerobacter* and *Thermotogales* (Andrews & Patel, 1996; Cayol *et al.*, 1995; Fardeau *et al.*, 1997, 2000; Jeanthon *et al.*, 1995; Ravot *et al.*, 1995; Slobodkin *et al.*, 1999; Stetter *et al.*, 1993; Wynter *et al.*, 1996). The bacteria isolated were generally thermophilic and some isolates were able to grow at up to 85 °C (Kim *et al.*, 2001; Stetter *et al.*, 1993).

Recently, we isolated a novel anaerobic, thermophilic, thiosulfate-reducing bacterium from geothermal water at Toyoha Mine (Hokkaido, Japan). Toyoha Mine is a Ag/Pb/Zn/Cu polymetallic vein-type deposit and the mineralization of the deposit occurred from 3 to 0.5 million years ago. Geothermal water was collected from cracks at 550 m below the land surface. The temperature and pH of the water were 71 °C and pH 5.8. The water seeped from the wall at a rate of 2 l min⁻¹.

Published online ahead of print on 14 June 2002 as DOI 10.1099/ijs.0.02201-0.

The GenBank/EMBL/DBJ accession number for the 16S rDNA sequence of strain ToBE^T is AB062280.

Fig. 1. Cell morphology of strain ToBE^T grown in AP4E medium (70 °C and pH 6.5). (a) Phase-contrast micrograph of strain ToBE^T. (b, c) Ultrathin sections of strain ToBE^T at late-exponential phase. Transmission electron microscopy (Hitachi model H-7000) was performed as described previously (Hattori *et al.*, 2000).

For enrichment and isolation, we used AP4E medium containing (l^{-1}) 0.8 g $Na_2S_2O_3$, 0.5 g glucose and 0.5 g Bacto yeast extract (Difco) in basal medium. The basal medium was composed of the following materials (l^{-1}): KH_2PO_4 , 0.75 g; K_2HPO_4 , 0.78 g; NH_4Cl , 0.53 g; $NaHCO_3$, 5.0 g; trisodium EDTA, 0.041 g; $FeSO_4 \cdot 7H_2O$, 0.011 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $CaCl_2 \cdot 2H_2O$, 0.029 g; NaCl, 0.23 g; trace element solution DSM 334 (DSMZ, 1993), 10 ml; vitamin solution DSM 141 (DSMZ, 1993), 10 ml. The medium was dispensed into vials and bottles and sealed with butyl-rubber stoppers and aluminium caps under a N_2/CO_2 (4:1, v/v) atmosphere and autoclaved. Prior to inoculation, the medium was reduced with a sterile stock solution of cysteine hydrochloride (final concentration 0.5 g l^{-1}). The pH of the medium was around 7.0. For primary enrichment, 2 ml inoculum (a hot water sample concentrated 200-fold with a centrifuge) was added to 20 ml AP4E medium. After incubation for 1 week at 70 °C under N_2/CO_2 , significant growth of morphologically uniform microbes was observed, and the enrichment culture was transferred to the same medium five times. White colonies, approximately 1 mm in diameter, were formed on AP4E medium solidified with 0.6% (w/v) Gelrite and 10 mM $MgCl_2 \cdot 6H_2O$ (final concentrations) after 3 days incubation, and strain ToBE^T was isolated successfully.

Morphologically, cells of strain ToBE^T were straight rods (about 0.6 μm wide and 2–6 μm long) showing no motility (Fig. 1a). Although the Gram-reaction was negative, observation by transmission electron microscopy indicated that the isolate had a Gram-positive type of cell wall (Fig. 1b). Terminal endospores were often observed in the cells (Fig. 1c).

Strain ToBE^T was a strictly anaerobic bacterium, unable to grow under aerobic conditions. Temperature, pH and NaCl ranges for growth were determined using 25 ml Hungate tubes containing 10 ml AP4E medium (Hattori *et al.*, 2000). The temperature for growth of strain ToBE^T at pH 7.0 ranged from 55 to 73 °C, with an optimum of 70 °C (Fig. 2a). The pH range for growth at 70 °C was 5.5–8.5 and optimum growth was observed at pH 6.5 (Fig. 2b). The isolate grew optimally with no added NaCl and growth did not occur above 1% (w/v) NaCl. Strain ToBE^T could not grow autotrophically with sulfate, sulfite, thiosulfate, elemental sulfur, nitrate, nitrite or Fe^{3+} under H_2/CO_2 . Yeast extract and trypticase were not required for growth of strain ToBE^T. The doubling time under optimum growth conditions (70 °C, pH 6.5 and no addition of NaCl) was 6.4 h.

The utilization of substrates and electron acceptors was tested according to the methods of Hattori *et al.*

Fig. 2. Effects of temperature (a) and pH (b) on growth of strain ToBE^T. Specific growth rates were calculated as means from duplicate cultures in AP4E medium.

(2000) and Slobodkin *et al.* (1997). Results were recorded 1 week after inoculation. With 5 mM thiosulfate, the isolate grew on the following substrates: arabinose (10 mM), cellobiose (10 mM), fructose (10 mM), glucose (10 mM), inositol (10 mM), maltose (10 mM), mannose (10 mM), sucrose (10 mM), trehalose (10 mM), xylose (10 mM), yeast extract (1 g l⁻¹), formate (20 mM), lactate (20 mM) and pyruvate (20 mM). The isolate also used all these substrates except formate by fermentation, but the growth yields were clearly lower than those with addition of thiosulfate. No growth was observed on galactose (10 mM), lactose (10 mM), mannitol (10 mM), melibiose (10 mM), raffinose (10 mM), rhamnose (10 mM), starch (1 g l⁻¹), chitin (1 g l⁻¹), glycogen (1 g l⁻¹), inulin (1 g l⁻¹), pectin (1 g l⁻¹), acetate (20 mM), citrate (20 mM), fumarate (20 mM), malate (20 mM), succinate (20 mM) or acetate (20 mM) + H₂/CO₂, with or without thiosulfate. Strain ToBE^T reduced sodium nitrate (5 mM) and sodium nitrite (2.5 mM) in the presence of sodium lactate (20 mM) as a sole substrate. Sodium sulfate (5 mM), sodium sulfite (2.5 mM), elemental sulfur (20 g l⁻¹), iron(III) citrate (5 mM) (Heising *et al.*, 1999) and sodium fumarate (5 mM) were not used as electron acceptors in the presence of lactate.

The major quinone (Shintani *et al.*, 2000) of strain ToBE^T was menaquinone 7 (MK-7). A trace amount of MK-8 was also detected. Fatty acid methyl ester analysis with a GC/MS system (Hanada *et al.*, 2002) revealed that the main cellular fatty acids were iso-C_{15:0} (60 % of the total fatty acids) and iso-C_{17:0} (30 %). The strain also contained C_{16:0} (8 %) as a

Fig. 3. Phylogenetic tree based on 16S rDNA sequences of strain ToBE^T and its relatives. Bootstrap percentages are indicated at branch points. Accession numbers are shown in parentheses. Bar, 0.03 substitutions per compared nucleotide.

Table 1. Comparison of properties among genera related to strain ToBE^T

Data for reference taxa were taken from Huber *et al.* (1996) (*A. degensii*), Sokolova *et al.* (2001) (*C. pacificum*) and Fardeau *et al.* (2000), Kim *et al.* (2001) and Xue *et al.* (2001) (*Thermoanaerobacter* spp.). +, Positive; –, negative; ±, positive or negative; ND, no data available. All taxa are positive for fermentation.

Character	Strain ToBE ^T	<i>A. degensii</i>	<i>C. pacificum</i>	<i>Thermoanaerobacter</i>
Morphology	Rod	Rod	Rod (sometimes branching)	Rod
Cell size (µm)	0.6 × 2–6	0.6 × 2–8.5	0.3 × 4–10	0.4–0.8 × 1–10
Habit	Thermal aquifer	Hot spring	Submarine thermal vent	Hot spring, oil well and lake
Temperature range for growth (°C)	55–73	57–77	50–80	35–85
16S rDNA similarity to strain ToBE ^T	(100)	87.0	89.1	86.2–89.5
DNA G + C content (mol %)	49.6	54	33	29–41
Spore formation	+	–	ND	+ *
Chemolithotrophic growth	–	+ †	+ ‡	–
Electron acceptors:				
SO ₄ ^{2–}	–	+	–	– *
SO ₃ ^{2–}	–	–	ND	±
S ₂ O ₃ ^{2–}	+	–	ND	+ *
S ⁰	–	+	–	+ *
Reduction of:				
NO ₃ [–]	+	+	ND	– *
NO ₂ [–]	+	–	ND	– *

* With some exceptions.

† With molecular hydrogen, nitrate is reduced to ammonium and sulfate or sulfur to hydrogen sulfide.

‡ Chemoautotrophic growth is observed on CO as the sole energy source.

minor component. The DNA G + C content (Mori *et al.*, 2000) of strain ToBE^T was 49.6 mol %.

An almost complete 16S rDNA sequence (Hattori *et al.*, 2000) was obtained for strain ToBE^T (accession no. AB062280). Phylogenetic analysis based on the neighbour-joining method (Saitou & Nei, 1987; Thompson *et al.*, 1994) demonstrated that strain ToBE^T was a member of the low-G + C group of the Gram-positive bacteria (Fig. 3). The phylogenetic tree indicated that the isolate was closely related to *Ammonifex degensii*, *Carboxydibrachium pacificum* and *Thermoanaerobacter* species, with a high bootstrap value (85.1%). However, 16S rDNA sequence similarities between strain ToBE^T and these related species were less than 90% (Table 1). These low values suggest that a new genus should be created for this isolate (Stackebrandt & Goebel, 1994).

There were obvious differences in phenotypic features between the novel isolate and related species. Two related species, *A. degensii* and *C. pacificum*, are chemolithotrophic bacteria (Table 1). *A. degensii* is able to grow on H₂/CO₂ as the sole energy and carbon source (Huber *et al.*, 1996). *C. pacificum* has a peculiar metabolism for acquiring energy, by oxidation of CO (Sokolova *et al.*, 2001). Strain ToBE^T did not show chemolithotrophic growth under any conditions that we tested. The lack of chemolithotrophy is a significant trait that differentiates the isolate from these two

related species. Members of the remaining related genus, *Thermoanaerobacter*, are anaerobic, thermophilic, rod-shaped bacteria, isolated from freshwater environments, that use saccharides for fermentation or thiosulfate respiration (Ben-Bassat & Zeikus, 1981; Bonch-Osmolovskaya *et al.*, 1997; Cook *et al.*, 1996; Fardeau *et al.*, 2000; Jin *et al.*, 1988; Kozianowski *et al.*, 1997; Larsen *et al.*, 1997; Slobodkin *et al.*, 1999; Wiegel & Ljungdahl, 1981; Wiegel *et al.*, 1979; Xue *et al.*, 2001; Zeikus *et al.*, 1979). Strain ToBE^T resembled *Thermoanaerobacter* species in morphological and physiological respects. However, the genomic DNA G + C content of strain ToBE^T was 49.6 mol %, whereas those of the *Thermoanaerobacter* species are less than 41 mol % (Table 1). The isolate was able to grow on formate in the presence of thiosulfate, while all *Thermoanaerobacter* species, except *Thermoanaerobacter kivui*, are unable to use this substrate as an energy source. Lactose is a good substrate for growth of all *Thermoanaerobacter* species, but it could not support growth of the novel isolate. Although the novel isolate reduced nitrate and nitrite, reduction of nitrogen oxides is not observed in almost all *Thermoanaerobacter* species (the only exception was *Thermoanaerobacter yonseiensis*). These differences in characteristics sufficiently support the phylogenetically solitary position of strain ToBE^T, as inferred from the 16S rDNA comparison. On the basis of its physiological and phylogenetic findings, we propose a novel genus

and species, *Thermanaeromonas toyohensis* gen. nov., sp. nov.

Strain ToBE^T was incapable of autotrophic growth on H₂/CO₂, but was able to grow on formate as a sole energy and carbon source in the presence of thiosulfate as an electron acceptor. Formate, the simplest organic acid, is synthesized readily and abiotically under conditions of high temperature and pressure such as those found in subterranean environments. It was also reported that a significant amount of this organic acid is contained in mineral veins (0.08–4.06 µg of the total mineral deposit; Zeng & Liu, 2000). These facts suggest that the novel isolate can grow independently of other autotrophic micro-organisms in the subsurface. It is probable that strain ToBE^T plays a leading ecological role in the subterranean biosphere as a primary producer and supplies organic materials for neighbouring heterotrophic bacteria.

Description of *Thermanaeromonas* gen. nov.

Thermanaeromonas (Ther.man.aer.mo'nas. Gr. n. *thermos* hot; Gr. pref. *an* not; Gr. n. *aer* air; Gr. n. *monas* unit, monad; N.L. fem. *Thermanaeromonas* thermophilic, anaerobic monad).

Cells are rod-shaped. Gram reaction is negative, but cells possess a Gram-positive cell-wall structure. Non-motile and terminal endospores are formed. Strictly anaerobic and thermophilic. Temperature range for growth is 55–73 °C. Optimum growth conditions are neutral pH. Chemo-organotrophic. Thiosulfate is used as an electron acceptor for growth. Nitrate and nitrite are also reduced. MK-7 is the major quinone and MK-8 also occurs as a minor quinone. Major cellular fatty acids are iso-C_{15:0} and iso-C_{17:0}. The genomic DNA G+C content of the type species is 49.6 mol% (as determined by HPLC). Phylogenetic position based on 16S rDNA is in the Gram-positive bacteria. The type species is *Thermanaeromonas toyohensis*.

Description of *Thermanaeromonas toyohensis* sp. nov.

Thermanaeromonas toyohensis (to.yo.hen'sis. N.L. adj. *toyohensis* from Toyoha, referring to its isolation from the Toyoha Mines).

Cells are straight, non-motile rods, about 0.6 µm width and 2–6 µm long, with rounded ends. In addition to the properties given in the description of the genus, the following properties are observed. Grows at 55–73 °C; optimum growth at 70 °C. The pH range for growth is 5.5–8.5, with optimum growth at pH 6.5. Growth does not occur above 1% NaCl. The doubling time is 6.4 h under optimum growth conditions. Thiosulfate is used as an electron acceptor, but sulfate, sulfite, elemental sulfur, iron(III) and fumarate are not. Nitrate and nitrite are reduced. Substrates utilized in the presence of thiosulfate include arabinose, cellobiose, fructose, glucose, inositol, maltose, mannose, sucrose, trehalose,

xylose, yeast extract, formate, lactate and pyruvate. These substrates except for formate are also used for fermentation. No growth occurs with galactose, lactose, mannitol, melibiose, raffinose, rhamnose, starch, chitin, glycogen, inulin, pectin, acetate, citrate, fumarate, malate, succinate or acetate + H₂/CO₂. Autotrophic growth is not observed. The genomic DNA G+C content, quinone profile and cellular fatty acids are as described for the genus.

The habitat is a thermal aquifer in Toyoha Mines (Hokkaido, Japan). The type strain is ToBE^T (= DSM 14490^T = JCM 11376^T).

Acknowledgements

We thank Xian-Ying Meng (National Institute of Advanced Industrial Science and Technology) for operation of the electron microscope. The Ministry of Education, Science & Technology (MEST), Japan, funds this research through the Special Coordination Fund 'Archaean Park Project': International Research Project on Interaction Between Sub-Vent Biosphere and Geo-Environments.

References

- Andrews, K. T. & Patel, B. K. C. (1996). *Fervidobacterium gondwanense* sp. nov., a new thermophilic anaerobic bacterium isolated from nonvolcanically heated geothermal waters of the Great Artesian Basin of Australia. *Int J Syst Bacteriol* **46**, 265–269.
- Ben-Bassat, A. & Zeikus, J. G. (1981). *Thermobacteroides acetethylicus* gen. nov. and spec. nov., a new chemoorganotrophic, anaerobic, thermophilic bacterium. *Arch Microbiol* **128**, 365–370.
- Bonch-Osmolovskaya, E. A., Miroshnichenko, M. L., Chernykh, N. A., Kostrikina, N. A., Pikuta, E. V. & Rainey, F. A. (1997). Reduction of elemental sulfur by moderately thermophilic organotrophic bacteria and the description of *Thermoanaerobacter sulfurophilus* sp. nov. *Microbiology* (English translation of *Mikrobiologiya*) **66**, 483–489.
- Cayol, J.-L., Ollivier, B., Patel, B. K. C., Ravot, G., Magot, M., Ageron, E., Grimont, P. A. D. & Garcia, J.-L. (1995). Description of *Thermoanaerobacter brockii* subsp. *lactiethylicus* subsp. nov., isolated from a deep subsurface French oil well, a proposal to reclassify *Thermoanaerobacter finii* as *Thermoanaerobacter brockii* subsp. *finii* comb. nov., and an emended description of *Thermoanaerobacter brockii*. *Int J Syst Bacteriol* **45**, 783–789.
- Cook, G. M., Rainey, F. A., Patel, B. K. C. & Morgan, H. W. (1996). Characterization of a new obligately anaerobic thermophile, *Thermoanaerobacter wiegelii* sp. nov. *Int J Syst Bacteriol* **46**, 123–127.
- Davydovacharakhchyan, I. A., Mileeva, A. N., Mityushina, L. L. & Belyaev, S. S. (1992). Acetogenic bacteria from oil fields of Tataria and Western Siberia. *Microbiology* (English translation of *Mikrobiologiya*) **61**, 208–216.
- DSMZ (1993). *Catalogue of Strains*, 5th edn. Braunschweig: Gesellschaft für Biotechnologische Forschung.
- Fardeau, M.-L., Ollivier, B., Patel, B. K. C., Magot, M., Thomas, P., Rimbault, A., Rocchiccioli, F. & Garcia, J.-L. (1997). *Thermotoga hypogea* sp. nov., a xylanolytic, thermophilic bacterium from an oil-producing well. *Int J Syst Bacteriol* **47**, 1013–1019.
- Fardeau, M.-L., Magot, M., Patel, B. K. C., Thomas, P., Garcia, J.-L. & Ollivier, B. (2000). *Thermoanaerobacter subterraneus* sp. nov., a novel thermophile isolated from oilfield water. *Int J Syst Evol Microbiol* **50**, 2141–2149.
- Gold, T. (1992). The deep, hot biosphere. *Proc Natl Acad Sci U S A* **89**, 6045–6049.
- Hanada, S., Takaichi, S., Matsuura, K. & Nakamura, K. (2002). *Roseiflexus castenholzii* gen. nov., sp. nov., a thermophilic, filamentous,

- photosynthetic bacterium that lacks chlorosomes. *Int J Syst Evol Microbiol* **52**, 187–193.
- Hattori, S., Kamagata, Y., Hanada, S. & Shoun, H. (2000). *Thermacetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. *Int J Syst Evol Microbiol* **50**, 1601–1609.
- Heising, S., Richter, L., Ludwig, W. & Schink, B. (1999). *Chlorobium ferrooxidans* sp. nov., a phototrophic green sulfur bacterium that oxidizes ferrous iron in coculture with a “*Geospirillum*” sp. strain. *Arch Microbiol* **172**, 116–124.
- Huber, R., Rossmagel, P., Woese, C. R., Rachel, R., Langworthy, T. A. & Stetter, K. O. (1996). Formation of ammonium from nitrate during chemolithoautotrophic growth of the extremely thermophilic bacterium *Ammonifex degensii* gen. nov., sp. nov. *Syst Appl Microbiol* **19**, 40–49.
- Jeanthon, C., Reysenbach, A. L., L’Haridon, S., Gambacorta, A., Pace, N. R., Glenat, P. & Prieur, D. (1995). *Thermotoga subterranea* sp. nov., a new thermophilic bacterium isolated from a continental oil reservoir. *Arch Microbiol* **164**, 91–97.
- Jin, F., Yamasato, K. & Toda, K. (1988). *Clostridium thermocopriae* sp. nov., a cellulolytic thermophile from animal feces, compost, soil, and a hot spring in Japan. *Int J Syst Bacteriol* **38**, 279–281.
- Kim, B.-C., Grote, R., Lee, D.-W., Antranikian, G. & Pyun, Y.-R. (2001). *Thermoanaerobacter yonseiensis* sp. nov., a novel extremely thermophilic, xylose-utilizing bacterium that grows at up to 85 °C. *Int J Syst Evol Microbiol* **51**, 1539–1548.
- Kotelnikova, S., Macario, A. J. L. & Pedersen, K. (1998). *Methanobacterium subterraneum* sp. nov., a new alkaliphilic, eurythermic and halotolerant methanogen isolated from deep granitic groundwater. *Int J Syst Bacteriol* **48**, 357–367.
- Kozianowski, G., Canganella, F., Rainey, F. A., Hippe, H. & Antranikian, G. (1997). Purification and characterization of thermostable pectate-lyases from a newly isolated thermophilic bacterium, *Thermoanaerobacter italicus* sp. nov. *Extremophiles* **1**, 171–182.
- Larsen, L., Nielsen, P. & Ahring, B. K. (1997). *Thermoanaerobacter mathranii* sp. nov., an ethanol-producing, extremely thermophilic anaerobic bacterium from a hot spring in Iceland. *Arch Microbiol* **168**, 114–119.
- Liu, Y., Karnauchow, T. M., Jarrell, K. F., Balkwill, D. L., Drake, G. R., Ringelberg, D., Clarno, R. & Boone, D. R. (1997). Description of two new thermophilic *Desulfotomaculum* spp., *Desulfotomaculum putei* sp. nov., from a deep terrestrial subsurface, and *Desulfotomaculum luciae* sp. nov., from a hot spring. *Int J Syst Bacteriol* **47**, 615–621.
- Mori, K., Yamamoto, H., Kamagata, Y., Hatsu, M. & Takamizawa, K. (2000). *Methanocalculus pumilus* sp. nov., a heavy-metal-tolerant methanogen isolated from a waste-disposal site. *Int J Syst Evol Microbiol* **50**, 1723–1729.
- Motamedi, M. & Pedersen, K. (1998). *Desulfovibrio aespoensis* sp. nov., a mesophilic sulfate-reducing bacterium from deep groundwater at Äspö hard rock laboratory, Sweden. *Int J Syst Bacteriol* **48**, 311–315.
- Nilsen, R. K. & Torsvik, T. (1996). *Methanococcus thermolithotrophicus* isolated from North Sea oil field reservoir water. *Appl Environ Microbiol* **62**, 728–731.
- Ollivier, B., Fardeau, M.-L., Cayol, J.-L., Magot, M., Patel, B. K. C., Prensier, G. & Garcia, J.-L. (1998). *Methanocalculus halotolerans* gen. nov., sp. nov., isolated from an oil-producing well. *Int J Syst Bacteriol* **48**, 821–828.
- Ravot, G., Magot, M., Fardeau, M.-L., Patel, B. K. C., Prensier, G., Egan, A., Garcia, J.-L. & Ollivier, B. (1995). *Thermotoga elfii* sp. nov., a novel thermophilic bacterium from an African oil-producing well. *Int J Syst Bacteriol* **45**, 308–314.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shintani, T., Liu, W.-T., Hanada, S., Kamagata, Y., Miyaoka, S., Suzuki, T. & Nakamura, K. (2000). *Micropruina glycoenica* gen. nov., sp. nov., a new Gram-positive glycogen-accumulating bacterium isolated from activated sludge. *Int J Syst Evol Microbiol* **50**, 201–207.
- Slobodkin, A., Reysenbach, A.-L., Strutz, N., Dreier, M. & Wiegel, J. (1997). *Thermoterrabacterium ferrireducens* gen. nov., sp. nov., a thermophilic anaerobic dissimilatory Fe(III)-reducing bacterium from a continental hot spring. *Int J Syst Bacteriol* **47**, 541–547.
- Slobodkin, A. I., Tourova, T. P., Kuznetsov, B. B., Kostrikina, N. A., Chernyh, N. A. & Bonch-Osmolovskaya, E. A. (1999). *Thermoanaerobacter siderophilus* sp. nov., a novel dissimilatory Fe(III)-reducing, anaerobic, thermophilic bacterium. *Int J Syst Bacteriol* **49**, 1471–1478.
- Sokolova, T. G., González, J. M., Kostrikina, N. A., Chernyh, N. A., Tourova, T. P., Kato, C., Bonch-Osmolovskaya, E. A. & Robb, F. T. (2001). *Carboxydobrachium pacificum* gen. nov., sp. nov., a new anaerobic, thermophilic, CO-utilizing marine bacterium from Okinawa Trough. *Int J Syst Evol Microbiol* **51**, 141–149.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Stetter, K. O., Huber, R., Blochl, E., Kurr, M., Eden, R. D., Fielder, M., Cash, H. & Vance, I. (1993). Hyperthermophilic archaea are thriving in deep North Sea and Alaskan oil reservoirs. *Nature* **365**, 743–745.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Whitman, W. B., Coleman, D. C. & Wiebe, W. J. (1998). Prokaryotes: the unseen majority. *Proc Natl Acad Sci U S A* **95**, 6578–6583.
- Wiegel, J. & Ljungdahl, L. G. (1981). *Thermoanaerobacter ethanolicus* gen. nov., spec. nov., a new, extreme thermophilic, anaerobic bacterium. *Arch Microbiol* **128**, 343–348.
- Wiegel, J., Ljungdahl, L. G. & Rawson, J. R. (1979). Isolation from soil and properties of the extreme thermophile *Clostridium thermohydro-sulfuricum*. *J Bacteriol* **139**, 800–810.
- Wynter, C., Patel, B. K. C., Bain, P., de Jersey, J., Hamilton, S. & Inkerman, P. A. (1996). A novel thermostable dextranase from a *Thermoanaerobacter* species cultured from the geothermal waters of the Great Artesian Basin of Australia. *FEMS Microbiol Lett* **140**, 271–276.
- Xue, Y., Xu, Y., Liu, Y., Ma, Y. & Zhou, P. (2001). *Thermoanaerobacter tengcongensis* sp. nov., a novel anaerobic, saccharolytic, thermophilic bacterium isolated from a hot spring in Tengcong, China. *Int J Syst Evol Microbiol* **51**, 1335–1341.
- Zeikus, J. G., Hegge, P. W. & Anderson, M. A. (1979). *Thermoanaerobium brockii* gen. nov. and sp. nov., a new chemoorganotrophic, caldactive, anaerobic bacterium. *Arch Microbiol* **122**, 41–48.
- Zeng, Y. & Liu, J. (2000). Short-chain carboxylates in fluid inclusions in minerals. *Appl Geochem* **15**, 13–25.