Thermoanaerobacter pseudethanolicus sp. nov., a thermophilic heterotrophic anaerobe from Yellowstone National Park

Rob U. Onyenwoke,[1] Vadim V. Kevbrin,[2] Anatolly. M. Lysenko[2] and Juergen Wiegel[1]

Correspondence
Juergen Wiegel
jwiegel@uga.edu

[1]Department of Microbiology, The University of Georgia, Athens, GA 30602-2605, USA

[2]Winogradsky Institute of Microbiology, Russian Academy of Sciences, Prospect 60-letiya Oktyabrya 7/2, 117 312 Moscow, Russia

Strain 39E^T, originally characterized as *Clostridium thermohydrosulfuricum* strain 39E and later renamed as *Thermoanaerobacter ethanolicus* strain 39E, shows less than 97 % 16S rRNA gene sequence similarity with the type strain of the type species of the genus *Thermoanaerobacter*, *T. ethanolicus* strain JW 200^T. On the basis of a polyphasic analysis that included DNA–DNA hybridization studies with the subspecies of *Thermoanaerobacter brockii*, its closest phylogenetic relatives, strain 39E^T represents a novel species of the genus *Thermoanaerobacter*, for which the name *Thermoanaerobacter pseudethanolicus* sp. nov. is proposed. The type strain is 39E^T (=DSM 2355^T=ATCC 33223^T).

Strain 39E^T was isolated and characterized by Zeikus *et al.* (1980) as a strain of *Clostridium thermohydrosulfuricum* (Hollaus & Sleytr, 1972) and was later described as a *Thermoanaerobacter ethanolicus* strain, 39E (Lee *et al.*, 1993).

As first reported by Bateson *et al.* (1989) and Rainey *et al.* (1993), the 16S rRNA gene sequence data clearly places strain 39E^T closer phylogenetically (>98 % similarity) to the subspecies of *Thermoanaerobacter brockii* (Zeikus *et al.*, 1979), i.e. *T. brockii* subsp. *brockii* DSM 1457^T (Zeikus *et al.*, 1979; Lee *et al.*, 1993; Cayol *et al.*, 1995), *T. brockii* subsp. *finnii* DSM 3389^T (Schmid *et al.*, 1986; Cayol *et al.*, 1995) and *T. brockii* subsp. *lactiethylicus* DSM 9801^T (Cayol *et al.*, 1995), than to *T. ethanolicus* strain JW 200^T, the type strain of the type species of the genus *Thermoanaerobacter* (Wiegel & Ljungdahl, 1981; Fig. 1). To clarify the relationship between strain 39E^T and the subspecies of *T. brockii*, DNA–DNA hybridization experiments were carried out.

DNA–DNA hybridization experiments were performed spectrophotometrically as described by De Ley *et al.* (1970) and modified by Huß *et al.* (1983). Chromosomal DNA for DNA–DNA hybridizations was isolated according to Marmur (1961). The results of DNA–DNA hybridizations between strain 39E^T and *T. brockii* subsp. *brockii* DSM 1457^T, *T. brockii* subsp. *finnii* DSM 3389^T and *T. brockii* subsp. *lactiethylicus* DSM 9801^T gave reassociation values of 56, 51 and 45 %, respectively (see Supplementary Table S1 available in IJSEM Online). The fact that all of the values were less than 70 % indicates that strain 39E^T is not related to any of the *T. brockii* subspecies at species level (Wayne *et al.*, 1987). This result was corroborated by the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany (P. Schumann, personal communication), where a DNA–DNA reassociation value of 23–34 % was obtained (using the spectrophotometric method of De Ley *et al.*, 1970) between strain 39E^T and *T. brockii* subsp. *brockii* DSM 1457^T. Our DNA–DNA hybridizations between the known subspecies of *T. brockii* (*T. brockii* subsp. *brockii* DSM 1457^T to *T. brockii* subsp. *finnii* DSM 3389^T and *T. brockii* subsp. *lactiethylicus* DSM 9801^T) gave values of 65 and 67 %, as compared with reported values of 89–97 % and 76–85 %, respectively. Our value for DNA–DNA hybridization between *T. brockii* subsp. *finnii* DSM 3389^T and *T. brockii* subsp. *lactiethylicus* DSM 9801^T was 62 %, while the reported value is 76–85 %. All of the values obtained in this study were significantly lower than the results obtained by Cayol *et al.* (1995) (see Supplementary Table S1). However, these aberrations might be due to the fact that Cayol *et al.* (1995) employed the tritium-labelled nucleotide method for determining DNA–DNA relatedness, whereas the results obtained in this work relied upon the spectrophotometric protocol of De Ley *et al.* (1970). Since our DNA–DNA hybridization results between the various *T. brockii* subspecies are not substantially below 70 % (see Supplementary Table S1), no changes in the status of the subspecies *T. brockii* subsp.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 39E^T is L09164.

The results of DNA–DNA hybridizations between strain 39E^T and three *Thermoanaerobacter brockii* subspecies are presented in a supplementary table available with the online version of this paper.

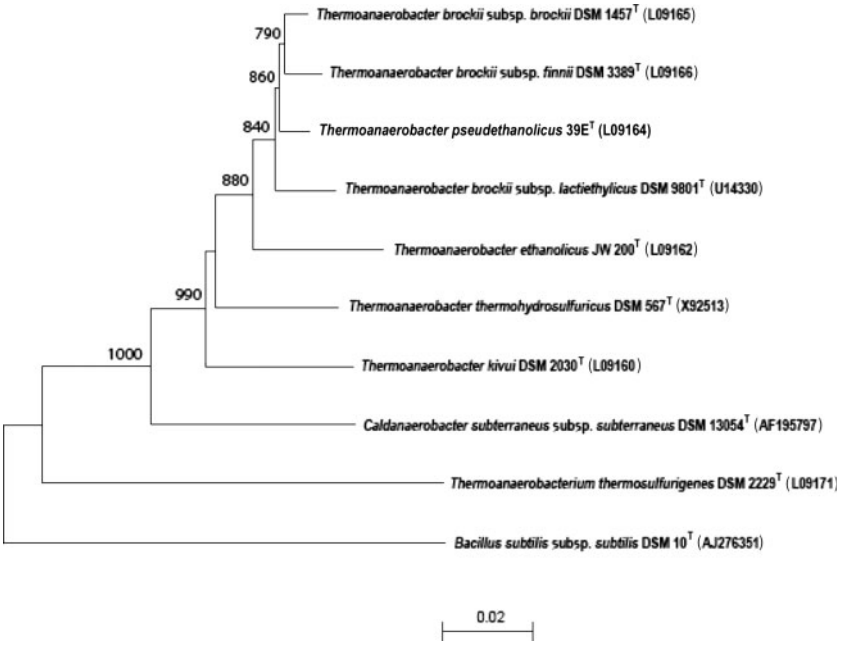

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequence data with maximum-likelihood correction for synonymous changes, showing the estimated relationships between strain 39E^T and related taxa. The 16S rRNA gene sequence data used represents *Escherichia coli* DSM 30083^T nucleotide positions 23–1450. Numbers at nodes indicate bootstrap percentages (based on 1000 replicates). The GenBank accession numbers for each reference strain are shown in parentheses. Bar, 0.02 nucleotide substitutions per site.

brockii DSM 1457^T, *T. brockii* subsp. *finnii* DSM 3389^T and *T. brockii* subsp. *lactiethylicus* DSM 9801^T (Cayol *et al.*, 1995) are proposed, although the data raise some doubts as to the validity of these subspecies, especially *T. brockii* subsp. *lactiethylicus* (Fig. 1). However, on the basis of the fact that the DNA–DNA hybridization values obtained for strain 39E^T and the T. *brockii* subspecies are significantly below 70 %, strain 39E^T does not represent a novel subspecies of *T. brockii* but instead represents a novel species of the genus *Thermoanaerobacter*, for which the name *Thermoanaerobacter pseudethanolicus* sp. nov. is proposed.

The classification of strain 39E^T as a novel species is mainly based on previously published physiological properties (Table 1), 16S rRNA gene sequence analysis (Fig. 1) and DNA–DNA hybridization results. The name was chosen because strain 39E^T (the proposed type strain of *T. pseudethanolicus* sp. nov.) produces fermentation products in proportions similar to those of strain JW 200^T (the type strain of *T. ethanolicus*), with high levels of ethanol being formed per mole of glucose utilized.

Description of *Thermoanaerobacter pseudethanolicus* sp. nov.

Thermoanaerobacter pseudethanolicus [pseud′e.tha.no′li. cus. Gr. adj. *pseudes* false; N.L. adj. *ethanolicus* a bacteria-specific epithet; N.L. masc. adj. *pseudethanolicus* a false (*Thermoanaerobacter*) *ethanolicus*].

Other names include *Thermoanaerobacter ethanolicus* strain 39E (Lee *et al.*, 1993) and *Clostridium thermohydrosulfuricum* strain 39E (Zeikus *et al.*, 1980).

The description is based mainly on those given by Zeikus *et al.* (1980) and Lee *et al.* (1993) for strain 39E^T. Cells are rod-shaped and form round, terminal, mother-cell-distending (drumstick-shaped) spores during growth on xylose-containing medium. Gram-stain reaction is variable, but the cell wall is Gram-type positive (Wiegel, 1981). No polymyxin B–lipopolysaccharide interaction is found (Wiegel & Quandt, 1982). Cells are motile and reduce thiosulfate to H_2S. Fermented carbohydrates include xylose, cellobiose, starch, glucose, maltose and sucrose. No growth is observed using CO_2/H_2. The temperature optimum is 65 °C. The doubling time at 65 °C is 75 min. The DNA G+C content of the type strain is 34.4± 0.3 mol% (T_m).

The type strain, 39E^T (=DSM 2355^T=ATCC 33223^T), was isolated from the Octopus Spring algal–bacterial mat in Yellowstone National Park, WY, USA, using modified

Table 1. Differential phenotypic characteristics for some species of the genus *Thermoanaerobacter*

Strains: 1, *T. brockii* subsp. *brockii* DSM 1457^T; 2, *T. brockii* subsp. *finnii* DSM 3389^T; 3, *T. brockii* subsp. *lactiethylicus* DSM 9801^T; 4, *T. pseudethanolicus* 39E^T; 5, *T. ethanolicus* JW 200^T. Data for reference strains were taken from Cayol *et al.* (1995). +, Positive; −, negative; v, variable result.

Characteristic	1	2	3	4	5
Sporulation	+*	+	+	+	−†
Motility	−	+	+	+	+
Gram stain	+	v	+	v	v
Optimum growth temperature (°C)	65–70	65	55–60	65	69

*Data from Cook *et al.* (1991).
†Contains the major sporulation genes (Onyenwoke *et al.*, 2004).

Trypticase-yeast extract-glucose medium (containing 5 % xylose instead of glucose) at 65 °C.

The genome sequence for strain 39E^T is presently available under the name *T. ethanolicus* 39E at http://genome.ornl.gov/microbial/teth_39e/.

References

Bateson, M. M., Weigel, J. & Ward, D. M. (1989). Comparative analysis of 16S ribosomal RNA sequences of thermophilic fermentative bacteria isolated from hot spring cyanobacterial mats. *Syst Appl Microbiol* **12**, 1–7.

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 finnii* as *Thermoanaerobacter brockii* subsp. *finnii* comb. nov., and an emended description of *Thermoanaerobacter brockii*. *Int J Syst Bacteriol* **45**, 783–789.

Cook, G. M., Janssen, P. H. & Morgan, H. W. (1991). Endospore formation by *Thermoanaerobium brockii* HTD4. *Syst Appl Microbiol* **14**, 240–244.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Hollaus, F. & Sleytr, U. (1972). On the taxonomy and fine structure of some hyperthermophilic saccharolytic clostridia. *Arch Microbiol* **86**, 129–146.

Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Lee, Y. E., Jain, M. K., Lee, C., Lowe, S. E. & Zeikus, J. G. (1993). Taxonomic distinction of saccharolytic thermophilic anaerobes: description of *Thermoanaerobacterium xylanolyticum* gen. nov., sp. nov., and *Thermoanaerobacterium saccharolyticum* gen. nov., sp. nov.; reclassification of *Thermoanaerobium brockii*, *Clostridium thermosulfurogenes*, and *Clostridium thermohydrosulfuricum* E100-69 as *Thermoanaerobacter brockii* comb. nov., *Thermoanaerobacterium thermosulfurigenes* comb. nov., and *Thermoanaerobacter thermohydrosulfuricus* comb. nov., respectively; and transfer of *Clostridium thermohydrosulfuricum* 39E to *Thermoanaerobacter ethanolicus*. *Int J Syst Bacteriol* **43**, 41–51.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Onyenwoke, R. U., Brill, J. A., Farahi, K. & Wiegel, J. (2004). Sporulation genes in members of the low G+C Gram-type positive phylogenetic branch (Firmicutes). *Arch Microbiol* **182**, 182–192.

Rainey, F. A., Ward, N. L., Morgan, H. W., Toalster, R. & Stackebrandt, E. (1993). Phylogenetic analysis of anaerobic thermophilic bacteria: aid for their reclassification. *J Bacteriol* **175**, 4772–4779.

Schmid, U., Giesel, H., Schoberth, S. M. & Sahm, H. (1986). *Thermoanaerobacter finnii* spec. nov., a new ethanologenic sporogenous bacterium. *Syst Appl Microbiol* **8**, 80–85.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Wiegel, J. (1981). Distinction between the Gram reaction and the Gram type of bacteria. *Int J Syst Bacteriol* **31**, 88.

Wiegel, J. & Ljungdahl, L. J. (1981). *Thermoanaerobacter ethanolicus* gen. nov., spec. nov., a new, extreme thermophilic, anaerobic bacterium. *Arch Microbiol* **128**, 343–348.

Wiegel, J. & Quandt, L. (1982). Determination of the Gram type using the reaction between polymyxin B and lipopolysaccharides of the outer cell wall of whole bacteria. *J Gen Microbiol* **128**, 2261–2270.

Zeikus, J. G., Hegge, P. W. & Anderson, M. A. (1979). *Thermoanaerobacter brockii* gen. nov. and sp. nov., a new chemoorganotrophic, caldoactive, anaerobic bacterium. *Arch Microbiol* **122**, 41–48.

Zeikus, J. G., Ben-Bassat, A. & Hegge, P. (1980). Microbiology of methanogenesis in thermal, volcanic environments. *J Bacteriol* **143**, 432–440.