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

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Strain 39Eᵀ, 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 200ᵀ. On the basis of a polyphasic analysis that included DNA–DNA hybridization studies with the subspecies of *Thermoanaerobacter brockii*, its closest phylogenetic relatives, strain 39Eᵀ represents a novel species of the genus *Thermoanaerobacter*, for which the name *Thermoanaerobacter pseudethanolicus* sp. nov. is proposed. The type strain is 39Eᵀ (=DSM 2355ᵀ=ATCC 33223ᵀ).

Strain 39Eᵀ 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 39Eᵀ closer phylogenetically (>98 % similarity) to the subspecies of *Thermoanaerobacter brockii* (Zeikus et al., 1979), i.e. *T. brockii* subsp. brockii DSM 1457ᵀ (Zeikus et al., 1979; Lee et al., 1993; Cayol et al., 1995), *T. brockii* subsp. finnii DSM 3389ᵀ (Schmid et al., 1986; Cayol et al., 1995) and *T. brockii* subsp. lactiethylicus DSM 9801ᵀ (Cayol et al., 1995), than to *T. ethanolicus* strain JW 200ᵀ, the type strain of the type species of the genus *Thermoanaerobacter* (Wiegel & Ljungdahl, 1981; Fig. 1). To clarify the relationship between strain 39Eᵀ 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 39Eᵀ and *T. brockii* subsp. brockii DSM 1457ᵀ, *T. brockii* subsp. finnii DSM 3389ᵀ and *T. brockii* subsp. lactiethylicus DSM 9801ᵀ 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 39Eᵀ 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 39Eᵀ and *T. brockii* subsp. *brockii* DSM 1457ᵀ. Our DNA–DNA hybridizations between the known subspecies of *T. brockii* (*T. brockii* subsp. *brockii* DSM 1457ᵀ to *T. brockii* subsp. *finnii* DSM 3389ᵀ and *T. brockii* subsp. *lactiethylicus* DSM 9801ᵀ) 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 3389ᵀ and *T. brockii* subsp. *lactiethylicus* DSM 9801ᵀ 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 39Eᵀ is L09164.

The results of DNA–DNA hybridizations between strain 39Eᵀ and three *Thermoanaerobacter brockii* subspecies are presented in a supplementary table available with the online version of this paper.
brockii DSM 1457\textsuperscript{T}, T. brockii subsp. finnii DSM 3389\textsuperscript{T} and T. brockii subsp. lactiethylicus DSM 9801\textsuperscript{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\textsuperscript{T} and the T. brockii subspecies are significantly below 70\%, strain 39E\textsuperscript{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\textsuperscript{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\textsuperscript{T} (the proposed type strain of T. pseudethanolicus sp. nov.) produces fermentation products in proportions similar to those of strain JW 200\textsuperscript{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. masc. 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\textsuperscript{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\textsubscript{2}S. Fermented carbohydrates include xylose, cellobiose, starch, glucose, maltose and sucrose. No growth is observed using CO\textsubscript{2}/H\textsubscript{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\textsubscript{m}).

The type strain, 39E\textsuperscript{T} (DSM 2355\textsuperscript{T}=ATCC 33223\textsuperscript{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**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporulation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−†</td>
</tr>
<tr>
<td>Motility</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Gram stain</td>
<td>+</td>
<td>v</td>
<td>+</td>
<td>v</td>
<td>v</td>
</tr>
<tr>
<td>Optimum growth temperature (°C)</td>
<td>65–70</td>
<td>65</td>
<td>55–60</td>
<td>65</td>
<td>69</td>
</tr>
</tbody>
</table>

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 39ET is presently available under the name *T. ethanolicus* 39E at http://genome.ornl.gov/microbial/teth_39e/.

**References**


