Description of *Methanobrevibacter gottschalkii* sp. nov., *Methanobrevibacter thaueri* sp. nov., *Methanobrevibacter woeisei* sp. nov. and *Methanobrevibacter wolinii* sp. nov.

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Formal nomenclature is proposed for five methanogens, isolated from horse, pig, cow, goose and sheep faeces, that represent four novel species of the genus *Methanobrevibacter*. The four species, *Methanobrevibacter gottschalkii* sp. nov., *Methanobrevibacter thaueri* sp. nov., *Methanobrevibacter woeisei* sp. nov. and *Methanobrevibacter wolinii* sp. nov., are distinguished from each other by a lack of genomic DNA reassociation and from previously described members of the genus on the basis of differences in the sequences of the 16S rRNA genes.

Keywords: *Methanobrevibacter*, faecal methanogens

Six of the seven species of *Methanobrevibacter* were isolated from gastrointestinal ecosystems (Miller, 2001). *Methanobrevibacter ruminantium*, the type species, was isolated from the bovine rumen. *Methanobrevibacter smithii* is the predominant CO₂-reducing methanogen isolated from the human colon (Miller et al., 1986; Lin & Miller, 1998). The type strain of *M. smithii* was originally isolated from a municipal sewage digester and was probably present in human faeces entering the treatment facility. Ferrari et al. (1994) isolated *Methanobrevibacter oralis* from the human oral cavity. Three different species of *Methanobrevibacter* were isolated from termite hindguts: *Methanobrevibacter cuticularis*, *Methanobrevibacter curvatus* and *Methanobrevibacter filiformis* (Leadbetter & Breznak, 1996; Leadbetter et al., 1998). The type strain of the seventh species, *Methanobrevibacter arboriphilicus* [corrected to *Methanobrevibacter arborophilus* in Miller (2001); this correction has not yet been validly published], was isolated from decaying cottonwood trees, and four other strains were isolated from sewage digestors (Miller, 2001).

Miller et al. (1986) isolated and described methanogens from various animal faeces. The morphology, physiology, cell-wall chemistry, G + C content of the genomic DNA and immunology of the isolates are consistent with those of the genus *Methanobrevibacter* (Conway de Macario et al., 1987; König, 1986; Miller et al., 1986).

The coccobacilli isolated from animals are distinguished from *M. arboriphilicus*, *M. cuticularis*, *M. curvatus* and *M. filiformis* on the basis of differences in morphology and physiology (Table 1). However, the coccobacilli species of the genus *Methanobrevibacter* and the animal isolates are not easily distinguishable on the basis of biochemical and physiological characteristics (Table 1).

Lin & Miller (1998) showed that the isolates from horse, pig, cow, goose and sheep faeces probably represented four novel species on the basis of (i) genomic DNA reassociation studies and (ii) comparison of 16S rRNA gene sequences with the type strains of the species of the genus available for analysis at that time. Subsequent comparison of almost complete 16S rRNA gene sequences deposited in GenBank showed that the animal faecal isolates shared only 91–93% sequence similarity with *M. cuticularis*, *M. curvatus* and *M. filiformis* (data not shown). However, the relationship of the animal isolates to *M. oralis* was not known because a 16S rRNA gene sequence was not available for phylogenetic analysis. Recently, Kulik et al. (2001) included the animal faecal methanogen sequences and a partial sequence for *M. oralis* in a phylogenetic comparison of methanogen sequences amplified from human dental plaque. Their analysis of partial 16S rRNA gene sequences (599 bp) showed that the animal isolates were phylogenetically distinct from
Table 1. Some phenotypic traits of the species of the genus Methanobrevibacter

<table>
<thead>
<tr>
<th>Trait shape</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>cb</td>
<td>Short to long rods in aggregates</td>
<td>Curved rods with polar fibres</td>
<td>Short rods</td>
<td>Rods in filaments</td>
<td>cb</td>
<td>cb</td>
<td>cb</td>
<td>cb</td>
<td>cb</td>
<td>cb</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>31</td>
<td>26</td>
<td>30</td>
<td>37</td>
<td>30</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Optimum temperature for growth (°C)</td>
<td>38</td>
<td>30–37</td>
<td>30</td>
<td>37</td>
<td>30</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Growth with bile</td>
<td>–</td>
<td>–</td>
<td>NK</td>
<td>NK</td>
<td>NK</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Formate used for growth</td>
<td>AcH, AA, CoM</td>
<td>v</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Medium additions for good growth</td>
<td>AcH, AA, CoM</td>
<td>Bvits, CO₃</td>
<td>NutB, RF</td>
<td>AA, RF, YE</td>
<td>DTT, YE</td>
<td>FaecX, AcH, Bvits</td>
<td>AcH, Ye</td>
<td>Trp + YE</td>
<td>Trp + YE</td>
<td>Trp + YE</td>
<td>CoM + VFA</td>
</tr>
</tbody>
</table>

M. oralis as well as from the methanogen sequences they amplified from dental plaque (Kulik et al., 2001).

All of the available molecular phylogenetic evidence supports the conclusion that the animal isolates represent novel species of the genus Methanobrevibacter. We propose a formal nomenclature for the animal isolates in this communication.

Description of Methanobrevibacter gottschalkii sp. nov.

*Methanobrevibacter gottschalkii* (gott.schal’ki.i. N.L. gen. n. gottschalkii of Gottschalk, named in honour of Gerhard Gottschalk for his notable contributions to the understanding of the biochemistry of methanogenesis).

Coccobacillus with rounded ends, about 0.7 μm in width and 0.9 μm in length, occurring in pairs or short chains. Gram-positive reaction. Cell walls are composed of pseudomurein (König, 1986). The peptide moiety contains glutamate, alanine and lysine (strains HO and PG) and may contain ornithine (strain HO). Galactosamine may be present at very low concentrations or absent from cell-wall hydrolysates (König, 1986). Cells are resistant to lysis by SDS. Optimum temperature 37 °C. Optimum pH 7.

Strict anaerobe. Cells grown in rumen fluid medium are catalase-negative. Grows and produces methane from H₂ and CO₂ (Miller et al., 1986). Does not grow or produce methane from formate, acetate, methanol, trimethylamines or methanol with H₂. Requires either acetate and/or one or more components of trypsin or yeast extract for growth. Does not require coenzyme M or branched-chain fatty acids for growth. Grows in medium with salt concentrations similar to sea water (medium 3 of Balch et al., 1979). Growth is not inhibited by bile salts (Miller et al., 1986). Neither strain HO nor strain PG reacts with polyvalent antibody probes raised against *M. ruminantium* (strain M1), *M. smithii* (strains PS, ALI), *M. arboriphilicus* (strains DH1, AZ, DC) or any other methanogen antisera in the antibody probe bank (Conway de Macario et al., 1987). The DNA G+C content is 29 mol% (Tm).

Strain HO was isolated from enrichments of horse faeces. Strain PG was isolated from enrichments of pig faeces. The two species share > 99% 16S rRNA gene sequence similarity and their genomic DNA reassociates at 73–108%, indicating that they are strains of the same species (Lin & Miller, 1998).

The type strain is strain HO (= DSM 11977= OCM 813). The GenBank accession number of its 16S rRNA sequence is U55238. Strain PG (= DSM 11978 = OCM 816) is a reference strain. The GenBank accession number of its 16S rRNA sequence is U55239.

Description of Methanobrevibacter thaueri sp. nov.

*Methanobrevibacter thaueri* (thau’e.r.i. N.L. gen. n. thaueri of Thauer, named in honour of Rolf K. Thauer for his fundamental contributions to the delineation of the biochemistry of methanogenesis).

Coccobacillus, with slightly tapered ends, about 0.5 μm in width and 0.6–1.2 μm in length, occurring in pairs and short chains. Some chains may have elongated cells. Gram-positive reaction. Cell walls are composed of pseudomurein (König, 1986). The peptide moiety contains glutamate, alanine and lysine. Galactosamine and glucosamine may be present at very low concentrations in cell-wall hydrolysates (König, 1986). Cells are resistant to lysis by SDS. Optimum temperature 37 °C. Optimum pH 7.

Strict anaerobe. Cells grown in rumen fluid medium are catalase-negative. Grows and produces methane
from H₂ and CO₂ (Miller et al., 1986). Does not grow or produce methane from formate, acetate, methanol, trimethylamines or methanol with H₂. Requires either acetate and/or one or more components of trypticase or yeast extract for growth. Does not require coenzyme M or branched-chain fatty acids for growth. Does not grow in medium with salt concentrations similar to sea water (medium 3 of Balch et al., 1979). Growth is inhibited by bile salts (Miller et al., 1986). Strain CW¹ does not react with polyvalent antibody probes raised against M. ruminantium (strain M¹), M. smithii (strains PS¹, ALI), M. arboriphilicus (strains DH1¹, AZ, DC) or any other methanogen antisera in the antibody probe bank (Conway de Macario et al., 1987). The DNA G + C content is 38 mol% (Tm).

Strain CW¹ was isolated from enrichments of cow faeces. The type strain is strain CW¹ (= DSM 11995¹ = OCM 817¹). The GenBank accession number of its 16S rRNA sequence is U55236.

Description of Methanobrevibacter woesei sp. nov.
Methanobrevibacter woesei (woe’s.e.i. N.L. gen. n. woesei of Woese, named in honour of Carl R. Woese for his pioneering contributions to the understanding of the phylogeny of methanogens and other microorganisms).

Coccobacillus with slightly tapered or rounded ends, about 0·6 µm in width and 1·0–1·4 µm in length, occurring in pairs or short chains. Gram-positive reaction. Cell walls are composed of pseudomurein (König, 1986). The peptide moiety contains glutamate, alanine and lysine. Galactosamine and glucosamine are present in cell-wall hydrolysates (König, 1986). Cells are resistant to lysis by SDS. Optimum temperature 37 °C. Optimum pH 7.

Strict anaerobe. Cells grown in rumen fluid medium are catalase-negative. Grows and produces methane from H₂ and CO₂ (Miller et al., 1986). Does not grow or produce methane from formate, acetate, methanol, trimethylamines or methanol with H₂. Requires either acetate and/or one or more components of trypticase or yeast extract for growth. Does not grow in medium with salt concentrations similar to sea water (medium 3 of Balch et al., 1979). Growth is inhibited by bile salts (Miller et al., 1986). Strain SH¹ does not react with polyvalent antibody probes raised against M. ruminantium (strain M¹), M. smithii (strains PS¹, ALI), M. arboriphilicus (strains DH1¹, AZ, DC) or any other methanogen antisera in the antibody probe bank (Conway de Macario et al., 1987). The DNA G + C content is 33 mol% (Tm).

Strain SH¹ was isolated from enrichments of sheep faeces (Miller et al., 1986). The type strain is strain SH¹ (= DSM 11976¹ = OCM 814¹). The GenBank accession number of its 16S rRNA sequence is U55240.

References


