NOTE

Description of *Methanobrevibacter gottschalkii* sp. nov., *Methanobrevibacter thaueri* sp. nov., *Methanobrevibacter woesei* 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 woesei* 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 arboriphilus* 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*

Taxa are indicated as: 1, *M. ruminantium* M1^T; 2, *M. arborophilicus* DH1^T; 3, *M. curvatus* RFM-2^T; 4, *M. cuticularis* RFM-1^T; 5, *M. filiformis* RFM-3^T; 6, *M. oralis* ZR^T; 7, *M. smithii* PS^T; 8, *M. gottschalkii* HO^T; 9, *M. thaueri* CW^T; 10, *M. woesei* GS^T; 11, *M. wolinii* SH^T. Abbreviations: NK, not known; AA, amino acid mixture; AcH, acetate; Bvits, B vitamin mixture; CB, coccobacillus; CoM, coenzyme M; DTT, dithiothreitol (cysteine and H₂S inhibit growth); FaecX, faecal extract; 2-MB, 2-methylbutyric acid; NutB, nutrient broth; RF, rumen fluid; Trp, Trypticase; v, strain-dependent; VFA, volatile fatty acid mixture; YE, yeast extract. All strains use H₂ + CO₂ for growth; no substrate other than formate and H₂ + CO₂ is used for growth by any of the strains shown.

Trait	1	2	3	4	5	6	7	8	9	10	11
Cell shape	CB	Short to long rods in aggregates	Curved rods with polar fibres	Short rods	Rods in filaments	CB	CB	CB	CB	CB	CB
DNA G+C content (mol%)	31	26	NK	NK	NK	28	30	29	38	30	33
Optimum temperature for growth (°C)	38	30–37	30	37	30	37	38	37	37	37	37
Optimum pH	7.2	7.5–7.8	7.2	7.7	7.2	6.9–7.4	6.9–7.4	7	7	7	7
Growth with bile	–	–	NK	NK	NK	+	+	+	–	+	–
Formate used for growth	+	v	–	+	–	–	+	–	–	+	–
Medium additions for good growth	AcH, AA, CoM, NH ₄ , 2-MB	Bvits, CO ₂	NutB, RF	AA, RF, YE	DTT, YE	FaecX	AcH, Bvits	AcH, Trp+YE	AcH, Trp+YE	AcH, Trp+YE	AcH, CoM+VFA

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^T and PG) and may contain ornithine (strain HO^T). 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 trypticase 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^T nor strain PG reacts with polyvalent

antibody probes raised against *M. ruminantium* (strain M1^T), *M. smithii* (strains PS^T, ALI), *M. arborophilicus* (strains DH1^T, 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% (*T_m*).

Strain HO^T 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^T (= DSM 11977^T = OCM 813^T). 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'er.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 CW^T does not react with polyvalent antibody probes raised against *M. ruminantium* (strain M1^T), *M. smithii* (strains PS^T, ALI), *M. arboriphilicus* (strains DH1^T, 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% (*T_m*).

Strain CW^T was isolated from enrichments of cow faeces.

The type strain is strain CW^T (= DSM 11995^T = OCM 817^T). The GenBank accession number of its 16S rRNA sequence is U55236.

Description of *Methanobrevibacter woesei* sp. nov.

Methanobrevibacter woesei (woe'se.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 micro-organisms).

Coccobacillus with slightly tapered or rounded ends, about 0.6 µm in width and 1.0 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). In comparison, formate is used poorly for growth and methane production. Does not grow or produce methane from 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. 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). Strain GS^T reacts with the polyvalent antibody probe raised against *M. smithii* strain ALI, but not with the probe against strain PS^T. It does not react with *M. ruminantium* (strain M1^T), *M. arboriphilicus* (strains DH1^T, AZ, DC) or any other methanogen antisera in the antibody probe bank (Conway de Macario *et al.*, 1987). The DNA G+C content is 31 mol% (*T_m*).

Strain GS^T was isolated from enrichments of goose faeces (Miller *et al.*, 1986).

The type strain is strain GS^T (= DSM 11979^T = OCM 815^T). The GenBank accession number of its 16S rRNA sequence is U55237.

Description of *Methanobrevibacter wolinii* sp. nov.

Methanobrevibacter wolinii (wo.lin'i.i. N.L. gen. n. *wolinii* of Wolin, named in honour of Meyer J. Wolin for his singular contributions to the physiological understanding of the role of methanogens and inter-species hydrogen transfer in anaerobic habitats).

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. Cell walls are composed of pseudomurein (König, 1986). The peptide moiety contains glutamate, alanine, lysine and ornithine. Galactosamine is absent or present at low concentrations and glucosamine is 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. Growth does not occur in medium 1 (Balch *et al.*, 1979) except when it is emended with coenzyme M and branched-chain fatty acids. May require either or both of the nutrients. 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 SH^T does not react with polyvalent antibody probes raised against *M. ruminantium* (strain M1^T), *M. smithii* (strains PS^T, ALI), *M. arboriphilicus* (strains DH1^T, 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% (*T_m*).

Strain SH^T was isolated from enrichments of sheep faeces (Miller *et al.*, 1986).

The type strain is strain SH^T (= DSM 11976^T = OCM 814^T). The GenBank accession number of its 16S rRNA sequence is U55240.

References

- Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R. & Wolfe, R. S. (1979). Methanogens: reevaluation of a unique biological group. *Microbiol Rev* 43, 260–296.
- Conway de Macario, E., Macario, A. J. L., Miller, T. L. & Wolin, M. J. (1987). Antigenic diversity of methanogenic bacteria from intestinal tracts of animals. *Syst Appl Microbiol* 9, 210–213.
- Ferrari, A., Brusa, T., Rutili, A., Canzi, E. & Biavati, B. (1994). Isolation and characterization of *Methanobrevibacter oralis* sp. nov. *Curr Microbiol* 29, 7–12.
- König, H. (1986). Chemical composition of cell envelopes of methanogenic bacteria isolated from human and animal feces. *Syst Appl Microbiol* 8, 159–162.
- Kulik, E. M., Sandmeier, H., Hinni, K. & Meyer, J. (2001). Identifi-

cation of archaeal rDNA from subgingival dental plaque by PCR amplification and sequence analysis. *FEMS Microbiol Lett* **196**, 129–133.

Leadbetter, J. R. & Breznak, J. A. (1996). Physiological ecology of *Methanobrevibacter cuticularis* sp. nov. and *Methanobrevibacter curvatus* sp. nov. isolated from the hindgut of the termite *Reticulitermes flavipes*. *Appl Environ Microbiol* **62**, 3620–3631.

Leadbetter, J. R., Crosby, L. D. & Breznak, J. A. (1998). *Methanobrevibacter filiformis* sp. nov., a filamentous methanogen from termite hindguts. *Arch Microbiol* **169**, 298–292.

Lin, C. & Miller, T. L. (1998). Phylogenetic analysis of *Methanobrevibacter* isolated from feces of humans and other animals. *Arch Microbiol* **169**, 397–403.

Miller, T. L. (2001). Genus II. *Methanobrevibacter* Balch and Wolfe 1981, 216^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 218–226. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.

Miller, T. L., Wolin, M. J. & Kusel, E. (1986). Isolation and characterization of methanogens from animal feces. *Syst Appl Microbiol* **8**, 234–238.