Reclassification of *Methanogenium tationis* and *Methanogenium liminatans* as *Methanofollis tationis* gen. nov., comb. nov. and *Methanofollis liminatans* comb. nov. and description of a new strain of *Methanofollis liminatans*

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Sequencing of 16S rRNA genes and phylogenetic analysis of *Methanogenium tationis* DSM 2702T (OCM 43) (T = type strain) and *Methanogenium liminatans* GKZPZT (= DSM 41400) as well as other members of the family *Methanomicrobiaceae* revealed that both species belong to a separate line of descent within this family. In addition, a new strain of *Methanogenium liminatans*, strain BM1 (= DSM 10196), was isolated from a butyrate-degrading, fluidized bed reactor and characterized. Cells of both species are mesophilic, highly irregular cocci that use H₂/CO₂ and formate for growth and methanogenesis. In addition, *Methanogenium liminatans* strains GKZPZT and BM1 used 2-propanol/CO₂, 2-butanol/CO₂ and cyclopentanol/CO₂. Both species contained diether and tetraether lipids. The polar lipids comprised aminophosphopentanetetrol derivatives, which appear to be characteristic lipids within the family *Methanomicrobiaceae*. The pattern of glycolipids, phosphoglycolipids and amino-phosphoglycolipids was consistent with the assignment of these two species to a taxon within the family *Methanomicrobiaceae*, but also permitted them to be distinguished from other higher taxa within this family. The G+C contents of the DNA of *Methanogenium tationis* and *Methanogenium liminatans* were 54 and 60 mol% (Tₘ and HPLC), respectively. On the basis of the data presented, the transfer of *Methanogenium tationis* and *Methanogenium liminatans* to the genus *Methanofollis* gen. nov. as *Methanofollis tationis* comb. nov. and *Methanofollis liminatans* comb. nov., respectively, is proposed, with *Methanofollis tationis* as the type species.

Keywords: *Methanogenium tationis*, *Methanogenium liminatans*, *Methanofollis* gen. nov., lipids, phylogeny

INTRODUCTION

The DNA of irregularly coccoid methanogens has a wide range of G+C contents (38–61 mol%), which indicates a wide evolutionary diversity among these methanogenic *Archaea*. However, only a limited number of phenotypic characteristics of irregularly coccoid methanogens are available for taxonomic purposes. In addition to morphology and substrate spectrum, characteristics such as polyamine content (Zellner et al., 1989a; Blotevogel et al., 1991; Boone et al., 1993), cellular lipids (Grant et al., 1985; Zellner et al., 1989a, b, c; Blotevogel et al., 1991; Koga et al., 1993) and whole-cell protein patterns (Maestrojuan et al.,

Abbreviations: PAS, periodic acid–Schiff; UASB, upflow anaerobic sludge blanket.

The GenBank/EMBL accession numbers for the 16S rDNA and rRNA sequences reported in this paper are Y16428, Y16429, AF095266–AF095272.
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1990) have been used. The S-layer architecture and the apparent molecular mass of glycoprotein subunits have also been used (Zellner et al., 1989a, c, 1990; Messner & Seytr, 1992; Seytr et al., 1993). Due to the fact that few physiological tests can be used to differentiate taxa within the methanogenic Archaea, other phenotypic characters (e.g. cell wall structure, chemical composition, polyamines) and genotypic characters (e.g. 16S rDNA sequence) must be used to delineate the higher taxonomic ranks. Recent developments in the taxonomy of both Archaea and Bacteria indicate that this combination of phenotypic and genotypic methods is suitable for the delineation of taxa in a system which seeks to base these on the phyletic groupings detected by such data.

Analysis of the sequences of 16S rRNA or its genes revealed a high diversity of irregularly coccoid methanogenic strains, with some deeply rooted lines of descent in phylogenetic trees (Zellner et al., 1989a; Rouviere et al., 1992; Boone et al., 1993). As a consequence, several coccoid methanogens were reclassified on the basis of DNA–DNA hybridization studies and/or 16S rDNA sequence analysis (Xun et al., 1989; Zellner et al., 1989c; Maestrojuan et al., 1990; Boone et al., 1993). This resulted in the description of the five current genera of hydrogenotrophic, irregularly coccoid methanogens of the order Methanomicrobiales: Methanogenium, Methanoculleus, Methanolacinia, Methanoplanus and Methanocorpusculum.

Recently, the reclassification of *Methanogenium tationis* (Zabel et al., 1984) as *Methanofollis tationis* was suggested (Boone et al., 1993) but not formally proposed. In this paper, the transfer of *Methanogenium tationis* and *Methanogenium liminatans* as *Methanofollis tationis* gen. nov., comb. nov. and *Methanofollis liminatans* comb. nov. is proposed on the basis of 16S rDNA sequencing and chemotaxonomic and physiological data. In addition, the isolation and characterization of a new *Methanofollis liminatans* strain, BM1, is reported.

**METHODS**

**Reference strains.** *Methanogenium tationis* strain (DSM 2702T) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). *Methanogenium liminatans* strain GZKPZT (= DSM 4140T) was isolated and (after deposition in the DSMZ) subcultured in this laboratory. The newly isolated strain BM1 has been deposited at the DSMZ as DSM 10196. The following strains were obtained from the Oregon Collection of Methanogens (Beaverton, OR, USA): *Methanoculleus olentangyi* RC/ER1 (OCM 52T), *Methanoculleus bourgensis* MS2T (OCM 15T), *Methanocorpusculum bavaricum* SZSXXZT (OCM 127T), *Methanocorpusculum sinense* China Z1T (OCM 128T) and *Methanocorpusculum labreanum* Z1T (OCM 1T).

**Media, media preparation and cultivation.** A synthetic basal medium (WHP medium) containing tungstate, with acetate and l-cysteine (the latter as a component of the reductant) as the sole organic carbon sources, was used for enrichment cultures, isolation procedures and cultivation of *Methanogenium liminatans* strains. WHP medium was based on the recipe described by Zellner & Jargen (1997) except that yeast extract, peptone and additional sodium sulfate were omitted. WHP medium contained 1 μM sodium tungstate and was used for the cultivation of strains DSM 2702T, GZKPZT and BM1. Substrates (e.g. sodium formate, sodium acetate, 2-propanol, 2-butanol, cyclopentanol) were added from anaerobic, sterile stock solutions (20%, w/v or v/v) as indicated to give concentrations of 0.5% (w/v or v/v) in the serum bottles. Cells were grown under H2/CO2 (4:1, v/v, 1,000 kPa) in 120 ml serum bottles on a rotary shaker (150 r.p.m.). When other substrates were used instead of hydrogen, N2/CO2 (4:1, v/v, 300 kPa) served as the gas atmosphere. For the preparation of large masses of cells, cells were grown in H2/CO2 (4:1, v/v) at a flow rate of 50 l h-1 in a 12 l fermenter (Biostat S; Braun).

*Methanogenium tationis* was grown as described previously (Zabel et al., 1984). *Methanoculleus olentangyi* and *Methanoculleus bourgensis* were grown as described by Corder et al. (1983) and Ollivier et al. (1986), respectively. *Methanocorpusculum labreanum* was grown as described by Zhao et al. (1989). *Methanocorpusculum sinense* and *Methanocorpusculum bavaricum* were grown as described by Zellner et al. (1989a).

**Source and habitat of strain BM1.** Strain BM1 (= DSM 10196) was isolated from a butyrate-degrading, fluidized bed reactor inoculated with a culture originally derived from the upflow anaerobic sludge blanket (UASB) reactor from the wastewater treatment plant at a sugar refinery in Bruhl, Germany (Zellner et al., 1991). The predominant methanogen in this bioreactor was an autofluorescent irregular coccus, similar in appearance to the new isolate (strain BM1) described here, and several other methanogens were identified immunologically (Zellner et al., 1991, 1997).

**Enrichment and isolation of strain BM1.** WHP medium with H2/CO2 (4:1, v/v, 300 kPa) as substrate was inoculated with 1% (v/v) bioreactor liquid and incubated at 37 °C. The gas phase was regularly replaced and autofluorescent, irregularly coccoid cells increased in number simultaneously with the formation of methane. This enriched culture was serially diluted into WHP medium and the highest dilution showing growth of the fluorescent, irregularly coccoid cells was again serially diluted. Aliquots (0.1 ml) of the dilutions were then streaked on agar plates with WHP medium plus 2.5% (w/v) Oxoid agar (Unipath). All manipulations were performed in an anaerobic chamber (M. Braun) under a nitrogen atmosphere. The plates were transferred into a stainless steel anaerobic jar, pressurized to 300 kPa with H2/CO2 (4:1, v/v) and incubated at 37 °C. After 20 d incubation, the gas pressure had dropped to 150 kPa, indicating gas consumption. The anaerobic jar was transferred into the anaerobic chamber. Cells were picked with a syringe needle from a well-isolated colony, injected into a 120 ml serum bottle containing 20 ml WHP medium under H2/CO2 (4:1, v/v, 300 kPa) and incubated at 37 °C. This procedure resulted in the isolation of strain BM1.

**Microscopy.** Phase-contrast and epifluorescence microscopy were carried out by using a Zeiss Axioscope microscope as described previously (Zellner et al., 1991). Motility was checked microscopically.

**Analyses.** Volatile fatty acids, alcohols, hydrogen and methane were analysed by GC (Zellner & Winter, 1987). The Colour Gram 2 staining set from bioMérieux was used for Gram staining according to the manufacturer’s instructions.
**RESULTS AND DISCUSSION**

**Morphology of strains, S-layer architecture and composition**

Cells of the newly isolated strain BM1 were highly irregular coccii. Some cells also had a ring-shaped appearance under phase-contrast microscopy. The cells were 1.25–2.0 μm in diameter, stained Gram-negative and were non-motile. The addition of 1% (w/v) SDS led to lysis of cells of *Methanogenium limitatans* strain GKZPZT and strain BM1, indicating a cell envelope with a proteinaceous cell wall. With the exception of *Methanogenium limitatans* strains, which appear as ring-shaped cells under phase-contrast microscopy (Zellner et al., 1990), the species described to date of the genera *Methanogenium* and *Methanoculleus* are irregular cocci and are indistinguishable from each other (Boone et al., 1993). However, some differences in cell envelope composition have been observed (Table 1). All irregularly coccoïd methanogens within the order *Methanomicrobiales* possess hexagonal S-layer lattices consisting of glycoprotein subunits with Mr ranging from 90000 to 155000. However, only methanogens belonging to the family *Methanocorpusculaceae* have an S-layer glycoprotein with Mr ranging from 90000 to 94000. The Mr of S-layer glycoprotein subunits of genera of the family *Methanomicrobiaceae* range from 101000 to 155000.

During the course of compiling this paper, the incorrect orthography of *Methanoculleus thermophilicus* was noticed which, according to Rule 61 of the...
Table 1. S-layers of irregularly coccoid methanogens of the families Methanomicrobiaceae, Methanoplanaceae and Methanocorpusculaceae

<table>
<thead>
<tr>
<th>Strain</th>
<th>DSM no.</th>
<th>Lattice constant c/c (nm)*</th>
<th>Glycoprotein subunit M₂ (x 10⁻³)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus Methanofollis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. tationis Chile 9T</td>
<td>2970T</td>
<td>ND</td>
<td>120</td>
<td>Zabel et al. (1984); Zellner et al. (1998)</td>
</tr>
<tr>
<td>M. liminatans GKPZT</td>
<td>4140T</td>
<td>15.4</td>
<td>118</td>
<td>Zellner et al. (1990, 1998)</td>
</tr>
<tr>
<td>Genus Methanoculleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. marisnigri JR1T</td>
<td>1498T</td>
<td>ND</td>
<td>138</td>
<td>Zellner et al. (1990)</td>
</tr>
<tr>
<td>M. olentangyi RC/ER</td>
<td>2772T</td>
<td>15.4</td>
<td>132</td>
<td>Zellner et al. (1990)</td>
</tr>
<tr>
<td>M. bourgensis MS2T</td>
<td>3045T</td>
<td>15.4</td>
<td>101</td>
<td>Zellner et al. (1990)</td>
</tr>
<tr>
<td>M. thermophilus CR-1T</td>
<td>2373T</td>
<td>ND</td>
<td>130</td>
<td>Zabel et al. (1985)</td>
</tr>
<tr>
<td>M. thermophilus UCLA</td>
<td>2624</td>
<td>ND</td>
<td>130</td>
<td>Zabel et al. (1985)</td>
</tr>
<tr>
<td>M. thermophilus Ratisbona</td>
<td>2640</td>
<td>ND</td>
<td>130</td>
<td>Zabel et al. (1985)</td>
</tr>
<tr>
<td>M. palmolei INSUZT</td>
<td>4273T</td>
<td>15.2</td>
<td>120</td>
<td>Zellner et al. (1998)</td>
</tr>
<tr>
<td>Genus Methanogenium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. cariaci JR1T</td>
<td>1497T</td>
<td>14.0</td>
<td>117</td>
<td>Zellner et al. (1990)</td>
</tr>
<tr>
<td>M. frittoni FR-4T</td>
<td>2832T</td>
<td>15.4</td>
<td>106</td>
<td>Zellner et al. (1990)</td>
</tr>
<tr>
<td>Genus Methanolacinia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. paynteri G2000T</td>
<td>2545T</td>
<td>15.3</td>
<td>155†</td>
<td>Zellner et al. (1989c)</td>
</tr>
<tr>
<td>Genus Methanoplanus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. limicola M3</td>
<td>2279T</td>
<td>14.0</td>
<td>143</td>
<td>Zellner et al. (1989a)</td>
</tr>
<tr>
<td>M. endosymbiosus MCI</td>
<td>3599T</td>
<td>ND</td>
<td>110</td>
<td>Zellner et al. (1989a)</td>
</tr>
<tr>
<td>Genus Methanocorpusculum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. parvum XII</td>
<td>3823T</td>
<td>14.3</td>
<td>90</td>
<td>Zellner et al. (1989a)</td>
</tr>
<tr>
<td>M. bavaricum SZXXZ</td>
<td>4179T</td>
<td>16.0</td>
<td>94</td>
<td>Zellner et al. (1989a)</td>
</tr>
<tr>
<td>M. sinense CHINAZ</td>
<td>4274T</td>
<td>15.8</td>
<td>92</td>
<td>Zellner et al. (1989a)</td>
</tr>
<tr>
<td>M. aggregans MSt</td>
<td>3027T</td>
<td>15.4</td>
<td>92</td>
<td>P. Messner &amp; G. Zellner, unpublished</td>
</tr>
</tbody>
</table>

ND, Not determined.
* Centre-to-centre spacing of hexagonally arranged protein subunits of S-layer.
† A second, weaker band with an M₂ of 135000 that reacted with PAS stain presumably represented a degradation product or a less glycosylated precursor.

Bacteriological Code (Lapage et al., 1992), may be corrected to Methanoculleus thermophilus (corrig.).

Culture conditions and substrates for growth and methanogenesis

The optimal growth temperature of strain BM1 was about 37-40 °C. No growth was obtained below 20 °C or above 45 °C. The optimal pH was about 7. Growth and methane production were observed on H₂/CO₂, formate, 2-propanol/CO₂ and 2-butanol/CO₂. Growth and methane production by strain BM1 on potential substrates was as follows (maximal OD₅₇₈ obtained/total methane produced per 20 ml culture in a 120 ml serum bottle): H₂/CO₂ (0.86/3285 μmol), formate (0.14/172 μmol), 2-propanol/CO₂ (0.06/52 μmol), 2-butanol/CO₂ (0.04/44 μmol) and cyclopentanol/CO₂ (0.00 1 /2 1 μmol). Thus, except for cyclopentanol/CO₂, which supported only methanogenesis, all these substrates supported both growth and methanogenesis. The secondary and cyclic alcohols were oxidized to the respective ketones. Strain BM1 did not grow or produce methane in media with acetate, methanol, methanamines, ethanol or 1-propanol as substrate. In contrast, Methanogenium tationis DSM 2702T did not grow or produce methane on 2-propanol/CO₂ or 2-butanol/CO₂ (Zellner & Winter, 1987) or cyclopentanol/CO₂. Acetate was required for growth of strain BM1 on formate. Thus, while strain BM1 resembled Methanogenium liminatans more closely in terms of substrate utilization, it could not be distinguished from other species of the genera Methanoculleus and Methanogenium on this basis (Table 2).

Cell physiology

The original descriptions of Methanogenium tationis and Methanogenium liminatans were supplemented by material on the utilization of secondary and cyclic
Table 2. Phenotypic features of irregularly coccoid strains of the families Methanomicrobiaceae and Methanoplanaceae

<table>
<thead>
<tr>
<th>Strain</th>
<th>DSM no.</th>
<th>Cell size (μm)</th>
<th>Flagella</th>
<th>Substrates*</th>
<th>Min. tp (h)</th>
<th>Growth temp. (°C)</th>
<th>G+C content (mol%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus Methanoplanus</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. maricasii JR17</td>
<td>2702^T</td>
<td>1.5-3.0</td>
<td>+</td>
<td>+</td>
<td>12</td>
<td>37-40/25-45</td>
<td>54</td>
<td>Zabel et al. (1984)</td>
</tr>
<tr>
<td>M. niederleinii GS2</td>
<td>4146^T</td>
<td>1.25-2.0</td>
<td>+</td>
<td>+</td>
<td>7.5</td>
<td>40/25-45</td>
<td>59</td>
<td>Zellner et al. (1990)</td>
</tr>
<tr>
<td>M. liminatans BM1</td>
<td>10196</td>
<td>1.25-2.0</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>40/20-45</td>
<td>69°</td>
<td>This paper</td>
</tr>
<tr>
<td>Genus Methanoculleus</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>M. bourgensii RS2</td>
<td>1245^T</td>
<td>1.1-1.2</td>
<td>+</td>
<td>+</td>
<td>10</td>
<td>20-25/10-45</td>
<td>61-62°</td>
<td>Romener et al. (1979)</td>
</tr>
<tr>
<td>M. thermophiles CR1^T</td>
<td>2373^T</td>
<td>0.8-1.3</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>37/25-55</td>
<td>59°</td>
<td>Ollivier et al. (1984)</td>
</tr>
<tr>
<td>M. thermophiles UCLA</td>
<td>2624</td>
<td>0.7-1.8</td>
<td>+</td>
<td>+</td>
<td>1.8</td>
<td>55-60/60</td>
<td>56, 59°</td>
<td>Ferguson &amp; Mah (1983)</td>
</tr>
<tr>
<td>M. thermophiles ZEPR1</td>
<td>2640</td>
<td>0.9-1.5</td>
<td>+</td>
<td>+</td>
<td>3.4</td>
<td>58/30-60</td>
<td>57</td>
<td>Zabel et al. (1985)</td>
</tr>
<tr>
<td>M. thermophiles TCI</td>
<td>3915</td>
<td>0.6-1.5</td>
<td>NR</td>
<td>+</td>
<td>+</td>
<td>55/30-60</td>
<td>54°</td>
<td>Wildgruber et al. (1988)</td>
</tr>
<tr>
<td>M. olenburgensis</td>
<td>6216^T</td>
<td>1.0</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>45/20-50</td>
<td>48°</td>
<td>Biotevogl et al. (1991)</td>
</tr>
<tr>
<td>M. palmodis DNSLZ^T</td>
<td>4273^T</td>
<td>1.25-2.0</td>
<td>+</td>
<td>+</td>
<td>13.5</td>
<td>40/21-51</td>
<td>59°</td>
<td>Zellner et al. (1998)</td>
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<td>Genus Methanoculleus</td>
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<tr>
<td>M. caracci JR17</td>
<td>1497^T</td>
<td>&lt;2.6, 1.3</td>
<td>-</td>
<td>+</td>
<td>11.0</td>
<td>37-45/15-50</td>
<td>51.6°</td>
<td>Romener et al. (1979)</td>
</tr>
<tr>
<td>M. titeri GC2</td>
<td>3596^T</td>
<td>0.5-1.5</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>30-35/15-39</td>
<td>46.7°</td>
<td>Wildgruber et al. (1988)</td>
</tr>
<tr>
<td>M. frisiai FL4^T</td>
<td>2838</td>
<td>1.2-2.5</td>
<td>+</td>
<td>+</td>
<td>1.2</td>
<td>57/26-62</td>
<td>49°</td>
<td>Harris et al. (1984)</td>
</tr>
<tr>
<td>M. friisii FL4^T</td>
<td>[SMCC459W]</td>
<td>1.2-2.5</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>69.6</td>
<td>15/0-17</td>
<td>Främzmann et al. (1997)</td>
</tr>
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<td>Genus Methanoculleus</td>
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</tr>
<tr>
<td>M. paynteri G-2000^T</td>
<td>2545^T</td>
<td>0.6 x 1.5-2.5</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>40/20-45</td>
<td>45°</td>
<td>Zellner et al. (1986)</td>
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<tr>
<td>Genus Methanoculleus</td>
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<tr>
<td>M. conradti M3^T</td>
<td>2279^T</td>
<td>1.3 x 0.1-0.25</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>40/17-41</td>
<td>47°</td>
<td>Wildgruber et al. (1982)</td>
</tr>
<tr>
<td>M. endosymbionica MC1^T</td>
<td>3599^T</td>
<td>1.6-3.4</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>32/16-36</td>
<td>38°</td>
<td>van Bruggen et al. (1986)</td>
</tr>
</tbody>
</table>

NR, Not reported; ND, not determined.
*H, H₂/CO₂; F, formate; 2P, 2-propanol/CO₂; 2B, 2-butanol/CO₂. None of the strains utilized acetate, methanol or methylamines. Methanogenium organophilum utilized ethanol, 1-propanol and 1-butanol in addition. Data on alcohol utilization partly taken from Zellner & Winter (1987). In addition, both Methanofollis liminatans strains, GZKZPZ and BM1, Methanoculleus palmodis and Methanoculleus bourgensis utilized cyclopentanol/CO₂ with concomitant production of cyclopentanone and CH₄, while Methanofollis tationis could not utilize cyclopentanol/CO₂. (+), Weakly positive.
†Growth temperature is given as optimal temperature/temperature range.
‡G+C content of DNA was determined by T₂ₐ, unless otherwise indicated, in which case it was determined by buoyant density (a) or HPLC (b).
§Methanoculleus olenburgensis and Methanoculleus bourgensis are apparently subjective synonymes (Xun et al., 1989; Boone et al., 1993).

Lipids of Methanogenium tationis and Methanogenium liminatans

Both diether- and tetraether-linked glycerol lipids were present in Methanogenium tationis DSM 2702^T and Methanogenium liminatans GZKZPZ^T (data not shown). The diether lipids were diphytanyl glycerol diethers, while the tetraether lipids showed no evidence of cyclization and only acyclic C_{40},C_{40} dibiphytanyl tetraethers were present. These results are consistent with the results from other members of the family Methanomicrobiaceae examined to date (Grant et al., 1985; Zellner et al., 1989c; Biotegovt et al., 1991; B. J. Tindall & G. Zellner, unpublished data).

The major polar lipids present in Methanogenium tationis DSM 2702^T and Methanogenium liminatans GZKZPZ^T were glycolipids, phospholipids and phosphoglycolipids (Fig. 1). The major phospholipids present were ether derivatives of phosphatidyl glycerol, phosphopentanetetrol amine and phosphopentanetetrol trimethylamine. The major glycolipid present was a diglycosyl ether lipid, the nature of the sugars and their mode of linkage not being determined. A single major phosphoglycolipid was also detected. The acids from Zellner & Winter (1987) and Bleicher et al. (1989). Methanogenium liminatans contains a secondary alcohol dehydrogenase activity that is devoid of zinc and is dependent on factor F_{420} (Bleicher & Winter, 1991).

The pterin of Methanogenium tationis has a structural modification not found in other methanogens and is called tationopterin. Tationopterin lacks a 7-methyl substituent but has both a glutamyl and an aspartyl residue conjugated to the phosphoglutaryl moiety (Raemakers-Franken et al., 1989) and can be distinguished from methanopterin and sarcinapterin (Keltjens et al., 1983; DiMarco et al., 1990).

Presumptive respiratory lipoquinones of Methanogenium tationis and Methanogenium liminatans

Examination of Methanogenium tationis DSM 2702^T and Methanogenium liminatans GZKZPZ^T for the presence of respiratory lipoquinones indicated that none were present, a finding consistent with results obtained for all other members of the methanogenic Archaea examined to date (B. J. Tindall, unpublished data).
presence of aminopentanetetrol-containing phospholipids is characteristic of members of the family Methanomicrobiaceae. Moreover, a detailed analysis of the total polar lipid composition of the various taxa within this group indicates that there are clear differences between the various taxa currently described (Grant et al., 1985; Zellner et al., 1989a, c; Blotevogel et al., 1991; B. J. Tindall & G. Zellner, unpublished data). Thus, the chemical composition of the cell may be a useful way of differentiating genera in this phyletic group.

Although it is not known whether members of the Methanocorpusculaceae also contain these unusual aminopentanetetrol-containing phospholipids, the polar lipid composition of members of this family is sufficiently different to distinguish them from the members of the family Methanomicrobiaceae (Zellner et al., 1989a). The fact that members of the family Methanocorpusculaceae have a distinct polar lipid pattern would appear to contradict the 16S rDNA sequence data, since this family appears to branch within the family Methanomicrobiaceae. However, the branching order of some groupings within the members of the order Methanomicrobiales is uncertain. Such uncertainties in branching order, particularly where the pendant edges of the dendrogram are long in relation to the length of internal branches, has been already documented.

The presence of identical polar lipids in both Methanogenium tationis and Methanogenium liminatans suggests that these features are not characteristic of the individual species but indicative of a higher taxonomic group. Although certain aspects of their polar lipid pattern are similar to other members of the family Methanomicrobiaceae, there are differences in the details of the polar lipid patterns which may be regarded as genus-specific markers.

**Phylogenetic position**

Sequencing of the 16S rRNA and rDNA was performed to determine the phylogenetic relationship of Methanogenium tationis strain DSM 2702T and Methanogenium liminatans strains GKZPZT (= DSM 4140T) and Methanogenium liminatans BM1 (= DSM 10196) within the family Methanomicrobiaceae. Numbers at branch points represent percentage bootstrap support. Bar, 10% sequence divergence.

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**Fig. 1.** Thin-layer chromatograms of the polar lipids of (a) Methanogenium tationis DSM 2702T and (b) Methanogenium liminatans GKZPZT. Spots are identified as: 1, phosphatidyl glycerol; 2, phosphatidyl pentanetetrol amine; 3, diglycosyl diether; 4, phosphatidyl pentanetetrol trimethylamine; 5, phosphoglycolipid; 6, phosphoglycolipid; 7, amino-phosphoglycolipid; 8, amino-phospholipid.

**Fig. 2.** Dendrogram showing the phylogenetic positions of Methanofollis tationis DSM 2702T, Methanofollis liminatans GKZPZT (= DSM 4140T) and Methanofollis liminatans BM1 (= DSM 10196) within the family Methanomicrobiaceae. Numbers at branch points represent percentage bootstrap support. Bar, 10% sequence divergence.
Methanofollis gen. nov.

Methanofollis (Me.tha.no.fol’lis. M.L. n. methanum methane; M.L. n. follis a bag; Methanofollis a methane-producing bag).

Irregular cocci, 1.5–3.0 μm in diameter, with a proteinaceous, SDS-sensitive S-layer. Cells stain Gram-negative. Some strains are motile. Obligately anaerobic, no microaerophilic or aerobic growth. Meso-philic (range 15 to 45 °C). Substrates for growth and methane production are H₂/CO₂ and formate. Methanol, methylamines, ethanol, 2-propanol/CO₂, 2-butanol/CO₂ and cyclopentanol/CO₂ are not utilized. Cells contain taripterin, a modified pterin distinct from methanopterin and sarcinapterin. Lipid composition as that described for the genus. The G+C content of DNA of the type strain is 60 mol% (Tₘ). The type strain is DSM 4140T, isolated from an industrial wastewater bioreactor (Biohochreaktor, Hoechst, Kelsterbach, Frankfurt, Germany). Strain BM1 (= DSM 10196) is a reference strain, isolated from a methane-producing fluidized bed reactor operated with synthetic wastewater containing butyrate and present in a full-scale UASB reactor treating sugar refinery wastewater anaerobically in Brühl, Germany.

ACKNOWLEDGEMENTS

This investigation was supported in part by grants of the Deutsche Forschungsgemeinschaft. Part of the practical work of G.Z. was performed in the Institute of Microbiology, University of Hannover, Germany, and was completed during a period as a Senior Research Associate of the Wageningen Agricultural University, financially supported by WIMEK (Wageningen Institute for Environment and Climate, Wageningen, The Netherlands). W.B.W. received support from the US Environmental Protection Agency, contract AERL-9003. C.R.W. received support from the...
National Science Foundation, grant DEB-9306171. The G+C content of DNA of strain BM1 was determined by Mrs Inge Reupke. We wish to thank Mrs Ulrike Mendrock for her help with the molecular work.

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