Methanogenium frittonii Harris et al. 1996 is a later synonym of Methanoculleus thermophilus (Rivard and Smith 1982) Maestrojua´ n et al. 1990

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The 16S rRNA gene sequence of [Methanogenium] frittonii DSM 2832T was determined and was found to be 99-9 % similar to the sequence of Methanoculleus thermophilus DSM 2373T. DNA–DNA hybridizations between both strains revealed 86 % DNA–DNA binding, indicating that both strains belong to the same species. The determination of the DNA G+C content of both type strains, DSM 2832T and DSM 2373T, revealed values of 56-1 and 59-1 mol%, respectively. Based on the phenotypic and genotypic characteristics, it is proposed to unite the species [Methanogenium] frittonii and Methanoculleus thermophilus under the name Methanoculleus thermophilus, which is the earlier synonym and hence has priority. Emended descriptions of the species Methanoculleus thermophilus and the genus Methanogenium are also given.

The genera Methanogenium and Methanoculleus are phylogenetically related within the family Methanomicrobiaceae, which represent methanogens of highly irregular coccoid shape. The genus Methanogenium currently comprises five species with validly published names: Methanogenium cariaci, Methanogenium frigidum, [Methanogenium] frittonii, Methanogenium marinum and Methanogenium organophilum. Previously, the species [Methanogenium] bourgens, [Methanogenium] marisnigri, [Methanogenium] olentangi and [Methanogenium] thermophilicum were transferred to the genus Methanoculleus by Maestrojua´ n et al. (1990). Later, Asakawa & Nagaoka (2003) united Methanoculleus bourgensis, Methanoculleus olentangi and Methanoculleus oldenburgensis in one species under the name Methanoculleus bourgensis. All described species of the genus Methanogenium, with the exception of [Methanogenium] frittonii, are psychrophilic to mesophilic and require acetate for growth. In contrast, the original description of [Methanogenium] frittonii by Harris et al. (1984) reports that this species is thermophilic and does not require acetate as a carbon source for growth. However, because of the lack of a 16S rRNA gene sequence, the phylogenetic relationship of [Methanogenium] frittonii to other members of the genus Methanogenium remained unclear. To clarify the taxonomic position of [Methanogenium] frittonii, we determined the almost complete 16S rRNA gene sequence of the type strain DSM 2832T.

The strains [Methanogenium] frittonii DSM 2832T and Methanoculleus thermophilus DSM 2373T were grown under the conditions indicated in the DSMZ catalogue of strains (www.dsmz.de). For the isolation of DNA, each strain was cultured in about 10 l of the respective DSMZ medium. After incubation for 48 h at 55 °C, cells were harvested by centrifugation. It turned out that both strains were very sensitive to lysis during resuspension of the cell pellet, which was done under oxic conditions. However, the loss of DNA could be efficiently prevented by resuspending the pellet in 80 % (v/v) 2-propanol (aqueous solution), which stabilized the nucleic acids.

Genomic DNA extraction and amplification of the 16S rRNA gene of strain DSM 2832T was carried out as reported by Rainey et al. (1996). Purified PCR products were sequenced with the CEQ™ DTCS Quick Start kit (Beckman Coulter), as described in the manufacturer’s protocol. The CEQ™ 8000 Genetic Analysis System was used for the electrophoresis of the sequence reaction products. An almost complete 16S rRNA gene sequence of 1451 nucleotides was determined, and was phylogenetically analysed using the ARB software package (Ludwig et al., 2004). The phylogenetically most closely related species of [Methanogenium] frittonii turned out to be Methanoculleus thermophilus. The 16S rRNA gene sequences of both type strains were almost identical (99-9 % similarity), and had similarity values around 96 % with sequences of other type strains of the genus Methanoculleus, which suggested the affiliation of strain DSM 2832T to this genus. In contrast, [Methanogenium] frittonii was only distantly related to representatives of the genus Methanogenium, as indicated...
by similarity values of below 92% among 16S rRNA gene sequences.

Genomic DNA for the determination of DNA base composition and DNA–DNA hybridization experiments was isolated by using a French pressure cell, and was purified by chromatography on hydroxyapatite, as described by Cashion et al. (1977). DNA–DNA hybridization experiments between both strains were carried out in 2× SSC buffer containing 5% (v/v) formamide at 68°C, according to the method of De Ley et al. (1970). DNA renaturation rates were measured by using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6×6 multicell changer and a temperature controller with an in situ temperature probe (Varian). The estimated level of DNA–DNA binding between the strains DSM 2832T and DSM 2373T was 86%. According to Wayne et al. (1987), reassociation values above 70% indicate that the strains belong to the same species.

The affiliation of both strains to one species is supported by several morphological and physiological traits. The sole important physiological difference is the requirement for acetate as a carbon source by Methanoculleus thermophilus, whereas [Methanogenium] frittonii can grow autotrophically. However, Romesser et al. (1979) reported that Methanoculleus marisnigri, a species closely related to Methanoculleus thermophilus, can also grow without acetate as a carbon source. Consequently, within the genus Methanoculleus the requirement for acetate seems to be variable among strains.

A further differentiating characteristic between both type strains is the DNA G+C content. In the original species descriptions of Methanoculleus thermophilus and [Methanogenium] frittonii, G+C contents of 59 mol% (buoyant density method; Rivard & Smith, 1982) and 49 mol% (buoyant density method; Harris et al., 1984), respectively, were reported. Generally, the variability of G+C content among strains within the same species does not exceed 5 mol% (Rosselló-Mora & Amann, 2001). To check this discrepancy, we repeated the determination of the DNA G+C content of both strains by using reversed-phase HPLC of nucleosides, according to Mesbah et al. (1989). The G+C content of Methanoculleus thermophilus DSM 2373T and [Methanogenium] frittonii DSM 2832T was found to be 59.1 and 56.1 mol%, respectively. While the reported value for strain DSM 2373T could be confirmed by our results, the obtained value for strain DSM 2832T was inconsistent with the data reported by Harris et al. (1984), but was in good agreement with values obtained by Zabel et al. (1985) and Widdel et al. (1988) by using the thermal denaturation method for three other strains affiliated to the species Methanoculleus thermophilus (55–57 mol%). Thus, we conclude that the originally reported DNA G+C content of 49 mol% for strain DSM 2832T was too low, due to experimental error.

Based on the presented results, it is proposed to unite the species [Methanogenium] frittonii and Methanoculleus thermophilus. According to rules 38, 42 and 24b(2) of the Bacteriological Code (Lapage et al., 1992), the validly published name Methanoculleus thermophilus has priority and hence should be used for the unified taxon.

In addition, the presented data and a critical review of the relevant literature permit an emendation of the genus Methanogenium, thereby enabling a more distinct differentiation from the related genus Methanoculleus (Table 1).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Methanogenium</th>
<th>Methanoculleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (μm)</td>
<td>0.5–2.6</td>
<td>0.6–2.0</td>
</tr>
<tr>
<td>Optimal growth temperature (°C)</td>
<td>15–35</td>
<td>25–60</td>
</tr>
<tr>
<td>Optimal NaCl concentration (M)</td>
<td>0.35–1.25</td>
<td>0.0–0.4</td>
</tr>
<tr>
<td>Requirement for acetate</td>
<td>+</td>
<td>d</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>47–52</td>
<td>55–62</td>
</tr>
</tbody>
</table>

Table 1. Differentiation between the genera Methanogenium and Methanoculleus

Cells of both genera are Gram-negative and have an irregular cocoid shape. Flagella and pili (fimbriae) may be present but motility is rarely observed. Cell envelopes consist of a cytoplasmic membrane and an S-layer of hexagonally arranged glycoprotein subunits with M, values in the range 100 000–138 000. H₂ and formate can be used by all strains as substrates for methanogenesis. Alcohols may serve as alternative hydrogen donors in some strains. Data from Romesser et al. (1979), Rivard & Smith (1982), Harris et al. (1984), Zabel et al. (1985), Widdel et al. (1988), Maestrojuaün et al. (1990), Franzmann et al. (1997), Zellner et al. (1998), Dianou et al. (2001), Asakawa & Nagaoa (2003), Chong et al. (2002) and Mikucki et al. (2003). +, Positive in all strains; d, different reaction in different species.

The characteristics of this species are as described by Rivard & Smith (1982) and Zabel et al. (1985), with the following exceptions. Secondary alcohols might be used as electron donor by single strains (Widdel et al., 1988). Most strains require acetate as a carbon source for growth, but single strains might be autotrophic. Trypticase peptone or yeast extract stimulates growth in all strains. The DNA G+C content is 55–59 mol%; the type strain has a G+C content of 59 mol%.

The type strain is DSM 2373T (= OCM 174T).


The description of this genus is that given by Romesser et al. (1979), emended by Maestrojuaün et al. (1990), except that optimal temperatures for growth are between 15 and 35°C.
and optimal NaCl concentrations range from 0.35 to 1.25 M.

The type species is *Methanogenium cariacci*.

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### References


