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

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The 16S rRNA gene sequence of [*Methanogenium*] *frittonii* DSM 2832^T was determined and was found to be 99.9 % similar to the sequence of *Methanoculleus thermophilus* DSM 2373^T. 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 2832^T and DSM 2373^T, 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*] *bourgense*, [*Methanogenium*] *marisnigri*, [*Methanogenium*] *olentangyi* and [*Methanogenium*] *thermophilicum* were transferred to the genus *Methanoculleus* by Maestrojuán *et al.* (1990). Later, Asakawa & Nagaoka (2003) united *Methanoculleus bourgense*, *Methanoculleus olentangyi* and *Methanoculleus oldenburgensis* in one species under the name *Methanoculleus bourgense*. 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 2832^T.

The strains [*Methanogenium*] *frittonii* DSM 2832^T and *Methanoculleus thermophilus* DSM 2373^T 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 2832^T was carried out as reported by Rainey *et al.* (1996). Purified PCR products were sequenced with the CEQTM DTCS Quick Start kit (Beckman Coulter), as described in the manufacturer's protocol. The CEQTM 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 2832^T to this genus. In contrast, [*Methanogenium*] *frittonii* was only distantly related to representatives of the genus *Methanogenium*, as indicated

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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 2832^T and DSM 2373^T 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 2373^T and [*Methanogenium*] *frittonii* DSM 2832^T was found to be 59.1 and 56.1 mol%, respectively. While the reported value for strain DSM 2373^T could be confirmed by our results, the obtained value for strain DSM 2832^T 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 2832^T was too low, due to experimental error.

Based on the presented results, it is proposed to unite the species [*Methanogenium*] *frittonii* and *Methanoculleus*

Table 1. Differentiation between the genera *Methanogenium* and *Methanoculleus*

Cells of both genera are Gram-negative and have an irregular coccoid 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_r values in the range 101 000–138 000. 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), Maestrojuán *et al.* (1990), Franzmann *et al.* (1997), Zellner *et al.* (1998), Dianou *et al.* (2001), Asakawa & Nagaoka (2003), Chong *et al.* (2002) and Mikucki *et al.* (2003). +, Positive in all strains; d, different reaction in different species.

Characteristic	<i>Methanogenium</i>	<i>Methanoculleus</i>
Size (µm)	0.5–2.6	0.6–2.0
Optimal growth temperature (°C)	15–35	25–60
Optimal NaCl concentration (M)	0.35–1.25	0–0.4
Requirement for acetate	+	d
DNA G+C content (mol%)	47–52	55–62

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).

Emended description of the species *Methanoculleus thermophilus* (Rivard and Smith, 1982) Maestrojuán *et al.* 1990

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 2373^T (= OCM 174^T).

Emended description of the genus *Methanogenium* Romesser *et al.* 1981 emend. Maestrojuán *et al.* 1990

The description of this genus is that given by Romesser *et al.* (1979), emended by Maestrojuán *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 cariaci*.

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