Transfer of *Methanococcus frisius* to the Genus *Methanosarcina* as *Methanosarcina frisia* comb. nov.

KARL-HEINZ BLOTEVOGEL^{1*} AND ULRICH FISCHER²

*Fachbereich Biologie, AG Mikrobiologie,¹ and Fachbereich Biologie, AG Geomikrobiologie,²
Universität Oldenburg, D-2900 Oldenburg, Federal Republic of Germany*

Several methanogenic bacteria that utilize methanol, acetate, or methylamines have been isolated from marine habitats, including *Methanococcus frisius*. However, the 1984 description by Mah and Kuhn of the genus *Methanococcus* excludes methylotrophic methanobacteria such as *Methanococcus frisius*. Therefore, we propose to transfer this species to the genus *Methanosarcina*, naming it *Methanosarcina frisia* comb. nov. (type strain, DSM 3318 [= ATCC 43330]). Other species having similar properties (e.g., *Methanococcus halophilus*) should be assignable to *Methanosarcina*.

All species of the order *Methanococcales* (*Methanococcus vannielii*, *Methanococcus voltae*, *Methanococcus maripaludis*, *Methanococcus deltae*, *Methanococcus thermolithotrophicus*, *Methanococcus jannaschii*) share a coccoid morphology and a marine habitat (for a review see reference 4). With the exception of the thermophilic strains *Methanococcus thermolithotrophicus* and *Methanococcus jannaschii*, the species are mesophilic. Among these mesophilic species the physiological characteristics are very similar, including the utilization of H₂ and formate as the only methanogenic substrates. In addition, temperature optima and deoxyribonucleic acid base compositions (guanine-plus-cytosine contents) are similar. The cell envelopes are mainly composed of a protein monolayer (4).

The genus *Methanococcus* was described in detail by Mah and Kuhn (5). These authors pointed out that energy for

cumscriptum of this genus, because this organism utilizes mainly methanol and methylamines but not formate; this is a metabolic behavior that is also common to *Methanococcus halophilus* (9, 10). Additionally, no flagella were observed, in contrast to the presence of flagella on species of the *Methanococcaceae*. Both *Methanococcus frisius* and *Methanococcus halophilus* should be transferred to another genus, a suggestion supported recently by Rouvière and Wolfe (7). These authors showed that the molecular sizes of the methylreductase subunits of *Methanococcus frisius* are unequivocally similar to those of the members of the genus *Methanosarcina*. Antigenic fingerprinting has been undertaken (K.-H. Blotevogel and A. J. L. Macario, Syst. Appl. Microbiol., in press) and has shown no relationship of *Methanococcus frisius* to members of the *Methanococcaceae*; there were slight cross-reactions with *Methanosarcina*

TABLE 1. Comparison of the main characteristics of known species of coccoid methanogens

Species	Morphology	Substrates	Guanine-plus-cytosine content (mol%)	Temp optimum (°C)	pH optimum	Salt requirement (% NaCl)
<i>Methanococcus vannielii</i> ^a	Coccus	H ₂ , formate	32.5	36–40	7.0–9.0	1.2–4.8
<i>Methanococcus halophilus</i> ^b	Coccus	Methanol, methylamines	ND ^c	26–36	6.5–7.4	7.0–9.0
<i>Methanosarcina barkeri</i> ^d	Coccus (packets)	H ₂ , methanol, methylamines, acetate	39	35	7.0	NR ^e
<i>Methanosarcina frisia</i> ^f	Coccus	H ₂ , methanol, methylamines	38.2	36	6.5–7.2	2.0–2.5
<i>Methanosarcina acetivorans</i> ^g	Coccus (aggregates)	Acetate, methanol, methylamines	41	35–40	6.5–7.0	1.2

^a Data from reference 5.

^b Data from reference 9.

^c ND, Not determined.

^d Data from reference 4.

^e NR, Not required.

^f Data from reference 2.

^g Data from reference 8.

growth of members of the family *Methanococcaceae* is obtained only by oxidation of H₂ or formate coupled to the reduction of CO₂ to CH₄. They excluded methylotrophic growth for all species within the genus *Methanococcus*. Therefore, *Methanococcus frisius* (2) falls outside the cir-

barkeri MS and W, *Methanosarcina mazei* S6, and *Methanosarcina thermophila* TM1.

Therefore, we propose that *Methanococcus frisius* be placed in the genus *Methanosarcina* of the *Methanosarcinaceae*. Morphology and substrate range clearly place *Methanococcus frisius* outside the genus *Methanohalophilus*, and the optimum salinity for growth places it outside the genus *Methanohalophilus* (3, 6). Differentiation from *Methanococ-*

* Corresponding author.

coides and *Methanlobus* depends on the ability of *Methanococcus frisius* to use H_2 - CO_2 , methanol, and methylamines.

The main differences among *Methanococcus vannielii*, *Methanococcus halophilus*, *Methanosarcina barkeri*, *Methanosarcina frisia*, and *Methanosarcina acetivorans* are summarized in Table 1. As Table 1 shows, the major differences between *Methanosarcina acetivorans* and *Methanosarcina frisia* are (i) the formation of aggregates by *Methanosarcina acetivorans*, (ii) the ability of *Methanosarcina acetivorans* to use acetate, (iii) no use of H_2 - CO_2 and a lower salt requirement (1.2% NaCl) by *Methanosarcina acetivorans*, and (iv) a higher guanine-plus-cytosine molar ratio in *Methanosarcina acetivorans*.

On the basis of the above-mentioned arguments, we transfer *Methanococcus frisius* to the genus *Methanosarcina* and name it *Methanosarcina frisia* comb. nov.

Description of *Methanosarcina frisia* comb. nov. *Methanosarcina frisia* (fri'si.a. f. derivative of L. masc. adj. *frisius*, pertaining to Frisia, a region in the northwest of the Federal Republic of Germany). Cells are fragile, regular cocci (diameter, 0.5 to 2.0 μ m) and occur singly or in pairs, never in aggregates. The cells are gram negative and possess no flagella but exhibit fimbrialike structures. The cell envelope consists only of a single layer of protein subunits similar to that of *Methanosarcina acetivorans* (8). In addition, *Methanosarcina frisia* is serologically distinct from other coccoid methanogenic genera, such as *Methanogenium*, *Methanoplanus*, *Methanosphaera*, *Methanlobus*, and *Methanococcus* (Blotevogel and Macario, in press). *Methanosarcina frisia* grows in temperature range from 22 to 42°C, with optimum growth at 36°C. The pH optimum is between 6.5 and 7.2.

The DNA base composition is 38.2 ± 1.0 mol% guanine plus cytosine. The organism is strictly anaerobic and grows in a defined mineral medium with H_2 - CO_2 , methanol, or methylamines. Acetate and formate are not used. Yeast extract stimulates growth. Sodium chloride is required (at least 20 g/liter). The habitat is the shoal mud of marine environments.

The type strain of *Methanosarcina frisia* is strain DSM

3318 (= ATCC 43330), which was isolated from the black mud of the Leybucht (North Sea) (2).

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