Transfer of the Type Species of the Genus *Methanococcus* to the Genus *Methanosarcina*, Naming it *Methanosarcina mazei* (Barker 1936) comb. nov. et emend. and Conservation of the Genus *Methanococcus* (Approved Lists 1980) with *Methanococcus vannielii* (Approved Lists 1980) as the Type Species

Request for an Opinion

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Mah (Curr. Microbiol. 3:321–326, 1980) isolated *Methanococcus mazei*, a methanogen that was first described by Barker in 1936. The current characterization of this organism demands transfer of it to the genus *Methanosarcina*. We present an emended description of *Methanosarcina mazei* comb. nov. Since *Methanococcus mazei* (Approved Lists 1980) is the type species of the genus *Methanococcus* and its removal invalidates the genus, we request an Opinion from the Judicial Commission to remove the type species from the genus *Methanococcus* and to conserve this genus with *Methanococcus vannielii* as the type species, as suggested by Balch et al. (Microbiol. Rev. 43:260–296, 1979).

This Request for an Opinion is presented in the following two parts: (i) an emended description of *Methanosarcina mazei* comb. nov. based on the isolation and characterization of an axenic strain of the organism; and (ii) justification for the conservation of the genus *Methanococcus* demanded by the taxonomic transfer of the type species, *Methanococcus mazei*, to the genus *Methanosarcina*. This transfer of the type species would otherwise invalidate the genus *Methanococcus*.

***Methanosarcina mazei* comb. nov. et emend.** On the Approved Lists of Bacterial Names (11), which became effective on 1 January 1980, *Methanococcus mazei* Barker 1936 was listed as the type species of the genus *Methanococcus* Kluver and van Niel 1936 emend. Barker 1936; a type strain was not extant.

The genus *Methanococcus* was created by Kluver and van Niel (7) in 1936 to accommodate the relatively small methanogenic cocci described by Groeneweg (6) in 1920. These small cocci formed "considerable masses," were embedded in a "slimy matrix," and were thought "to ferment methyl and butyl alcohols and acetone in addition to ethyl alcohol" (3). In cultures with ethanol as the substrate, they overgrew "all other types of methane bacteria" (3). These cocci were morphologically distinct from the organisms for which Kluver and van Niel (7) created the genus *Methanosarcina*. At that time, no axenic culture of any methanogenic bacterium existed.

When Kluver and van Niel (7) named the genus *Methanococcus*, they did not name a species; therefore, this genus name was not validly published according to the *International Code of Nomenclature of Bacteria* (8), and a nomenclatural type species of the genus did not exist. However, Kluver and van Niel stated in their description of the genus, "The type species to be assigned in the near future" (7). Later that year, Barker (3), who was working in the laboratory of

Kluver and van Niel, described a methanogenic coccoid organism that was present in enriched but not axenic cultures, recognized the generic name *Methanococcus* published by Kluver and van Niel (7), and named the organism *Methanococcus mazei* (3). Thus, in 1936 *Methanococcus mazei* was the first and only named species of the genus *Methanococcus* (*Methanococcus vannielii* was not named until 1951 by Stadtman and Barker [12]), and with "later general acceptance" (Rule 20e, Opinion 20 of the *International Code* [8]), *Methanococcus mazei* was cited as the type species of the genus *Methanococcus* by Barker in 1957 in *Bergey's Manual of Determinative Bacteriology*, 7th ed. (4), and by Bryant in 1974 in *Bergey's Manual*, 8th ed. (5). The Approved Lists of Bacterial Names (11), which became effective on 1 January 1980, included *Methanococcus mazei* Barker 1936 as type species of the genus *Methanococcus* Kluver and van Niel 1936 emend. Barker 1936.

The original description of *Methanococcus mazei* by Barker (3) contained detailed photomicrographs of various stages of the life cycle, including single small spherical cells, irregular masses, and "cysts" of various sizes and shapes. However, because the description of *Methanococcus mazei* of Barker was not based on axenic cultures, some doubt was cast on these and other characteristics, especially the ability of the organism to utilize alcohols and acetone as substrates.

Recent isolation of *Methanococcus mazei* in axenic culture (9) confirmed most of the findings of Barker, particularly some of the characteristic morphological stages. This axenic culture differs from the description of Barker in substrate utilization. In addition to acetate, the following substrates are used for methanogenesis: methanol, methylamine, trimethylamine, and H₂-CO₂. Butyrate, higher alcohols, and acetone are not used.

Morphologically, the axenic culture of *Methanococcus mazei* exhibits a life cycle (9). In young cultures, individual coccoid cells develop into large *Methanosarcina*-like aggregates. These become cysts, which in turn can release individual coccoid elements. These stages in the life cycle of

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Methanococcus mazei resemble two of the biotypes (biotypes 1 and 3) described by Zhilina and Zavarzin (14). Zhilina (13) identified three biotypes (called biotypes or morphotypes by Zhilina) in the genus *Methanosarcina*. Biotype 1 is composed of large, irregular multiunit aggregates; biotype 2 is made up of smaller clusters; and biotype 3 is composed of small individual coccoid elements. Barker (3) chose the name *Methanococcus mazei* because of the predominance of individual coccoid cells (biotype 3) which occurred in his enrichment cultures. However, the irregular multiunit aggregates (biotype 1) which characterize the young, axenic cultures of *Methanococcus mazei* (9) were not mentioned by Barker (3) or by Zhilina and Zavarzin (14). These forms (biotype 1) were like those previously described by Mazé as "pseudo-sarcine" (10). Mah (9) showed that axenic cultures of *Methanococcus mazei* biotype 1 (which resemble *Methanosarcina* sp. strain TM-1 described by Zinder and Mah [15]) gives rise to oval bodies described as cysts by Barker (3) (called macrocysts by Zhilina and Zavarzin [14]), which can be ruptured to release individual coccoid elements (biotype 3 [13]). Thus, during its early growth cycle, *Methanococcus mazei* is morphologically indistinguishable from some *Methanosarcina* strains (e.g., strain TM-1, which has biotype 1 morphology). Apparently, *Methanosarcina barkeri* and other previously reported methanosarcinae do not release their individual constituent elements and cannot undergo complete life cycles.

Physiologically, the axenic strain of *Methanococcus mazei* metabolizes methanogenic substrates (acetate, methanol, methylamine, trimethylamine) similar to those metabolized by all known strains of *Methanosarcina barkeri*. However, *Methanococcus mazei* differs significantly from these strains by its ability to use acetate or methanol much more rapidly than H_2 - CO_2 , which it uses very slowly or not at all. In most of the remaining genera of methanogenic bacteria, including the two species of the genus *Methanococcus* (*Methanococcus vannielii* and *Methanococcus voltae*), only H_2 - CO_2 and occasionally formate are used for methanogenesis.

Finally, the recent finding by Woese and Wolfe (personal communication) of an S_{AB} value of 0.8 (an association coefficient [2] based on the degree of 16S ribosomal ribonucleic acid homology) in a binary comparison of *Methanococcus mazei* DSM 2053^T (T = type strain) and *Methanosarcina barkeri* DSM 800^T leaves little doubt regarding the close relationship between these two species. For these reasons, we propose transfer of *Methanococcus mazei* to the genus *Methanosarcina*, and we present the following emended description of *Methanosarcina mazei* comb. nov.

Methanosarcina mazei (Barker 1936) comb. nov. et emend. (*Methanococcus mazei* Barker 1936, 433 (3); "Pseudosarcina" Mazé 1915, 398 [4, 5]) (ma'ze.i. N.L. gen. n. *mazei* of Mazé, named for P. Mazé, the French bacteriologist who first studied the organism). Individual, irregular coccoid cells 1.0 to 3.0 μ m in diameter form irregular clumps 20 to 100 μ m or more in diameter. These irregular clumps may cluster together in large rafts 1,000 μ m or more in diameter. The clumps later become cysts, which may give rise to individual coccoid cells. A life cycle involving these morphological stages may occur.

In roll tubes, colonies on acetate medium are buff white to tannish yellow, with a grainy appearance in young cultures (<7 days); older colonies (>7 days) have a smooth appearance, and surface colonies are circular, glistening, mucoid, and pulvinate with entire margins. Occasionally, gas is trapped in the mucoid colony surface, fulminating into clusters of bubbles.

Acetate, methanol, methylamine, and trimethylamine are converted to methane. Either acetate or methanol is used more rapidly than H_2 - CO_2 . H_2 - CO_2 is used very slowly or not at all. Butyrate, ethanol, and butanol are not methanogenic.

Methanogenic substrates are utilized as sole energy sources in the presence of yeast extract and Trypticase (BBL Microbiology Systems); stimulated by sludge supernatant. Ammonia may serve as a nitrogen source.

Grows between 30 and 40°C and between pH 6.1 and 8.0; optimum growth at pH 7.0.

The estimated generation times on substrates during exponential methane formation are as follows: 1% methanol, 7.7 h; 0.05% methanol in combination with 1% acetate, 7.7 h; 1% acetate, 16.6 h.

The deoxyribonucleic acid base composition of strain DSM 2053^T (= strain S-6^T) is 42 mol% guanine plus cytosine, as determined by the buoyant density method. The S_{AB} value in a binary comparison with *Methanosarcina barkeri* DSM 800^T is 0.8.

Habitat: garden soil, sewage sludge, black mud, and feces of herbivorous animals; also isolated from urban solid waste and various sewage and animal waste digesters, swine waste lagoons, and kelp enrichments.

The type strain of *Methanococcus mazei* is strain DSM 2053 (= strain S-6), which was isolated from an anaerobic sewage digester.

Conservation of the genus *Methanococcus*. Transfer of *Methanococcus mazei* to the genus *Methanosarcina* removes the type species from the genus *Methanococcus*. Thus, according to Rule 37a of the *International Code* (8), the genus *Methanococcus* is invalidated unless an Opinion from the Judicial Commission of the International Committee on Systematic Bacteriology rules to the contrary. We present here a Request for an Opinion to conserve the genus *Methanococcus* in its current taxonomic conception by adopting *Methanococcus vannielii* as the type species.

In 1979, Balch et al. (2) listed *Methanococcus vannielii* Stadtman and Barker 1951 (12) as the type species in their emended description of the genus *Methanococcus*, probably because *Methanococcus mazei* was not isolated axenically until 1980 (9) and had been omitted from the first draft of the Approved Lists of Bacterial Names published in 1976 (1). The inclusion of *Methanococcus mazei* on the definitive Approved Lists of Bacterial Names, which became effective on 1 January 1980, validates its nomenclatural standing (Rule 24a). We propose that the emended description of the genus *Methanococcus* by Balch et al. (2) be accepted as the concept of the genus *Methanococcus*. According to Rules 20e and 36, the genus *Methanococcus* is ascribed to Stadtman and Barker (12) as emended by Balch et al. (2) instead of Kluyver and van Niel 1936 emend. Barker 1936 (Approved List 1980) (11).

Methanococcus Stadtman and Barker 1951, 269 emend. Balch et al. 1979, 285 (Me.tha.no.coc'cus. N.L. n. *methanum* methane; N.L. n. *coccus* spherical cell; N.L. masc. n. *Methanococcus* methane coccus). Cells are fragile, regular to irregular cocci that are 0.5 to 5 μ m in diameter and occur singly or in pairs. Cells are highly motile (flagella may be inserted in clusters) and gram negative. There is a single layer of protein subunits external to the plasma membrane. Muramic acid not detected. Ether-linked polyisoprenoid (branched) chain lipids are the predominant lipid components.

Very strict anaerobes. Energy for growth is obtained by oxidation of H_2 or formate with reduction of CO_2 to CH_4 .

Cells contain coenzyme M and coenzyme F₄₂₀. Carbohydrates, proteinaceous material, and organic compounds other than formate are not used as energy sources. Yeast extract is stimulatory, and Se and W may be stimulatory for growth. May require 1.2 to 4.8% NaCl. May be osmotically fragile.

The optimal growth temperature is between 32 and 40°C; the optimal pH varies with species from pH 6.7 to 9.0.

The deoxyribonucleic acid base composition is 31 mol% guanine plus cytosine (determined for *Methanococcus voltae* DSM 153^T and *Methanococcus vanniellii* DSM 1224^T), as determined by the buoyant density method.

The type species is *Methanococcus vanniellii* Stadtman and Barker 1951, 269 (Approved Lists 1980).

This emended description of the genus *Methanococcus* does not include the previous morphological description of cocci occurring in masses and restricts the utilization of substrates to H₂-CO₂ and formate. Acetate is no longer included as a methanogenic substrate. Thus, any aceticlastic coccoid methanogens which may be isolated in the future will be excluded from the genus *Methanococcus*. The proposal of *Methanococcus vanniellii* as the type species is consistent with this emended description of morphology and substrate utilization for members of the genus *Methanococcus*.

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