Request for an Opinion

Replacement of the Type Strain of Methanobacterium formicicum and Reinstatement of Methanobacterium bryantii sp. nov. nom. rev. (ex Balch and Wolfe, 1981) with M.o.H. (DSM 863) as the Type Strain

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Methanobacterium formicicum and Methanobacterium bryantii were inadvertently assigned the same type strain, strain M.o.H. (DSM 863). Because M. formicicum has temporal precedence over M. bryantii, the latter species has no taxonomic standing and is an objective synonym of the former. I request an opinion rejecting the type strain M. formicicum DSM 863, replacing it with strain MF (DSM 1535) as the neotype strain of M. formicicum, and reinstatement of M. bryantii with strain M.o.H. (DSM 863) as the holotype strain.

When Methanobacterium formicicum was placed on the Approved Lists of Bacterial Names (10), the strain on which the original description (C. G. T. P. Schnellen, Ph.D. thesis, University of Delft, Delft, The Netherlands, 1947) was based had been lost. Strain MF, isolated by Marvin P. Bryant, was the culture which conformed most closely to the original description. It has been suggested as the type strain (1), but a description of its isolation was not published until later (4). Of the strains whose isolation and characteristics had been formally described, strain M.o.H. (DSM 863) most closely matched the characteristics of M. formicicum, although the differences were too great to place the strain in the species M. formicicum (5). Perhaps because there were no other suitable strains, the Approved Lists (10) named M.o.H. the type strain of M. formicicum.

When Methanobacterium bryantii was described (1), strain M.o.H. was proposed as the holotype strain and was recorded as such on the Approved Lists (10). However, this strain was already the type strain of M. formicicum. Therefore, M. bryantii is an objective synonym of M. formicicum.

This Request for an Opinion is presented in two parts: (i) rejection of the type strain of M. formicicum, which does not conform to the published description of the species, replacing it with strain MF as the neotype strain; and (ii) reinstatement of the species M. bryantii with strain M.o.H. as the type strain.

Replacement of strain M.o.H. with strain MF as type strain of M. formicicum. In 1947, Schnellen isolated and described M. formicicum (Schnellen, M.S. thesis), but the strains on which he based his description have been lost. Later, Mylroie and Hungate (9) isolated 15 strains of M. formicicum, but these were also lost. In 1966, Marvin P. Bryant isolated strain MF, whose characteristics closely agree with the description of M. formicicum. This is the oldest extant strain of M. formicicum, although the description of its isolation was published only recently (4). Since its isolation in 1966, this strain has been widely but unofficially regarded among the scientific community as the type strain of M. formicicum, and it is the sole reference strain distributed by Paul H. Smith and Marvin P. Bryant (listed in the eighth edition of Bergey’s Manual of Determinative Bacteriology [3] as the sources for reference strains of M. formicicum).

Later, Bryant et al. isolated strain M.o.H. from a culture of “Methanobacillus omelianskii” (5) and described its characteristics. Strain M.o.H. shows some resemblance to M. formicicum, but morphological and physiological differences place strain M.o.H. in a separate species. The major distinguishing characteristics of strain M.o.H. are its colonial morphology, the presence of some gram-positive-staining cells, its larger width, and its failure to catabolize formate. In each of these features, strain MF more closely resembles the original description of M. formicicum. In 1979, Balch et al. (1) showed by 16S ribosomal ribonucleic acid cataloging that strain MF was phylogenetically different from strain M.o.H. and named strain MF as the type strain of M. formicicum. However, this designation was not in accordance with Rule 18e (now Rule 18c; see Int. J. Syst. Bacteriol. 32:142–143) of the Bacteriological Code (8). Strain M.o.H. (DSM 863) is the type strain because it was named as such, albeit incorrectly, in the Approved Lists of Bacterial Names (6). I propose that strain MF replace strain M.o.H. as the type strain of M. formicicum. The proposed species description follows:

Methanobacterium formicicum. Schnellen 1947, 85 (Schnellen, Ph.D. thesis) (for.mi’ci.cum. N.L. n. acidum formicum; N.L. adj. formicum pertaining to formic acid). Original description supplemented by material from Mylroie and Hungate 1954, 58 (9), Smith 1966, 159 (11), Langenberg, Bryant and Wolfe 1968, 1124 (7), and Zeikus and Bowen 1975, 373 (12).

Cells are slender, cylindrical, and irregularly crooked, with blunt, rounded ends. Rods are long and often form chains or filaments. Cells are 0.4 to 0.8 μm wide and 2 to 15 μm long. Each strain has a relatively constant diameter, but its length may vary. Nonmotile. Endospores not formed. Has intracytoplasmic membranous elements and fimbriae.

Surface colonies are white to gray, flat, and filamentous. Deep colonies are profusely filamented spheroids. In roll tubes with H₂-CO₂, colonies appear after 3 to 5 days, growth...
is complete within 2 weeks, and colonies attain a diameter up to 5 mm. Appearance of liquid cultures depends on the strain: medium may be uniformly turbid, or growth may occur as highly granular clumps which do not break up even with vigorous agitation (7).

Some strains may be auxotrophic; acetate and cysteine may be stimulatory.

Good growth at 37 and 45°C but not at 55°C.

May be numerous in anaerobic digestors or anaerobic freshwater sediments; may be present in low numbers in the rumen of cattle.

Mol% G + C of the DNA is 41 to 42, determined by buoyant density.

Type strain: MF (DSM 1535), isolated from a sewage sludge digester.

Reference strain: MS1 (DSM 3636).

**Reinstatement of M. bryantii.** The species *M. bryantii* has no taxonomic standing because its type strain is the same as that of *M. formicicum*. *M. formicicum* has precedence because it was published earlier (10) than *M. bryantii* (6). The latter is therefore an objective synonym of the former. However, with the designation of strain MF as the type strain of *M. formicicum*, strain M.o.H. becomes available as the type strain for *M. bryantii*. I therefore propose reinstatement of *M. bryantii* with strain M.o.H. as the type strain.

The following description is proposed:

*Methanobacterium bryantii* sp. nov. nom. rev. (ex Balch and Wolfe in Balch, Fox, Magrum, Woese, and Wolfe 1979, 269 [1]) (bry. an'ti.i. N.L. gen. n. bryantii of Bryant, named for Marvin P. Bryant for his pioneering work on the separation and characterization of this methanogen from the "Methanobacillus omelianskii" syntrophic culture). Original description supplemented by material from Bryant and Boone (4), Bryant et al. (5), Langenberg et al. (7), and Balch et al. (1).

Cells are slender and cylindrical with blunt, rounded ends, often forming chains and filaments which are irregularly crooked. Cells are 0.5 to 1.0 μm wide, and chains and filaments are 10 to 15 μm long. Gram stain results are variable. Nonmotile. Fimbriae are present.

Surface colonies, which can reach 1 to 5 mm in diameter, are flat with diffuse to filamentous edges and have a characteristic gray to light gray-green appearance. Deep colonies are rounded and filamentous. Cells tend to clump in liquid culture.

Ammonia is used as a nitrogen source. Acetate, cysteine, and B vitamins are highly stimulatory for growth.

Good growth at 37 and 45°C but not at 55°C.

Mol% G + C of the DNA is 33 to 38 (determined by buoyant density).

Type strain: M.o.H. (DSM 863) was isolated from the coculture "Methanobacillus omelianskii," which was originally isolated from an anaerobic digestor.

Reference strain: M.o.H.G. (DSM 862).

May be found in anaerobic digestors and anaerobic freshwater sediments.

Within the genus *Methanobacterium*, *M. bryantii* most closely resembles *M. formicicum*. These two species can be distinguished from most other *Methanobacterium* species by their Gram stain reaction: they stain mainly gram negative. Also, these two species are the only ones in the genus which are both mesophilic and grow as long rods and, frequently, filaments (2). The easiest way to distinguish *M. bryantii* from *M. formicicum* is the inability of the former to catabolize formate; all known strains of *M. formicicum* can use formate. Also, *M. bryantii* cells are slightly narrower than those of *M. formicicum*, and the colonies of *M. bryantii* are less filamentous and more greenish than those of *M. formicicum*.

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**LITERATURE CITED**