NOTES

Transfer of *Methanlobus siciliae* to the Genus *Methanosarcina*, Naming It *Methanosarcina siciliae*, and Emendation of the Genus *Methanosarcina*

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A sequence analysis of the 16S rRNA of *Methanlobus siciliae* T4/M^T (T = type strain) showed that this strain is closely related to members of the genus *Methanosarcina*, especially *Methanosarcina acetivorans* C2A^T. *Methanlobus siciliae* T4/M^T and HI350 were morphologically more similar to members of the genus *Methanosarcina* than to members of the genus *Methanlobus* in that they both formed massive cell aggregates with pseudosarcinae. Thus, we propose that *Methanlobus siciliae* should be transferred to the genus *Methanosarcina* as *Methanosarcina siciliae*.

Strain T4/M, the type strain of *Methanlobus siciliae*, was isolated from marine sediment, briefly characterized, and named (18). Later, a more complete characterization of this strain and reference strain HI350 (14) revealed physiological characteristics that supported placement of this species in the genus *Methanlobus*. However, in this paper we present 16S rRNA sequence data for *Methanlobus siciliae* T4/M^T (T = type strain) which indicate that this species should be transferred to the genus *Methanosarcina*. Strain T4/M^T (= OCM 156^T) was grown in MSH medium (14) by using modifications of the Hungate techniques (5). Cells were grown on 20 mM trimethylamine in a 2-liter bottle containing 1,200 ml of medium and were collected by centrifugation at 10,000 × g. The 16S rRNA was sequenced by the reverse transcriptase method. The sequence was then aligned by using a sequence editor with a representative collection of archaeal 16S rRNA sequences (2, 20). Because of the degree of similarity among these sequences, the alignment procedure was a straightforward manual one. Previously aligned close relatives of the new sequence, established secondary structural constraints, and sequence conservation patterns were used to guide the process (20). Corrected pairwise distances (expressed as estimated number of changes per 100 nucleotides) were computed from percentages of similarity by using the Jukes-Cantor correction (6) as modified by Olsen (19). A dendrogram (Fig. 1) was constructed from the evolutionary distance matrix by using the algorithm of De Soete (4). Figure 1 shows that strain T4/M^T is phylogenetically more closely related to the genus *Methanosarcina* than to the genus *Methanlobus*. Therefore, we propose that *Methanlobus siciliae* should be transferred to the genus *Methanosarcina* and named *Methanosarcina siciliae* comb. nov.

As previously defined, the genera *Methanlobus* (18) and *Methanosarcina* (12) differ in substrate range, morphology, and halophily (2). All *Methanlobus* species grow exclusively on simple methylated compounds, such as methanol, methylamines, and sometimes methyl sulfides (dimethylsulfide and methane thiol) (7, 14–16). In contrast, all species currently assigned to the genus *Methanosarcina* grow on H₂-CO₂ or acetate in addition to methanol and methylamines (2, 10), but no strain currently assigned to the genus *Methanosarcina* has been reported to grow on dimethylsulfide or methane thiol (15). *Methanosarcina siciliae* T4/M^T and HI350 can grow on dimethylsulfide and methane thiol in addition to methanol and trimethylamine, but *Methanosarcina mazei* LYC and C 16 cannot (14, 15). We found that *Methanosarcina acetivorans* C2A^T, which had not been tested previously for growth on dimethylsulfide, was also able to catabolize dimethylsulfide, as shown in the experiment described below. A culture of *Methanosarcina acetivorans* C2A^T (= OCM 95^T) was obtained from the Oregon Collection of Methanogens, Portland, Oreg., grown in MS medium (1) supplemented with 50 mM methanol, and inoculated (inoculum volume, 2.5%) into medium containing 4.5 mM dimethylsulfide, as well as three control media containing the following growth substrates: (i) none; (ii) 25 mM methanol; and (iii) 25 mM methanol plus 4.5 mM dimethylsulfide. We used large bottles (volume, 600 ml) containing 300 ml of media so dimethylsulfide could be added directly as a liquid and to minimize the fraction of dimethylsulfide lost by diffusion into the stoppers. Within days, cultures containing methanol produced approximately the amount of methane stoichiometrically expected from methanol. After a lag of about 1 week, the bottles containing dimethylsulfide began to produce methane, and this continued until the amount of methane produced was approximately equal to the amount expected stoichiometrically from the dimethylsulfide. Methane production was accompanied by an obvious increase in the turbidity of the medium and an increase in cells visible by epifluorescence microscopy. This finding indicates that *Methanosarcina acetivorans* and *Methanosarcina siciliae* are the

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FIG. 1. Phylogenetic relationships of *Methanobacterium* and *Methanosarcina* species, based on 16S rRNA sequences.

only dimethylsulfide-degrading species of the genus *Methanosarcina*.

Methanobacterium cells also differ morphologically from *Methanosarcina* cells. *Methanobacterium* cells are irregular cocci and occur either singly or in small aggregates in all growth stages. In contrast, the cells of many *Methanosarcina* strains undergo morphological changes at different growth phases (3, 11, 13, 17). In young *Methanosarcina* cultures, individual coccoid cells form aggregates having pseudosarcinal shapes, which then become "cysts" of various sizes; the cysts have a common external wall composed of heteropolysaccharide. These large cell aggregates and cysts may be disrupted either physically or enzymatically. Physical disruption yields myriads of smaller irregular elements, whereas enzymatic action by disaggregatase can turn the cysts or aggregates into single cells, thereby dispersing the rapidly settling clumps and producing turbid suspensions (9, 21).

We found that *Methanosarcina siciliae* T4/M^T and HI350 (= OCM 210) cells often grew in large aggregates. This characteristic is more typical of members of the genus *Methanosarcina* than of members of the genus *Methanobacterium*. During the initial characterization of these strains, we observed only irregular coccoid cells that occurred singly or in small aggregates (14). However, when the cultures were later transferred repeatedly in MSH medium containing dimethylsulfide as a catabolic substrate, the cells grew in massive clumps consisting of pseudosarcinae (Fig. 2A). Similar clumps were also observed in cultures growing on methanol or trimethylamine if relatively young cultures were transferred repeatedly during the mid-exponential growth phase. These clumps could be disrupted into smaller cell aggregates by physical shaking. Prolonged incubation after exhaustion of substrate induced spontaneous disaggregation, releasing single cells and causing the culture to become turbid. Media having pH values below 7 favored clump formation. Electron microscopy of thin sections, however, did not reveal a continuous wall structure surrounding the pseudosarcinae, as is often seen in *Methanosarcina barkeri* and *Methanosarcina mazei*; instead, there was only amorphous wall material at the surfaces of single cells (Fig. 2B), reminiscent of the cell walls of *Methanobacterium tindarius* Tindari 3^T (8). Cells were not as tightly stuck together as typical *Methanosarcina* cells in their clumps. Both individual

FIG. 2. (A) Phase-contrast micrograph of cell aggregates of *Methanosarcina siciliae* HI350. (B) Thin-section micrograph of *Methanosarcina siciliae* HI350. Cells for electron microscopy were fixed at room temperature for 30 min in 2.5% glutaraldehyde buffered to pH 7.4 with 0.2 M sodium cacodylate. They were then postfixed in osmium tetroxide for 30 min at 4°C, dehydrated, and embedded in Spurr low-viscosity resin. Sections were poststained with uranyl acetate and lead citrate, examined, and photographed with a Zeiss model EM-10 transmission electron microscope at 60 kV.

cells and massive cell clumps of *Methanosarcina siciliae* T4/M^T and HI350 lysed immediately when 2 g of sodium dodecyl sulfate per liter was added, and no visible cellular framework remained. In contrast, when *Methanosarcina barkeri* clumps or some *Methanosarcina mazei* clumps are treated with dodecyl sulfate, the intact cell shapes are microscopically visible (9). This finding suggested that *Methanosarcina siciliae* T4/M^T and HI350 had proteinaceous cell walls similar to those of *Methanosarcina acetivorans* C2A^T and *Methanosarcina mazei* C 16 (= *Methanosarcina frisia* C 16^T [10]) and Gö1. Cysts, commonly known as the third morphological state of many *Methanosarcina* strains (12), were not observed in cultures of strain T4/M^T or HI350.

Our results showed unequivocally that strain T4/M^T is more closely related to *Methanosarcina* species than to *Methanobolus* species. Among the *Methanosarcina* strains compared, strain T4/M^T was most closely related to *Methanosarcina acetivorans* C2A^T. *Methanosarcina acetivorans* C2A^T differs physiologically from *Methanosarcina siciliae* T4/M^T and HI350. *Methanosarcina acetivorans* C2A^T can grow on acetate in addition to methanol, methylamines (17), and dimethylsulfide. This species is now considered halotolerant (10), although strains have been isolated only from marine sediments (17). Phylogenetically, *Methanosarcina acetivorans* and *Methanosarcina siciliae* are closely related; the 16S rRNA sequences of their type strains exhibit 98.3% similarity. This level of difference is typical of closely related species (2) or even strains within the same species. Thus, in the absence of DNA-DNA reassociation data, we consider *Methanosarcina siciliae* and *Methanosarcina acetivorans* separate *Methanosarcina* species.

All previously described *Methanosarcina* strains are nonhalophilic and catabolize either acetate or H₂-CO₂ (or both) in addition to methanol and methylamines. With the inclusion of *Methanosarcina siciliae* in this genus, however, these characteristics cannot be considered diagnostic criteria for the genus *Methanosarcina* (12). Thus, we hereby emend the description of the genus *Methanosarcina* to include slightly halophilic, nonaceticlastic, obligate methylophils. The range of catabolic substrates for *Methanosarcina* species is also expanded to include dimethylsulfide and methane thiol.

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