NOTES

*Methanosarcina vacuolata* sp. nov., a Vacuolated Methanosarcina

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The validation of gas-vacuolated strains of methanosarcinae at the species level under the name of *Methanosarcina vacuolata* sp. nov. is presented, based on chemotaxonomic as well as morphological characteristics. *M. vacuolata* is shown to be related but distinct from the type species *M. barkeri* and the other species of freshwater methanosarcinae, *M. mazei* and *M. thermophila*.

Methanosarcinae have been described as a distinct group of methanogens characterized by specific morphological features and a broad spectrum of utilized substrates (3, 4). At present, there are a few species in the genus of *Methanosarcina* which are differentiated mainly by their morphology and physiology. The type species suggested by H. Barker, *M. methanica*, and described only as an enrichment culture, have been substituted by *Methanosarcina Barkeri* (16, 22). *M. thermophila* is known to develop at 55°C (32). The slightly halophilic *M. acetivorans* has seawater as its habitat (23). *M. mazei* was formerly the type species of the genus of *Methanococcus* (4, 15), but is now accepted as belonging to *Methanosaeta* (31) after appropriate emendation of the description of the genus (17) and the designation of a neotype for the formate-utilizing marine type species of *Methanococcus*. In 1971, a new morphological form of *Methanosaeta* having gas vacuoles was discovered (24) and effectively published as a new species "*M. vacuolata*" (30), which was not, however, included in the Approved Lists.

The validity of the vacuolated species of *Methanosarcina* has been challenged on the grounds that the difference in $S_{AB}$ values of 0.8 between two strains of *M. barkeri* (2, 27) and the vacuolated W strain (3) should not justify a new species. Thus the question remained open. Also, a few new vacuolated strains were assigned to *M. Barkeri* (1, 2, 18). Vacuolated strains of methanosarcinae, including the type strain *M. vacuolata* Z-761 (DSM 1232) have been a subject of a number of comparative studies with *M. Barkeri* (2, 6, 7, 10-12, 14, 21). The available data clearly indicate that vacuolated strains represent a separate species, close to but distinct from *M. barkeri*. In fact, *M. vacuolata* differs from other species not only morphologically but also in some chemotaxonomic characteristics (Table 1).

The present communication should be viewed as a fulfillment of the requirements of the Code of Nomenclature of Bacteria and validation of *Methanosarcina vacuolata*.

**Enrichment and cultivation.** Gas-vacuolated methanosarcinae have been isolated from several different habitats but mainly from subaerial soils of marshes and wetlands. They have not been reported from brackish or thermal habitats. The type strain *M. vacuolata* Z-761 was isolated from a mesophilic anaerobic digester in Moscow (27, 30). For freshwater strains, a bicarbonate-buffered medium by N. Plennig is recommended, as modified for methanogens with acetate, methanol, or methylamines serving as substrates (29). Although H$_2$-CO$_2$ can also be utilized, it is not recommended for the enrichment of methanosarcinae. Among the substrates listed, acetate is preferable for enrichment, while methanol is effective for large-scale cultivation. The type strain Z-761 is very sensitive to atmospheric oxygen. Thus, the number of colony-forming units was observed to drop by half after just 4 min of exposure to air (25). Gas-vacuolated methanosarcinae remain viable at 4°C for at least 4 years, provided the containing vessel is sealed airtight. Storage in liquid nitrogen is recommended by Hippe (8). The purity of the cultures can be checked by phase-contrast microscopy and by inoculating organic media, i.e., a glucose-peptone medium. Glucose can be added directly to the medium for methanosarcinae because it does not inhibit growth at concentrations up to 0.5 g/liter (5); thus, contamination will be shown as turbidity. Specific contaminants of vacuolated methanosarcinae are species of *Desulfovibrio*, which form syntrophic cultures when grown on acetate, methanol, and methylamines; they are able to utilize hydrogen evolved by the methanosarcinae at concentrations up to 30 ppm as found for the type strain (28). The production of hydrogen sulfide in the medium supplemented with sulfates is an indication of contamination by sulfidogens.

**Morphology.** According to the classification proposed by Zhilina (26), gas-vacuolated methanosarcinae form aggregates of biotype II, and in this respect are similar to strains of *M. barkeri*. The absence of various types of cysts allows strains of gas-vacuolated methanosarcinae to be differentiated from those of *M. mazei* (14, 16, 30) and *M. acetivorans* (22), whereas *M. thermophila* is distinguished by its forming large pseudoparenchymatous aggregates of biotype I (32), which was earlier believed to be a characteristic feature of *M. methanica*, now rejected (16). The morphological biotypes in methanosarcinae are analogous to *Pleurocapsaceae* in cyanobacteria and are distinguished by a multilayered glycocalyx. Vacuolated strains are easily recognized in phase-contrast microscopy by glistening inclusions of gas vacuoles. Unlike "*Methanotrix thermoacetophila*," which has an unclustered distribution of gas vesicles (20), *M. vacuolata* shows gas vesicles tightly packed in the gas vacuoles, as in cyanobacteria (1, 18, 24). Despite the presence of gas vesicles, *M. vacuolata* Z-761 showed a fairly low buoyancy and was unable to float in the liquid medium; the cells sedimented, which indicated the high density typical of other methanosarcinae.

**Colonies.** Colonies of the type strain Z-761 are light yellow, angular, granular, and prone to dispersion, much like
M. barkeri. Under ultraviolet light, oxidized cells of M. vacuolata give out a typical greenish fluorescence.

**Multiplication.** Multiplication is by irregular fission of the cells in different planes, which is responsible for the compression and the irregular shape of minute cells (0.5 to 1 μm) within aggregates. The rounded appearance of large cocci (2 to 2.5 μm) is characteristic of “pskudococci,” whose cross sections are invisible in the phase-contrast microscope. M. vacuolata normally exhibits two types of aggregates of pseudococci, one formed by multiple fission of cells and one arranged in sarcinalike packets. Multiple fission, originally described (2), although they have never been observed in the type strains, W (18), FR-1 (1), and 2-761 (24, 30), were similar.

**Ultrastructure.** The ultrastructures of all vacuolated strains, W (18), FR-1 (1), and Z-761 (24, 30), were similar. The cell envelope consisted of a laminated heteropolysaccharide capsule or glycocalyx, and the cell structure was typical for procaryotic organisms. Up to 90% of the cell volume was often occupied by gas vacuoles. Gas vesicles are typical for procaryotic organisms. Up to 90% of the cell volume was often occupied by gas vacuoles. Gas vesicles have been isolated from M. vacuolata, with the C₃₀:₄ and C₃₀:₃ squalenes have been observed by using gas chromatography and mass spectrometry of extracts of whole cells of M. vacuolata, with the C₃₀:₄ and C₃₀:₃ squalenes amounting to 40 and 41%, respectively, of all nonpolar lipids. The detected isoprenes (C₂₇, C₂₆, C₂₅, and C₂₄) might well be the products of degradation of biphynyl (21). M. vacuolata DSM 1232 has been found to contain membrane-bound cytochrome b (12). M. vacuolata as well as of M. barkeri contain mostly factor III and not B₁₂ (6).

**Serology.** Immunological analysis (in collaboration with L. V. Bezrukova) has shown a weak reaction of immunofluorescence of M. vacuolata with the serum prepared against the type strains of M. barkeri (1+) and M. mazei (1+). Thus, M. vacuolata is serologically different from other methanocarciniae.

**Differentiation.** Differentiation between M. barkeri and M. vacuolata can be summarized as follows: morphology—the same type of aggregation (biotype 2) and cell structure, yet with the occurrence of gas vesicles in M. vacuolata (30); cell wall—a similar composition of heteropolysaccharides with a slight difference in the components concentrations (10, 11); lipid content—the main components in M. vacuolata are squalenes, not found in M. barkeri (13, 21); 61% DNA–DNA homology between M. vacuolata and M. barkeri (14); weak immunofluorescence (1+) with the antiserum to M. barkeri MS; different composition of ribosomal proteins (7); identical SₐB values (0.8) in 16S rRNA comparison of M. barkeri with M. mazei or M. barkeri and M. mazei with M. vacuolata (3, 17).

On these grounds it can be argued that M. barkeri and M. vacuolata are close but different species in the genus and they are readily differentiated by the presence or absence of gas vesicles.

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**TABLE 1. Differentiation of M. vacuolata and M. barkeri based on chemotaxonomic characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain Z-761 (DSM 1232)</th>
<th>Strain MS (DSM 800)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA homology (mol%) (14)</td>
<td>M. vacuolata Z-761</td>
<td>100</td>
</tr>
<tr>
<td>M. barkeri MS</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Lipid composition (21, 13)</td>
<td>Nonpolar isoprenoid hydrocarbons:</td>
<td></td>
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<tr>
<td></td>
<td>C₆₀</td>
<td></td>
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<tr>
<td></td>
<td>C₅₃</td>
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<td>Polar glycerol ethers:</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>C₃₀</td>
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<tr>
<td></td>
<td>C₂₀</td>
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<tr>
<td></td>
<td>C₁₀</td>
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<tr>
<td>Composition of heteropolysaccharides of the cell wall (11) (%b):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>2.2 (0.12)</td>
<td>3.75 (0.20)</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>30.8 (1.72)</td>
<td>27.5 (1.54)</td>
</tr>
<tr>
<td>D-Glucuronic acid</td>
<td>18.0 (0.92)</td>
<td>16.0 (0.82)</td>
</tr>
<tr>
<td>D-Galacturonic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetyl residues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASH</td>
<td>8.7 (2.0)</td>
<td>8.3 (1.9)</td>
</tr>
<tr>
<td>H₂O</td>
<td>2.5</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>14</td>
</tr>
</tbody>
</table>

* = Not detected; +, present.

b Millimoles per gram are given parenthetically.
**Description of Methanosarcina vacuolata sp. nov. Methanosarcina vacuolata** (vacuolum, M.L. diminutive of vacuum, an empty place; N.L. f. pl. vacuolata, referring to methanosarcinae with gas vesicles). Cells spherical or compressed, 1 to 2 μm in rounded aggregates arranged into sacharilike packets. Nonmotile. Gram positive. Not lysed by 0.07% sodium dodecyl sulfate at 23°C. Differentiating cyto logical characteristic: the presence of large vacuoles containing gas vesicles. Multiplication by uneven fission in different planes; septation may not be visible by phase-contrast microscopy. Rounded pseudococci observed in a light microscope are aggregates of polygonal cells with a common microcapsule.

Forms 0.5- to 1.0-mm-deep colonies in agar; light yellow, angular, fine granulation. Grows in liquid medium as a yellow sediment, easily dispersed. Strictly anaerobic. Methanogenic catabolism: methanol, mono-, di-, and trimethylamines, H₂-CO₂ are utilized with formation of methane. Anogenic catabolism: methanol, mono-, di-, and trimethy-ylamines, H₂-CO₂ are utilized with formation of methane. Formate is not utilized. Autotrophic anabolism. Organic compounds with growth factors enhance growth of some strains but are not obligately needed. Ammonium is the source of nitrogen and sulfide is the source of sulfur. Optimal temperature, 37 to 40°C; temperature range, 18 to 42°C. Optimal pH, 7.5; range, 6 to 8. Squalenes are major component of lipids. Cytochromes and factor III are present in the cells, while B₁₂ is absent. Guanosine plus cytosine content of DNA is 36.3 mol%. Habitat: soils of wetlands, swamps, freshwater sediments, anaerobic digestors.

Type strain is Z-761 (DSM 1232) isolated from an anaerobic digestor in Moscow, USSR. Deposited in the Institute of Microbiology of the Academy of Sciences of the USSR and in Deutsche Sammlung von Mikroorganismen, Göttingen, Federal Republic of Germany.

**LITERATURE CITED**


