Deoxyribonucleic Acid Hybridization Study of *Methanogenium* and *Methanocorpusculum* Species, Emendation of the Genus *Methanocorpusculum*, and Transfer of *Methanogenium aggregans* to the Genus *Methanocorpusculum* as *Methanocorpusculum aggregans* comb. nov.

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Deoxyribonucleic acid hybridization indicated that species of the genera *Methanogenium* and *Methanocorpusculum* are phylogenetically diverse. The 10 strains examined in this study fall into four phylogenetically defined groups. The first group (*Methanogenium marisnigri*, *Methanogenium bourgense*, *Methanogenium olentangyi*, and *Methanogenium thermophilicum*) is physiologically the most diverse, containing mesophiles and thermophiles and containing marine and nonmarine organisms. The second group (*Methanogenium aggregans*, *Methanocorpusculum parvum*, and *Methanocorpusculum labreanum*) contains very small mesophilic coccoid organisms from anaerobic digestors. The third group contains a single species, *Methanogenium tationis*, which was isolated from a solfataric mud pool, and the fourth group contains the type species, *Methanogenium cariaci*, which is a marine organism. Based on the high deoxyribonucleic acid sequence similarity between *Methanogenium aggregans* and *Methanocorpusculum parvum*, we propose to transfer *Methanogenium aggregans* to the genus *Methanocorpusculum*, naming it *Methanocorpusculum aggregans* comb. nov. The type strain is strain MSt (= DSM 3027 = OGC 21). The species in the other three major groups are placed in the genus *Methanogenium*, although the lack of phylogenetic relatedness indicates that some of these organisms should be separated from *Methanogenium cariaci* into new genera. However, we propose that cataloging or sequencing of ribosomal ribonucleic acid from these species must be completed before new genera are designated.

The genus *Methanogenium* was originally described as a group of irregularly coccoid, H₂-utilizing, marine methanogens with low temperature optima (20 to 25°C) and guanine-plus-cytosine (G+C) contents of 52 to 61 mol%. Several new coccoid, methanogenic species were assigned to this genus because their physiology and the G+C contents of their deoxyribonucleic acids (DNAs) suggested that they were more closely related to *Methanogenium* than to the coccoid genus *Methanococcus*. However, the description of the genus *Methanogenium* was never formally emended to contain these species (2, 5, 6, 8, 11, 12). More recently, *Methanocorpusculum* was established as a new genus when 16S ribosomal ribonucleic acid cataloging studies showed strong phylogenetic differences between *Methanocorpusculum parvum* and two species of *Methanogenium*, *Methanogenium cariaci* and *Methanogenium marisnigri* (12). However, *Methanocorpusculum parvum* was not compared with other *Methanogenium* species.

In this study we found that *Methanogenium aggregans* is phylogenetically closely related to *Methanocorpusculum parvum*. We also describe the DNA sequence similarities among species of *Methanogenium* and *Methanocorpusculum*.

(Some of the data have been presented previously [Xun, Boone, and Mah, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, abstr. I24, p. 185].)

MATERIALS AND METHODS

Bacterial strains and growth conditions. Strains were obtained from the Deutsche Sammlung von Mikroorganismen (DSM) or from the Oregon Graduate Center Collection of Methanogenic Archaeobacteria (OGC). An attempt was made to obtain and include all extant *Methanogenium* and *Methanocorpusculum* strains. For the following strains our source of each strain is listed first in parentheses, followed by an alternate source in most cases (accession numbers are indicated): *Methanocorpusculum labreanum* Z^T (OGC 1^T, DSM 4855^T) (T = type strain), *Methanocorpusculum parvum* XII^T (DSM 3823^T, OGC 63^T), *Methanogenium aggregans* MSt^T (OGC 21^T, DSM 3027^T), *Methanogenium bourgense* MS2^T (OGC 15^T, DSM 3045^T), *Methanogenium bourgense* LX1 (OGC 24), *Methanogenium cariaci* JR1^T (DSM 1497^T, OGC 49^T), *Methanogenium marisnigri* JR1^T (DSM 1498^T, OGC 56^T), *Methanogenium olentangyi* RC/ER^T (DSM 2772^T, OGC 52^T), *Methanogenium tationis* DSM 2702^T (DSM 2702^T, OGC 43^T), and *Methanogenium thermophilicum* UCLA (OGC 6, DSM 2624). *Methanogenium thermophilicum* UCLA has approximately 100% DNA sequence similarity with *Methanogenium thermophilicum* CR1^T (12); in the hybridization study of Zabel et al. (12), the strain referred to as strain Los Angeles is actually strain UCLA (3; T. J. Ferguson, Ph.D. thesis, University of California at Los Angeles, Los Angeles, 1983), and strain CR1^T is referred to by its DSM accession number (DSM 2373^T).

The media and growth conditions which we used were the same as those used for characterization of each organism (2, 5, 6, 9, 12–14). For mass culture, cells were grown in a 10-liter fermentor (1) or a 4-liter round-bottom flask.

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DNA isolation. Cultures were cooled to 4°C, and cells were harvested by centrifugation at 5000 × g for 15 min. DNA was immediately isolated by a modification of the Marmur method (4). When harvested cells were stored as a frozen paste, DNA isolation was more difficult, perhaps because of interactions between high-molecular-weight DNA and cell debris.

G+C content of the DNA. The density of each DNA preparation was determined by ultracentrifugation in CsCl gradients (7); DNAs from *Micrococcus lysodeikticus* (Sigma Chemical Co., St. Louis, Mo.; density was assumed to be 1.731 g/cm³) and *Clostridium perfringens* (Sigma; density was assumed to be 1.691 g/cm³) were used as standards. The G+C content of the DNA was calculated by using the formula of Schildkraut et al. (10).

DNA sequence similarity. DNA sequence similarities were determined by using the procedures of Johnson (4), including the S1 nuclease (Sigma) method for hydrolysis of single-stranded DNA; salmon sperm DNA (Sigma) was used as the reference DNA. Single-stranded DNA was labeled with Na¹²⁵I (New England Nuclear Corp., Wilmington, Del.), and, after hybridization, S1 nuclease-resistant fragments were precipitated with a 20% (vol/vol) HCl solution (10 g of NaH₂PO₄ and 10 g of Na₄P₂O₇ per liter of 1 N HCl) and collected on 0.45-μm nitrocellulose filters (type HA; Millipore Corp., Bedford, Mass.). The filters were washed with three 5-ml quantities of a 16.7% (vol/vol) HCl solution and 2 ml of 95% ethanol and then dried at room temperature in a fume hood and counted with a liquid scintillation counter.

RESULTS AND DISCUSSION

We determined DNA sequence similarities among 10 strains belonging to the genera *Methanogenium* and *Methanocorpusculum* (Table 1). Although these methanogens share many taxonomically important traits, such as methanogenesis from H₂-CO₂, irregular coccoid morphology, requirement for or toleration of moderate salt concentrations during growth, and a G+C content between 50 and 62 mol%, we found a great deal of phylogenetic diversity. For instance, the type species of the genus *Methanogenium*, *Methanogenium cariaci*, is only distantly related to other *Methanogenium* or *Methanocorpusculum* species. The data in Table 1 are displayed as a dendrogram in Fig. 1. Four major, distinct groups which have levels of intergroup sequence similarity of less than 10% are evident. The first group (*Methanogenium marisnigri*, *Methanogenium bourgense*, *Methanogenium olentangyi*, and *Methanogenium thermophilicum*) is physiologically the most diverse group, containing mesophiles, thermophiles, marine, and nonmarine organisms. The second group (*Methanocorpusculum aggregans*, *Methanocorpusculum parvum*, and *Methanocorpusculum labreanum*) contains very small mesophilic coccoid organisms isolated from anaerobic digestors. The third group contains a single species, *Methanogenium tationis*, which was isolated from a solfataric mud pool, and the fourth group contains the type species, *Methanogenium cariaci*, which is a marine organism.

Because *Methanogenium aggregans* is phylogenetically more closely related to *Methanocorpusculum parvum* than to any species of *Methanogenium*, we propose its transfer to the genus *Methanocorpusculum*, as *Methanocorpusculum aggregans* comb. nov. A description of the genus *Methanocorpusculum* has not been specifically proposed previously, so we recommend the description below.

***Methanocorpusculum* (Zellner, Stackebrandt, Conway de Macario, and Winter, 1987, 13).** The original description of

TABLE 1. Sequence similarities of DNAs as determined by hybridization techniques

Strain	% Sequence similarity 10 ² :									
	<i>Methanogenium</i> bourgense LX1	<i>Methanogenium</i> bourgense MS2 ^T	<i>Methanogenium</i> olentangyi RC/ER ^T	<i>Methanogenium</i> marisnigri JR1 ^T	<i>Methanocorpusculum</i> thermophilicum UCLA	<i>Methanocorpusculum</i> labreanum Z ^T	<i>Methanogenium</i> aggregans MS1 ^T	<i>Methanocorpusculum</i> parvum XII ^T	<i>Methanogenium</i> tationis DSM 2702 ^T	<i>Methanogenium</i> cariaci JR1 ^T
<i>Methanogenium</i> bourgense LX1	100									
<i>Methanogenium</i> bourgense MS2 ^T	78 (69, 86)	100								
<i>Methanogenium</i> olentangyi RC/ER ^T	68 (76, 59)	66 (81, 52)	100							
<i>Methanogenium</i> marisnigri JR1 ^T	24 (30, 18)	27 (ND, 27)	35 (40, 30)	100						
<i>Methanocorpusculum</i> thermophilicum UCLA	28	29	27 (23, 31)	23 (23, ND)	100					
<i>Methanocorpusculum</i> labreanum Z ^T	4 (-3, 12)	4 (-5, 13)	18 (4, 31)	-6 (-6, -5)	-6 (-10, -2)	100				
<i>Methanogenium</i> aggregans MS1 ^T	14 (10, 19)	32	35 (17, 53)	14 (6, 22)	22 (17, 27)	48 (59, 37)	100			
<i>Methanocorpusculum</i> parvum XII ^T	-4 (ND, -4) ^b	13 (ND, 13)	0 (ND, 0)	-4 (ND, -4)	22 (27, 16)	31 (ND, 31)	71 (ND, 71)	100		
<i>Methanogenium</i> tationis DSM 2702 ^T	8 (5, 12)	28 (26, 31)	17 (15, 19)	0 (-5, -5)	9 (-2, 20)	4 (5, 2)	7 (7, 7)	1 (1, ND)	100	
<i>Methanogenium</i> cariaci JR1 ^T	3 (ND, 3)	7 (ND, 7)	7 (ND, 7)	4 (ND, 4)	5 (ND, 5)	2 (ND, 2)	ND (ND, ND)	27 (ND, 27)	4 (ND, 4)	100

^a The values are the averages (when available) of reciprocal crosses. Within the parentheses, the first value is the percentage of hybridization when DNA from the strain indicated at the top of the column was labeled and hybridized with unlabeled DNA from the strain indicated on the left; the second value is the percentage of hybridization for the reciprocal cross.

^b ND, Not determined.

FIG. 1. Dendrogram of the phylogenetic relationships among strains of the genera *Methanogenium* and *Methanocorpusculum*. Based on DNA sequence similarities (from the values shown in Table 1), the most closely related organisms were grouped together, and the phylogenetic relationship between an organism and a group or between two groups was calculated as the average of all of the hybridization determinations between the two groups. The standard deviations are indicated by dashed lines.

Zellner et al. (13) was supplemented by data from Ollivier et al. (6) and Zhao et al. (14).

Cells are small (average diameter, $\leq 1 \mu\text{m}$) and coccoid, with a negative Gram reaction. Sensitive to lysis in the presence of 0.3 g of sodium dodecyl sulfate per liter.

Catabolic substrates include $\text{H}_2\text{-CO}_2$ and formate; 2-propanol- CO_2 may also be catabolized, but acetate, methylamines, methanol, propionate, butyrate, ethanol, and amino acids are not catabolized.

Grows most rapidly in medium with an Na^+ concentration of 50 to 250 mM or more. Acetate and yeast extract or Trypticase peptones are required.

The G+C content of the DNA is 48 to 52 mol%.

The type species is *Methanocorpusculum parvum*.

The species from the other three major groups are currently classified in the genus *Methanogenium*, although the lack of phylogenetic relatedness indicates that some of these should be separated from *Methanogenium cariaci* into new genera. However, we propose that such transfers should be postponed until additional data bearing on the phylogeny of these species are available.

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