Arch. Microbiol. 121, 147–153 (1979)

Archives of
Microbiology

Methanogenium, a New Genus of Marine Methanogenic Bacteria, and Characterization of *Methanogenium cariaci* sp. nov. and *Methanogenium marisnigri* sp. nov.

J. A. Romesser[1,*], R. S. Wolfe,[1,**], F. Mayer[2], E. Spiess[2], and A. Walther-Mauruschat[2]

[1] Department of Microbiology, University of Illinois, Urbana, Illinois 61801 U.S.A.
[2] Institut für Mikrobiologie der Universität Göttingen, D-3400 Göttingen, Federal Republic of Germany

Abstract. A new genus of marine methanogenic bacteria and two species within this genus are described. *Methanogenium* is the proposed genus and *Methanogenium cariaci* the type species. Cells of the type species are Gram-negative, peritrichously flagellated, irregular cocci with a periodic wall surface pattern. Colonies formed by these bacteria are yellow, circular and umbonate with entire edges. The DNA base composition is 52 mol% guanine plus cytosine. Formate or hydrogen and carbon dioxide serve as substrates for growth. Cells of *Methanogenium marisnigri* are of similar shape but smaller diameter than *M. cariaci*. The colonies of *M. marisnigri* are convex, and the DNA base composition is 61 mol% G + C. Formate or hydrogen and carbon dioxide are growth substrates. Sodium chloride is required for growth of both methanogens.

Key words: *Methanogenium cariaci* – *Methanogenium marisnigri* – Marine methanogenic bacteria – Ultrastructure – Taxonomy *Methanogenium* gen. nov.

Although the majority of the oceans are aerobic, several anoxic basins exist, and the largest of these are the Black Sea and the Cariaco Trench. These waters are characterized by high concentrations of methane (Lamontagne et al., 1973), but the presence of methane producing bacteria in these habitats has not been established. Indeed, only one methanogen, *Methanococcus vannielii*, appears to be unique to the marine environment, having been isolated from San Francisco Bay. We describe here the isolation and characterization of two methanogenic bacteria obtained from sediment collected in the Cariaco Trench and the Black Sea.

Materials and Methods

Source of Sediment. Sediment was collected at station 1355 on R. V. Chain cruise 120/1 in the Black Sea and from station 2030 on R. V. Atlantis II cruise 79 in the Cariaco Trench.

Media. The organisms were cultivated in 125 ml serum vials in sterile growth media prepared under a strictly anaerobic growth atmosphere (80% H_2 and 20% CO_2) by a modification of the Hungate technique (1950) as described by Bryant and Robinson (1961) and revised by Balch and Wolfe (1976). The maintenance medium for both organisms contained the following constituents in distilled water at the indicated final percentage compositions (wt/vol): KCl, 0.033; $MgCl_2 \cdot 6H_2O$, 0.26; $MgSO_4 \cdot 7H_2O$, 0.35; NH_4Cl, 0.025; $CaCl_2 \cdot 2H_2O$, 0.014; K_2HPO_4, 0.014; cysteine hydrochloride, 0.05; $Na_2S \cdot 9H_2O$, 0.05; $FeSO_4$ $(NH_4)_2SO_4 \cdot 6H_2O$, 0.001; yeast extract (Difco), 0.2; resazurin, 0.0001. In addition, 1% (vol/vol) each of vitamin solution and trace mineral solution was added to the medium (Wolin et al., 1963). Medium MC for *Methanogenium cariaci* also contained the following constituents at the indicated final percentage compositions (wt/vol): sodium acetate, 0.05; NaCl, 2.65; Na_2CO_3, 0.4. Medium MM for *M. marisnigri* also contained (%, wt/vol): NaCl, 0.9; Na_2CO_3, 0.2; Trypticase (BBL), 0.2. The final pH of the media equilibrated with 80% H_2 and 20% CO_2 at 206 kN/m^2 was 7.0 for medium MC and 6.6 for medium MM. Cultures of both methanogens were incubated at room temperature. The enrichment medium contained the following constituents in a mixture of 30% distilled water and 70% sea water at the indicated final concentration (wt/vol) in percent: ammonium acetate, 0.05: sodium formate, 0.1: Trypticase, 0.2; yeast extract, 0.1; ammonium 2-mercaptoethanesulfonate (HS-CoM), 0.0001; Na_2CO_3, 0.1; $NaHCO_3$, 0.2; cysteine hydrochloride, 0.05; $Na_2S \cdot 9H_2O$, 0.05; resazurin, 0.0001. In addition, 1% (vol/vol) each of vitamin solution, trace mineral solution and volatile fatty acid solution [isobutyric, α-methylbutyric, isovaleric, and valeric acids each at a final concentration of 0.05% (vol/vol)] were added. The pH of the medium after equilibration with the 80% H_2 and 20% CO_2 gas atmosphere at 137 kN/m^2 was 7.4. The enrichments were incubated at 17°C for *M. cariaci* and 8°C for *M. marisnigri*.

* Present address: Experimental Station, E. I. DuPont de Nemours and Co., Wilmington, Delaware 19898, U.S.A.
** To whom offprint requests should be sent

Abbreviations. SDS, sodium dodecylsulfate; PIPES, piperazine-N,N′-bis (2 ethanesulfonic acid); HS-CoM, coenzyme M, 2-mercaptoethanesulfonic acid

0302-8933/79/0121/0147/$01.40

Enrichments. Sediment cores (5 cm diameter) were collected with a gravity coring device. Sediment samples were obtained by burning a hole through the plastic core liner with a red hot cork borer. A 1 ml plastic syringe with the luer tip cut off was inserted into the center of the core and a 0.5 ml sediment sample was withdrawn. The sediment sample was injected into a 125 ml serum vial that was being flushed with 100% CO_2 and that contained 4.5 ml of sterile medium. The serum vial was stoppered, crimp-sealed, and then pressurized to 41 kN/m^2 CO_2. The vial was further pressurized to 206 kN/m^2 with H_2.

Isolation on Agar Plates. Isolation of the marine methanogens was achieved by streaking the enrichment cultures on agar plates in a Freter type anaerobic hood (Aranki and Freter, 1972). Agar medium for streak plates was prepared in round bottom flasks. A standard preparation to make 400 ml of agar contained 150 ml of distilled water, 8 g of agar, 0.1 g of cysteine hydrochloride, and 0.1 g of $Na_2S \cdot 9H_2O$ in one flask. In a second flask all additional components of the enrichment medium, including 0.1 g $Na_2S \cdot 9H_2O$ and 0.1 g cysteine hydrochloride, were dissolved in 50 ml of distilled water and combined with 200 ml of sea water. Both flasks were made anaerobic by use of the Hungate technique with an 80% N_2 and 20% CO_2 gas atmosphere. Each flask was autoclaved, cooled to 60°C, and then transferred into a Freter-type anaerobic hood. The contents of the flasks were mixed, and plates were poured inside the hood. Inoculated plates were incubated at room temperature in modified pressure cookers (Balch and Wolfe, 1976) under 206 kN/m^2 80% H_2 and 20% CO_2.

Methods. Photomicrographs were made with a Carl Zeiss universal photomicroscope. Wet mounts of cultures were prepared on slides which had been coated with 2% washed Noble agar (Difco) and dried.

Cells for electron microscopy were negatively stained with 4% uranyl acetate according to the procedure of Valentine et al. (1968). For sectioning the cells were fixed (without previous washing) with glutaraldehyde and then with OsO_4. Each solution was supplemented with 2% (wt/vol) NaCl. A block staining with uranyl acetate was included during the dehydration procedure in acetone. The cells were embedded in Spurr's (1969) low viscosity medium, sections were cut with a LKB Ultratome III ultramicrotome, and the sections were poststained with lead citrate. Electron micrographs were taken with a Philips EM 301 electron microscope and magnifications were calibrated both with a cross-lined grating replica and with catalase crystals. Freeze-etch studies were performed as described by Mayer et al. (1977).

Deoxyribonucleic acid (DNA) was purified from lysed cells by the method of Marmur (1961), and the buoyant density was determined by centrifugation in a cesium chloride density gradient in a Beckman Model E centrifuge. The base ratio (mol % G + C) was calculated by the method of Schildkraut et al. (1962). DNA from *Micrococcus luteus* was used as a standard and was a gift from C. L. Hershberger.

Methane was quantitated with a Packard 7800 series gas chromatograph equipped with a Porapak QS colum connected to a hydrogen flame detector.

Results

Methanogenium cariaci was isolated from an enrichment culture inoculated with sediment taken at a 4 cm depth from the top of a core. A survey of sediment samples taken at various depths in the core showed the presence of methane producing bacteria at a depth of 4, 25, 49, and 81 cm, but none at 100, 300, or 500 cm depth. The maximal number of methanogens in the sample as indicated by a most-probable-number dilution series was between 20 and 200 per cm^3 of sediment.

Surface colonies of *M. cariaci* were circular, umbonate, greenish yellow with entire edges and a shiny surface. The colonies were less than 0.5 mm in diameter after 2 weeks of incubation and less than 4 mm in 14 weeks. The colonies exhibited a dull greenish-blue fluorescence when illuminated with long-wave UV light.

Individual cells of *M. cariaci* (Fig. 1A, B) were highly irregular (almost raisen-shaped) cocci with a width up to 2.6 µm. The cells could be made to round up into more regular cocci by lowering the salt concentration, but the cell growth rate was reduced under those conditions. Although motility was never observed, electron micrographs revealed the cells to be peritrichously flagellated. The flagella were up to 18 µm long and were rather thin, when compared to flagella of typical bacteria (Table 1, Fig. 2A). *M. cariaci* also was observed to bear long thin pili about 4 nm in diameter. Although the cells stained Gram-negative, micrographs of ultrathin sections (Fig. 1, B and C) showed that the cell walls were neither Gram-negative as they lacked an outer membrane (Costerton et al., 1974), nor typically Gram-positive (Glauert and Thornley, 1969) as they were very thin (Table 1). Indeed, Kandler and Koenig (1978) and Kandler (personal communication) have examined cells of *M. cariaci* and have shown that they lack a rigid cell wall. The walls lacked muramic acid and were easily solubilized by sodium dodecylsulfate (SDS). This SDS sensitivity is similar to that reported by Jones et al. (1977) for *Methanococcus vannielii.* As revealed by freeze etching, the cell wall had a periodic surface pattern (Fig. 3, A) consisting of structural units about 14 nm in diameter, showing a similarity to *M. vannielii.* In cells from old cultures these particles seemed to dissociate easily from the surface. The particles seemed to be composed of several subunits (Fig. 2A). The periodicity of the cell wall could also be observed in negatively stained specimens (Fig. 2C).

Methanogenium marisnigri was isolated from sediment at depths of 0.5, 5, 10, 15, and 20 cm, but none were found at 30, 40, 60, 80, or 100 cm depth. Results of a dilution series showed there to be fewer than 20 methanogens per cm^3 of sediment.

Surface colonies of *M. marisnigri* exhibited fluorescence and a size similar to colonies of *M. cariaci.* The colonies were circular, convex, and yellow with entire edges and a shiny surface. Young colonies were transparent whereas old colonies had transparent edges with an opaque center.

Single cells of *M. marisnigri* (Fig. 4A, E) were irregular cocci and had a cell diameter up to 1.3 µm. *M. marisnigri* was peritrichously flagellated, the flagella

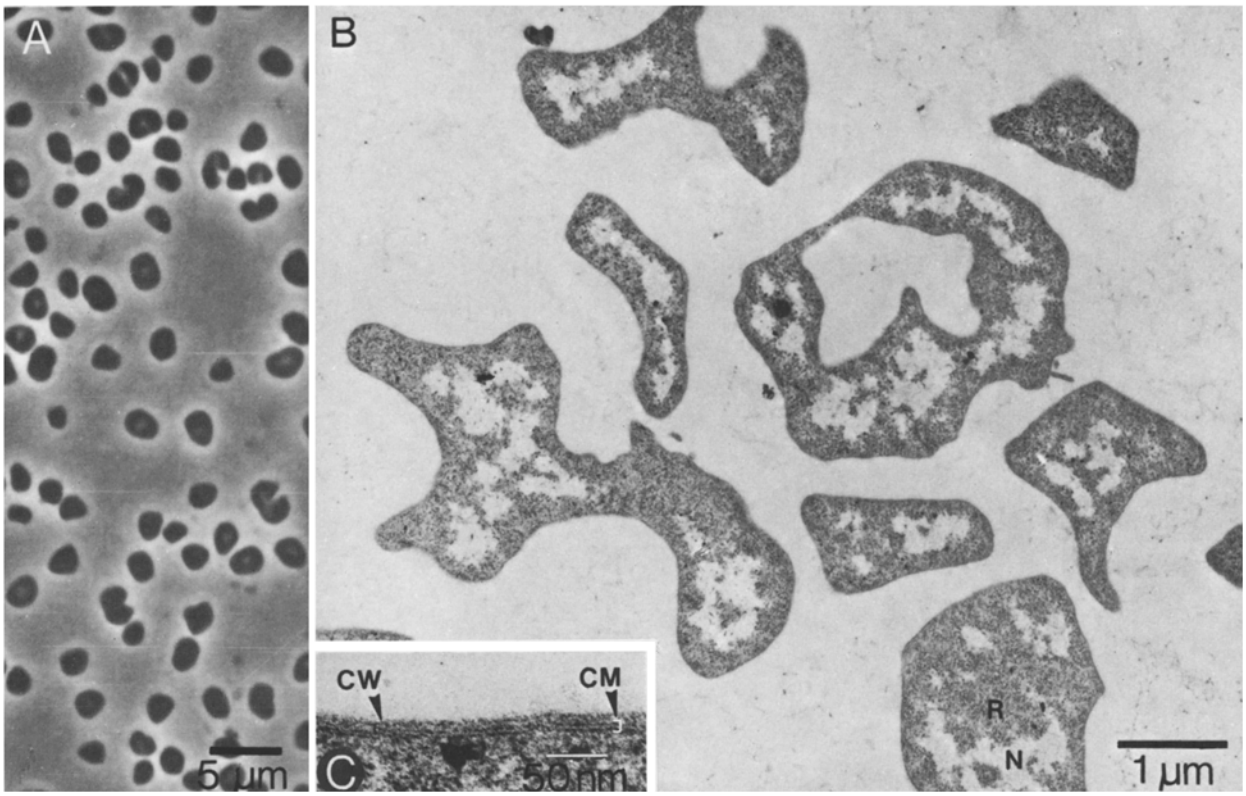

Fig. 1 A–C. *Methanogenium cariaci.* **A** Phase contrast photomicrograph of living cells; bar equals 5 µm; **B** ultrathin section (× 14,000); **C** section showing cell wall (*CW*) and cytoplasmic membrane (*CM*) (× 161,000)

Table 1. Dimensions of the cell envelope and flagella of the marine methanogens

	Thickness (nm)		
	Cytoplasmic membrane	Cell wall	Flagella
Methanogenium marisnigri	8.0[a]	10.4[a]	11.2[b]
		10.4[b]	
		11.4[c]	
M. cariaci	6.7[a]	9.5[a]	10.9[b]
		8.7[b]	
		11.4[c]	

[a] Ultrathin sections
[b] Negative staining
[c] Freeze etching

measuring up to 13 µm long and 11.2 nm in diameter (Fig. 2B, Table 1). The cytoplasmic membrane of *M. marisnigri* (Fig. 4B, C) appeared to be slightly thicker than that of *M. cariaci* (Fig. 1C), but the dimensions of the cell envelope layers depended on the type of preparation (Table 1). *M. marisnigri* had a similar periodic surface pattern (Figs. 3B; 2D) to *M. cariaci*. O. Kandler (personal communication) found no peptidoglycan in the cell walls of *M. marisnigri*, but found a surface layer of protein subunits and sugar moieties of only 0.8% galactose, 0.3% glucose and 0.1% mannose on a dry weight basis.

The optimal growth temperature for both methanogens was 20–25°C but *M. marisnigri* has a slightly broader growth temperature range (Fig. 5).

The effect of pH on growth of the marine methanogens was measured by varying the concentration of Na_2CO_3 that was added to the medium. The total Na^+ ion concentration was kept constant at 0.323 M (comparable to 1.9% NaCl) by adding NaCl. The optimal pH for growth of *M. marisnigri* was 6.2–6.6, and for *M. cariaci* it was 6.8–7.3 (Fig. 6).

The optimal concentration of added NaCl was determined to be about 0.1 M for *M. marisnigri* and 0.46 M for *M. cariaci* (Fig. 7). Na^+ ion was available from other medium components at a concentration of 0.09 M, making the total Na^+ ion concentration 0.19 M and 0.54 M respectively.

Results of growth studies showed that *M. cariaci* required acetate for growth and that maximal growth was achieved at a sodium acetate concentration of 0.1% (wt/vol). *M. cariaci* also required yeast extract, but not Trypticase for growth.

M. marisnigri did not require acetate or yeast extract for growth, nor were they stimulatory. Trypticase was

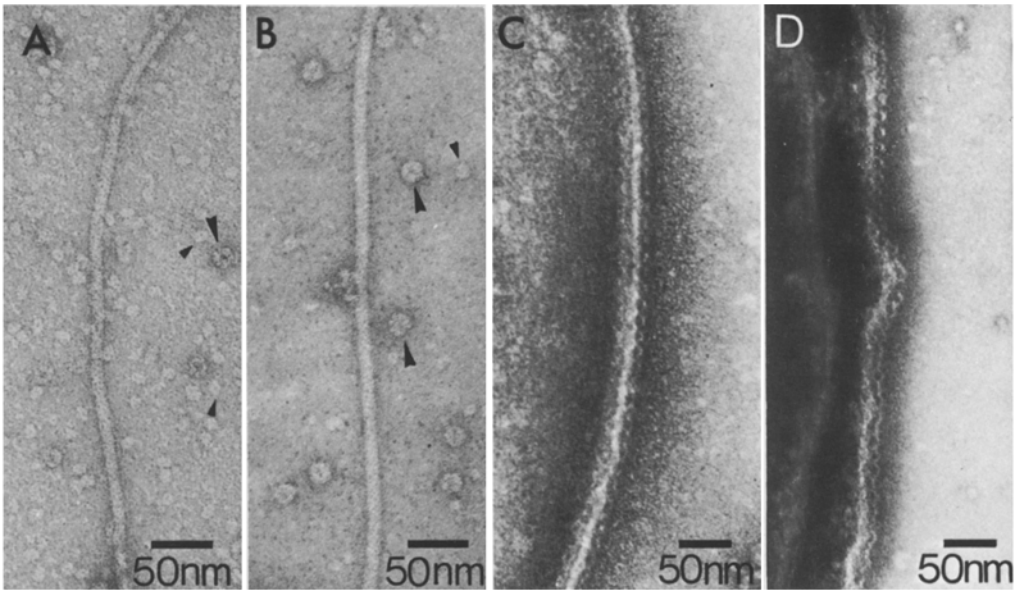

Fig. 2 A–D
Uranyl acetate-stained components of *M. cariaci* and *M. marisnigri.* **A** flagellum (×161,000); **C** cell wall structure (×135,000) of *M. cariaci*; **B** flagellum (×163,500) and cell wall structure (**D**) (×135,000) of *M. marisnigri.* Particles presumed to be structural units and subunits of the cell wall are marked by arrowheads

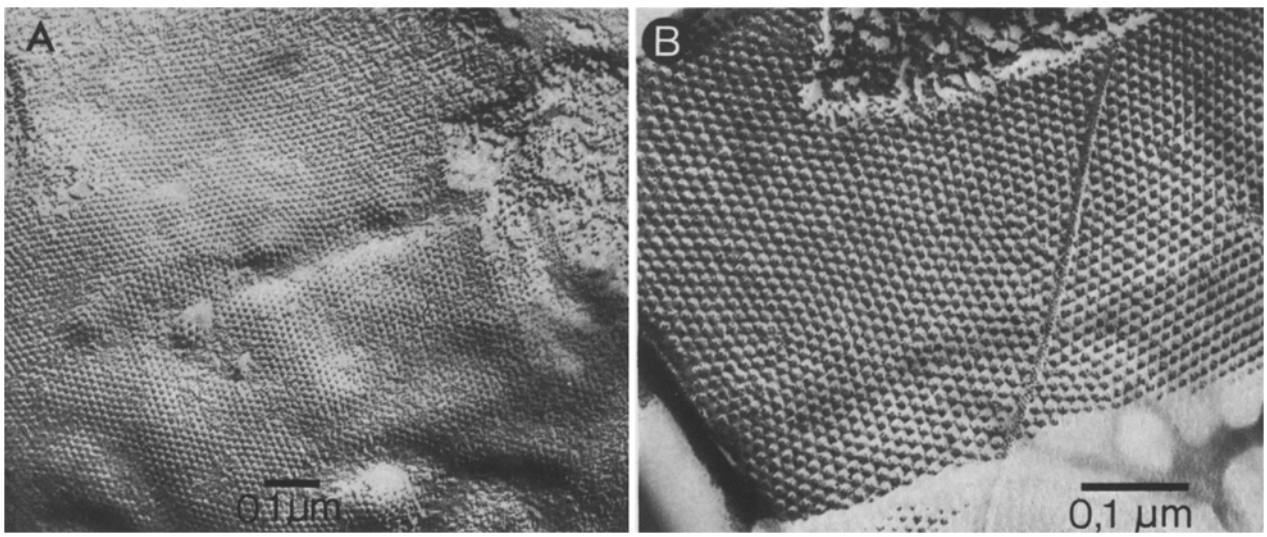

Fig. 3. Freeze-etch treatment of (**A**) *M. cariaci* (×72,000) and (**B**) *M. marisnigri* (×140,000) showing particulate nature of the cell walls

required, however, and the requirement could not be replaced by casamino acids (vitamin-free or technical), Casitone, Tryptone, Peptone, Tryptose or Neopeptone.

The mol percent G + C was determined to be 51.6% for *M. cariaci* and 61.2% for *M. marisnigri.* Coenzyme M (2-mercaptoethanesulfonate) was found in both organisms (Balch and Wolfe, 1979). Eirich examined cells of *M. marisnigri*, finding an electrophoretically typical F_{420} coenzyme (Personal communication).

Neither marine methanogen grew on acetate or methanol, but both grew on formate (Table 2). When each methanogen was incubated in a Na_2CO_3 buffered medium the production of CH_4 was observed in amounts proportional to the amount of formate added to the culture medium, but growth of each methanogen was not obtained. Not until a PIPES buffered-medium was used was growth on formate detected. The mechanism of this apparent Na_2CO_3 inhibition of formate-coupled growth is unknown.

Discussion

We propose that these marine methanogens be placed in the family Methanobacteriaceae Barker (1956) as presented in the eighth edition of Bergey's Manual of Determinative Bacteriology (Bryant, 1974). Though

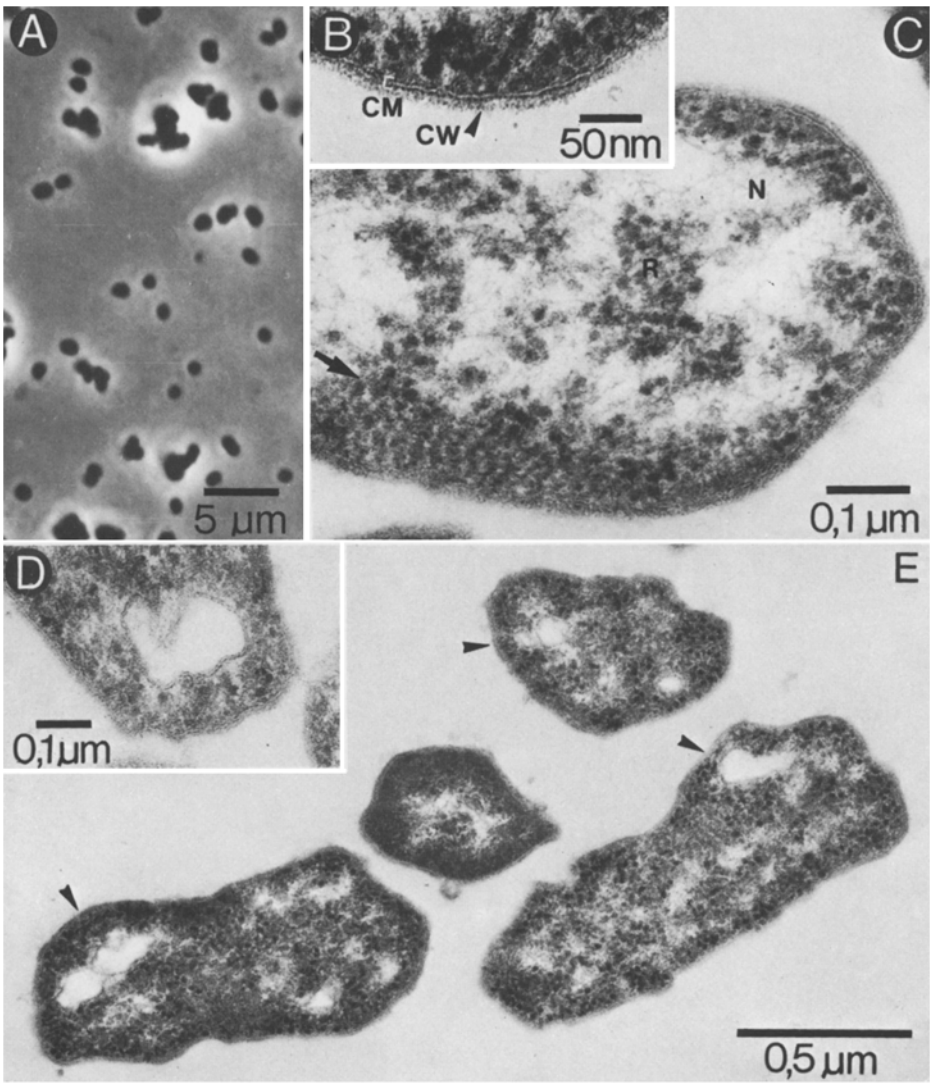

Fig. 4A–E. *Methanogenium marisnigri.* **A** Phase contrast micrograph of living cells; bar equals 5 µm; **B** ultrathin section showing the cell wall (*CW*) and cytoplasmic membrane (*CM*) (×161,000); **C** ultrathin section displaying a region of regularly arrayed particles (*arrow*) (×107,000); **D** ultrathin section showing a membrane-enclosed vesicle (×70,000); **E** ultrathin section showing vesicles or invaginations (*arrow*) *heads* and irregular nature of the cells (×45,000)

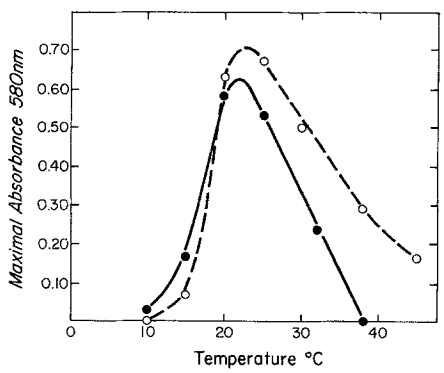

Fig. 5. Optimal temperature range for growth of the marine methanogens. (●) *M. cariaci;* (○) *M. marisnigri*

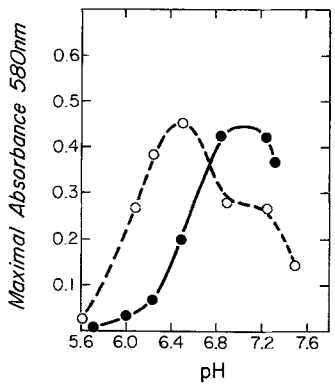

Fig. 6. Effect of pH on growth of the marine methanogens. (○) *M. marisnigri*; (●) *M. cariaci*

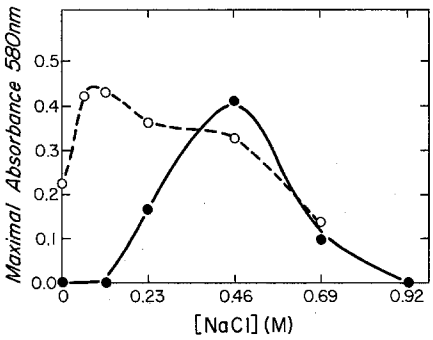

Fig. 7. Effect of NaCl concentration on growth of the marine methanogens. (○) *M. marisnigri;* (●) *M. cariaci*

Table 2. Effect of formate addition on growth of the marine methanogens

Addition[b]	Atmosphere	Final absorbance[a]	
		M. cariaci	*M. marisnigri*
None	80% H_2: 20% CO_2	0.180	0.180
None	80% N_2: 20% CO_2	0.000	0.000
Formate	H_2: CO_2	0.270	0.259
Formate	N_2: CO_2	0.080	0.059

[a] 580 nm; 15 mm path length; average of 3 tubes
[b] 0.25% (w/v). The basal medium was identical to the maintenance medium described in Materials and Methods, except Na_2CO_3 was omitted and PIPES buffer was added (2.6 g per 200 ml, pH = 6.4)
PIPES = piperazine-N,N′-bis(2-ethanesulfonic acid)

they are similar to *Methanococcus* in their morphology and cell wall structure, they are distinct because of their requirements for NaCl, their much higher G + C contents (31.1% for *M. vannielii*; W. E. Balch, personal communication) and a lack of relatedness when their 16S rRNA oligonucleotide catalogs are compared to *M. vannielii* (Fox et al., 1977; C. R. Woese, personal communication). It is suggested that these marine methanogens be placed in a new genus of methane-producing bacteria, for which the name *Methanogenium* is proposed. The type species for this genus is *M. cariaci* sp. nov., named for its source, the Cariaco Trench.

A formal generic description follows: *Methanogenium* gen. nov., Romesser and Wolfe. *Methanogenium*, M. L. n. *methanum* methane; Gr. v. suff. *genium* producing; M. L. neut. n. *methanogenium* methane producing. Cells are highly irregular cocci with a cell width 1.3 to 2.6 μm. Cells are peritrichously flagellated, have pili, are gram-negative, and are sensitive to 1% SDS. Cell walls have a periodic surface pattern. Colonies are circular, greenish-yellow to yellow with entire edges and a shiny surface. DNA base composition ranges from 52 to 61 mol % G + C. Temperature optimum is 20 to 25°C. pH optimum is 6.8 to 7.3. Organisms are found in anaerobic marine sediments. Cells are fastidious anaerobes; formate or H_2 and CO_2 serve as substrates for growth and methane production, acetate and methanol do not. Yeast extract and acetate or Trypticase as well as sodium ions are required for growth.

The formal species descriptions are: *Methanogenium cariaci* sp. nov., Romesser and Wolfe. *Cariaci.* L. gen. n. *cariaci* of the Cariaco.

Morphology: Highly irregular cocci; cell width 2.6 μm. Cells are peritrichously flagellated and have pili. Gram nagative; sensitive to 1% SDS. Cell wall has a periodic surface pattern.

Colony characteristics: Circular, umbonate, greenish-yellow with entire edges and a shiny surface.

DNA base composition: 52 mol % G + C.

Temperature relationship: Optimum, 20–25° C.

pH relationship: Optimum, 6.8 to 7.3.

Source: Sediment from Cariaco Trench.

Physiology: Fastidious anaerobe; formate or H_2-CO_2 serve as substrates for growth and methane production; acetate and methanol do not.

Nutrition: Optimal growth in 0.54 M Na^+; yeast extract and acetate required for growth.

Type strain: JR1. DSM number 1497 Göttingen, FRG.

Methanogenium marisnigri sp. nov., Romesser and Wolfe. *Marisnigri.* L. gen. n. *maris* of the sea; L. adj. *niger* black; *marisnigri* M. L. neut. n. Black Sea.

Morphology: Irregular cocci; cell width 1.3 μm. Cells are peritrichously flagellated. Gram-negative; sensitive to 1% SDS. Cell wall has a periodic surface pattern.

Colony characteristics: Circular, convex, yellow with entire edges and a shiny surface.

DNA base composition: 61 mol % G + C.

Temperature relationship: Optimum, 20–25° C.

pH relationship: Optimum 6.2 to 6.6

Source: Sediment from the Black Sea.

Physiology: Fastidious anaerobe; formate or H_2-CO_2 serve as substrates for growth and methane production; acetate and methanol do not.

Nutrition: Optimal growth at 0.19 M Na^+; Trypticase required for growth.

Type strain: JR1. DSM number 1498 Göttingen, FRG. The description of the type strain is the same as for the species.

Acknowledgements. The excellent technical assistance of D. Szurgot, R. Mangoff and V. Gabriel is gratefully acknowledged. We thank W. E. Balch for performing the mol % G + C determinations. We especially appreciate the invitation of H. Jannasch of the Woods Hole Oceanographic Institution to join research cruises (supported by the National Science Foundation) on R. V. Chain and R. V. Atlantis II. We thank Professor H. Merxmüller der Universität München for his interest and suggestions in naming these organisms. J. A. R. was the

recipient of a traineeship from the U. S. Public Health Service GM 07-283 (603). This work was supported by the National Science Foundation grant PCM 76-02652, by the U.S. Public Health Service Grant AI 12277 and by the Stiftung Volkswagenwerk.

References

Aranki, A., Freter, R.: Use of anaerobic glove boxes for the cultivation of strictly anaerobic bacteria. Am. J. Clin. Nutr. **25**, 1329–1334 (1972)

Balch, W., E., Wolfe, R. S.: New approach to the cultivation of methanogenic bacteria; 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl. Environ. Microbiol. **36**, 781–791 (1976)

Balch, W. E., Wolfe, R. S.: Specificity and biological distribution of coenzyme M (2-mercaptoethane sulfonic acid). J. Bacteriol. **137**, 256–263 (1979)

Barker, H. A.: Bacterial fermentations. New York: John Wiley and Sons (1956)

Bryant, M. P.: Methane-producing bacteria. In: Bergey's manual of determinative bacteriology, (Buchanan, R. E., Gibbons, N. E., ed.). 8th ed. pp. 472–477., Baltimore: MD, Williams and Wilkins Co., 1974

Bryant, M. P., Robinson, I. M.: An improved nonselective culture medium for ruminal bacteria and its use in determining diurnal variation in number of bacteria in the rumen. J. Dairy. Sci. **44**, 1446–1456 (1961)

Costerton, J. W., Ingram, J. M., Cheng, K.-J.: Structure and function of the cell envelope of Gram-negative bacteria. Bacteriol. Rev. **38**, 87–110 (1974)

Fox, G. E., Magrum, L. S., Balch, W. E., Wolfe, R. S., Woese, C. R.: Classification of methanogenic bacteria by 16S ribosomal RNA characterization. Proc. Natl. Acad. Sci. **74**, 4537–4541 (1977)

Glauert, A. M., Thornley, M. J.: The topography of the bacterial cell wall. Ann. Rev. Microbiol. **23**, 159–198 (1969)

Hungate, R. E.: The anaerobic mesophilic cellulolytic bacteria. Bacteriol. Rev. **14**, 1–49 (1950)

Jones, J. B., Bowers, B., Stadtman, T. C.: *Methanococcus vannielii:* ultrastructure and sensitivity to detergents and antibiotics. J. Bacteriol. **130**, 1357–1363 (1977)

Kandler, O., Koenig, H.: Chemical composition of the peptidoglycan-free cell walls of methanogenic bacteria. Arch. Microbiol. **118**, 141–152 (1978)

Lamontagne, R. A., Swinnerton, J. W., Linnenbom, V. J., Smith, W. D.: Methane concentrations in various marine environments. J. Geophys. Res. **78**, 5317–5323 (1973)

Marmur, J.: A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3**, 2317–2324 (1961)

Mayer, F., Lurz, R., Schoberth, S.: Electron microscopic investigation of the hydrogen-oxidizing acetate-forming anaerobic bacterium *Acetobaterium woodii*. Arch. Microbiol. **115**, 207–213 (1977)

Schildkraut, C. L., Marmur, J., Doty, P.: Determination of base composition of deoxyribonucleic acid from its buoyant density in CsCl. J. Mol. Biol. **4**, 430–443 (1962)

Spurr, A. R.: A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**, 31–43 (1969)

Valentine, R. C., Shapiro, B. M., Stadtman, E. R.: Regulation of glutamine synthetase. XII. Electron microscopy of the enzyme from *Escherichia coli*. Biochemistry **7**, 2143–2152 (1968)

Wolin, E. A., Wolin, M. J., Wolfe, R. S.: Formation of methane by bacterial extracts. J. Biol. Chem. **238**, 2882–2886 (1963)

Received December 13, 1978