

Genus IV. Methanococcoides Sowers and Ferry 1985, 223^{VP} (Effective publication: Sowers and Ferry 1983, 688)

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Me.tha'no.coc.coi'des. *Methanococcus* established genus; Gr. adj. suff. *-ides* similar to; M.L. neut. n. *Methanococcoides* organism similar to *Methanococcus*.

Extremely irregular cocci, $1 \pm 0.2 \mu\text{m}$ in diameter; occur singly or in pairs. Gram-negative. Nonmotile. **Strictly anaerobic**. Temperature range: 15–35°C; optimum: 30–35°C. NaCl and Mg^{2+} are required for growth; optimum NaCl concentration: 0.2–0.6 M; optimum Mg^{2+} concentration as MgSO_4 : 0.025–0.2 M. **Trimethylamine, dimethylamine, methylamine and methanol are substrates** for growth and methanogenesis; **acetate, formate and H_2 are not**.

The mol% G + C of the DNA from the only described strain, TMA-10, is 42. (T_m).

Type species: *Methanococcoides methylutens* Sowers and Ferry 1983, 688.

Further Descriptive Information

The irregularly shaped cells (Fig. 25.19) become spherical, as the NaCl or Mg^{2+} concentrations (as MgSO_4) are decreased, and lyse when either is eliminated (Sowers and Ferry, 1983). Cultures will not grow if NaCl is substituted with KCl or NaSO_4 , or if divalent cations such as Mn^{2+} are substituted for Mg^{2+} cations. Whole cells are immediately lysed by 0.01% sodium dodecyl sulfate or 0.001% Triton X-100. Electron microscopy of thin sections shows a monolayered cell wall approximately 10 nm thick. Acid hydrolysates of isolated cell walls yield a variety of amino acids, which indicates that the walls are protein. Aspartic and glutamic acids are predominant. Amino sugars are not detected. The membrane polar lipid fraction consists of 2,3-diphytanyl glycerol ethers; dibiphytanyl diglycerol tetraethers are not detected. Structures such as storage granules or internal membranes are not observed in the cytoplasm. Fimbrialike structures are occasionally observed in electron micrographs.

Surface colonies (0.5–1.5 mm) are yellow, circular and convex with entire edges. Colonies fluoresce blue-green under UV light.

Medium that contains seawater, mineral salts, biotin and substrate with an N_2 or $\text{N}_2\text{-CO}_2$ (80:20) atmosphere is required for growth of *M. methylutens* strain TMA-10 (Sowers and Ferry, 1985). A mixture of NaCl, MgSO_4 , KCl and CaCl_2 may be substituted for seawater. Yeast extract, Trypticase or rumen fluid may be substituted as sources of biotin. Essential trace metals include nickel, iron and cobalt. Strains of *M. methylutens* will grow in the presence of vancomycin (100 mg/l).

Strains of *M. methylutens* have been isolated from a marine trench that contained large deposits of organic material.

Enrichment and Isolation Procedures

Culture media are prepared under an O_2 -free atmosphere of $\text{N}_2\text{-CO}_2$ (80:20) (Balch et al., 1979). Enrichment medium contains a solution of 80% artificial seawater diluted with demineralized water plus minerals, vitamins, cysteine-sulfide-reducing agent and trimethylamine-HCl (Sowers and Ferry, 1983). Resazurin is added as an E_h indicator. The pH is

Differentiation of the genus *Methanococcoides* from other genera

The genus *Methanococcoides* is separated from *Methanosarcina* based on its inability to use acetate, comparative cataloging of the 16S rRNA (C. Woese, personal communication) and DNA/RNA homology values (Sowers et al., 1984). In addition, *Methanococcoides* lacks the thick (400 nm) heteropolysaccharide cell wall layer which is found among the *Methanosarcina* (Kandler, 1982), although one species of *Methanosarcina* also lacks a heteropolysaccharide layer (Sowers et al., 1984).

Unlike *Methanobolus*, *Methanococcoides* has no membranous internal structures or apparent storage granules (König and Stetter, 1982). The genera show no DNA/DNA homology and only 67% DNA/RNA homology. Immunological fingerprinting by indirect immunofluorescence

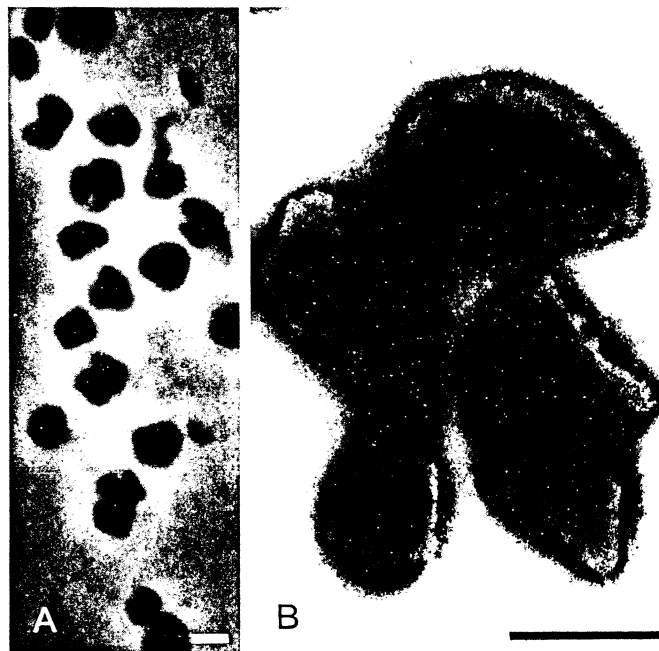


Figure 25.19. Phase-contrast micrograph (A) and ammonium molybdate-stained electron micrograph (B) of *M. methylutens* strain TMA-10 grown on trimethylamine. Bars, 1 μm .

maintained at 7.2 by a CO_2 -bicarbonate buffer system. Alternatively, medium 3 of Balch et al. (1979) may be used with the addition of suitable substrate. Enrichment cultures are incubated at 20–30°C and assayed for turbidity and methane production. Isolation medium is the same as enrichment medium, with the addition of 2% purified agar (BBL Microbiology Systems) or Noble agar (Difco). Inocula are streaked onto agar roll tubes, or serial dilutions are added to molten agar and rolled (Hungate, 1969). Isolates are identified as yellow colonies that fluoresce blue-green in long wave UV light (Mink and Dugan, 1977).

Maintenance Procedures

M. methylutens is maintained by biweekly transfer on agar slants that contain isolation medium or a modification with salts substituted for seawater (Sowers and Ferry, 1983). Cultures may also be maintained by freezing in serum vials that contain enrichment medium and glycerol (1:1).

(S probe) shows only slight cross-reactivity between *M. methylutens* strain TMA-10 and *M. tindarius* strain T3 (E. Conway de Macario, personal communication).

Taxonomic Comments

The most comprehensive phylogenetic study of the *Methanosarcinaceae* is based on DNA/RNA and DNA/DNA homology values of only seven strains (Sowers et al., 1984). The division of genera in this family is supported by rRNA hybridization values obtained by Tu et al. (1982) for division of genera in the archaeobacteria. Although the phylogenetic divisions appear distinct at this time, the current divisions may

become less distinct and warrant the merging of some genera as more methylophilic strains become available and their phylogenies are determined. The maintenance of separate genera may also be impractical for identification purposes if more distinguishing phenotypic characteristics are not found among other strains.

Further Reading

Conway de Macario, E., M.J. Wolin and A.J.L. Macario. 1982. Antibody analysis of relationships among methanogenic bacteria. *J. Bacteriol.* 149: 316-219.

List of species of the genus *Methanococcoides*

1. *Methanococcoides methylutens* Sowers and Ferry 1985, 223.^{VP}
(Effective publication: Sowers and Ferry 1983, 688.)

methylutens. mod. chem. word *methyl*-; L. part. adj. *utens* using; *methylutens* using methyl.

The description of the species is the same as that given for the genus.
Type strain: ATCC 33938; DSM 2657.

OTHER TAXA

FAMILY METHANOPLANACEAE WILDGRUBER, THOMM AND STETTER, 1984, 270^{VP}
(EFFECTIVE PUBLICATION: WILDGRUBER, THOMM AND STETTER *IN* WILDGRUBER, THOMM, KÖNIG, OBER, RICCHIUTO AND STETTER 1982, 36)

K. O. STETTER

Me.tha.no.pla.na'ce.ae. M.L. neut. n. *Methanoplanaceae* the *Methanoplanus* family.

Plate-shaped cells occurring as thin plates with sharp edges (Figs. 25.20 and 25.21). Gram-negative. The cell envelope shows a hexagonal surface pattern (Fig. 25.21). Strictly anaerobic. Chemolithotrophic. **H₂ and CO₂ or formate serve as energy source** for growth and methane formation. On hydrogen in the presence of molecular sulfur; H₂S is formed in addition to methane. The organism is mesophilic.

Habitat: free-living in anaerobic environments within swamps or as endosymbionts in marine ciliates.

The mol% G + C of the DNA is 38.7-47.5.

Comparison by DNA/RNA hybridization (Tu et al., 1982) with members of the genera *Methanosarcina* and *Methanogenium* showed a lower fractional stability (fs) between *Methanoplanus* and *Methanogenium* (fs = 0.59) than between *Methanogenium* and *Methanosarcina* (fs = 0.62), indicating phylogenetical distance.

Type genus: *Methanoplanus* Wildgruber, Thomm and Stetter 1984, 270.

Further Descriptive Information

Cells are fragile. They can be easily broken by detergents, e.g., SDS (2%) or by the French press. Cell division, possibly by constriction or budding, is indicated by the existence of Y-shaped cells. No septa forma-

tion visible. Grana of polyphosphate are visible within the cells (Wildgruber et al., 1982). Two species: *Methanoplanus limicola* was isolated from an anaerobic sample taken from a swamp composed of waste and water from steam drilling (pH 7; 19°C), and *Methanoplanus endosymbiosus* was isolated from homogenized cells of the marine ciliate *Metopus contortus*.

Enrichment and Isolation Procedures

Methanoplanus limicola can be enriched anaerobically in medium 3 (Balch et al., 1979) in pressurized (200 kPa H₂-CO₂ (80:20)) serum bottles at 30°C in the presence of vancomycin, penicillin and kanamycin with each at 150 µg/ml, and tetracycline at 100 µg/mg. It can be isolated by streaking onto polysilicate plates containing medium 3 (Balch et al., 1979). Round, smooth, bright ocher-colored colonies about 2 mm in diameter were visible after 3 months at 30°C. No growth on agar (Wildgruber et al., 1982). *Methanoplanus endosymbiosus* can be isolated from homogenized cells of *Metopus contortus* by plating the homogenate on solid media (van Bruggen et al., 1986a) containing penicillin (10³ IU/ml) or lysozyme (1 mg/ml). Incubation at 30°C for 3 weeks. Colonies about 2 mm in diameter were whitish yellow, convex and circular with entire margin.

Key to the genera of the family Methanoplanaceae

Only one genus exists: *Methanoplanus*.

Genus *Methanoplanus* Wildgruber, Thomm and Stetter 1984, 270^{VP} (Effective publication: Wildgruber, Thomm and Stetter *in* Wildgruber, Thomm, König, Ober, Ricchiuto and Stetter 1982, 36)

K.O. STETTER

Me.tha.no.pla'nus. M.L. n. *methanum* methane; M.L. adj. *planus* flat; M.L. masc. n. *Methanoplanus* the methane (-producing) plate.

Cells are angular, crystallike plates 0.07-0.30 µm thick, 1.6-3.4 µm long and 1.5 µm wide and occur singly (Figs. 25.20 and 25.21). The cells are sometimes branched, without septa. The cell envelope shows a hexagonal surface pattern (Fig. 25.21). No sacculus is present. **Flagellate**. Strictly anaerobic. Optimum temperature: 32-40°C; maximum: 41°C; minimum: 16°C. Growth occurs in 0.4 and 6% NaCl.

Chemolithotrophic, growing on H₂ and CO₂ or formate. No growth occurs on methanol or methylamines. The end product is methane (Wildgruber et al., 1982). In the presence of molecular sulfur,

H₂S is formed, in addition to H₂ and CO₂ (Stetter and Gaag, 1983). Cells are resistant to vancomycin, penicillin, kanamycin and tetracycline.

The mol% G + C of the DNA is 38.7-47.5.

Type species: *Methanoplanus limicola* Wildgruber, Thomm and Stetter 1984, 270.

Maintenance Procedures

Stock cultures of *Methanoplanus limicola* can be stored anaerobically at -20°C for several months after renewing the gas phase.