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Characterization of *Methanococcus maripaludis* sp. nov., a new methanogen isolated from salt marsh sediment

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Abstract. A predominant methanogenic bacterium was isolated from salt-marsh sediment near Pawley's Island, South Carolina. A habitat-simulating medium with H_2:CO_2 as substrate was used for enrichment and isolation. The methanogen is strictly anaerobic, weakly-motile, non-spore-forming, Gram negative, and a pleomorphic coccoid-rod averaging 1.2 by 1.6 μm. Colonies are circular, translucent, pale yellow, and have a smooth surface and an entire edge. The organism is a mesophile, growing between 18 and 47° C, with an optimum near 38° C. The pH optimum for growth is 6.8–7.2, and only formate or a mixture of H_2 plus CO_2 serve as substrates. Seawater (20–70 % v/v) is required, but it can be replaced by 15 mM, or greater, magnesium. Optimal growth occurs with 110 mM sodium. Growth rate is stimulated by selenium (10 μM) but organic compounds (acetate, vitamins, amino acids) are neither stimulatory nor required. The methanogen grows well in autotrophic medium with a doubling time of about 2 h. Cells are fragile, are lysed by aqueous solutions of low osmolality and by detergents, and they lack muramic acid. The cell wall is a single electron dense layer. The DNA base composition is 33 mol % guanine plus cytosine. Antigenic relationship of cells and the 16S ribosomal RNA catalog indicate that the salt marsh methanogen is a unique species of *Methanococcus*, for which we propose the name *Methanococcus maripaludis* sp. nov.

Key words: *Methanococcus maripaludis* – Marine methanogenic bacteria – Methanogen – Autotroph – Hydrogen oxidation – Archaebacteria

Barker (1940) was first to isolate a methanogen, *Methanobacterium omelianskii*, from a marine sediment. However, this culture was found later to be a symbiotic association of two bacterial species (Bryant et al. 1967). Stadtman and Barker (1951) isolated *Methanococcus vannielii* from San Francisco bay mud, but it was not determined if this was a predominant component of the sediment methanogens. *Methanococcus voltae*, the only other mesophilic member belonging to the order *Methanococcales* described to date, was isolated from a Florida estuarine mud (J. M. Ward, MS. thesis, Univ. of Florida, Gainesville 1970). Recently, Romesser et al. (1979) reported low populations of methanogens in deposits of the Black Sea and Cariaco Trench, and they described a new genus (*Methanogenium*) of methane producing bacteria inhabiting these sediments. We now report the isolation and characterization of a predominant methanogenic bacterium from the anaerobic sediment of a South Carolina salt marsh. Characteristics indicate that the isolate is a member of the genus *Methanococcus*, and we propose the name *Methanococcus maripaludis* sp. nov.

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Abbreviations: MPN, Most probable number; SDS, Sodium dodecylsulfate (sodium lauryl sulfate)

Materials and methods

Collection of sediment. Grab samples of sediment (top 10 cm) from the tall *Spartina alterniflora* region of Pawley's Island salt marsh, South Carolina, were stored for 3 days in sealed mason jars at 2–5° C before processing. Characteristics of the tall *Spartina* salt marsh were similar to those previously described by Jones and Paynter (1980).

Culture media and anaerobic techniques. The strict anaerobic techniques of Hungate (1950, 1969), with modifications and revisions by Balch et al. (1979), were used.

Sediment enrichments were made in a habitat-simulating medium and maintained under an atmosphere of H_2:CO_2 (4:1, v/v), as described by Jones and Paynter (1980) for the determination of methanogenic populations by the Most Probable Number (MPN) procedure. Solid medium for purification was the same as enrichment medium except that 2 % (w/v) agar (Difco, Detroit, MI, USA) was added.

Maintenance medium and defined medium for growth studies consisted of the following (g per l): K_2HPO_4, 0.14; KCl, 0.33; $MgCl_2 \cdot 6H_2O$, 2.8; $MgSO_4 \cdot 7H_2O$, 3.4; NH_4Cl, 0.25; $CaCl_2 \cdot 2H_2O$, 0.05; NaCl, 20.0; $Fe(NH_4)_2(SO_4)_2 \cdot 7H_2O$, 0.002; $NaHCO_3$, 5.0; L-cysteine · HCl · H_2O, 0.5; $Na_2S \cdot 9H_2O$, 0.5; resazurin, 0.001; vitamin solution, 10 ml; and trace mineral solution, 10 ml. Vitamin and trace mineral solutions have been described (Balch et al. 1979). Under an atmosphere of 80 % H_2 plus 20 % CO_2, the final pH was 6.8–7.0.

Growth studies were performed in 18 × 150 mm culture tubes containing 5 ml of medium, and pressurized to one, two, or three atmospheres of 80 % H_2 plus 20 % CO_2. The atmosphere was repressurized frequently during growth.

Assay methods for methane and bacterial growth. In some experiments with 80 % H_2 plus 20 % CO_2 as the substrate, methane production was assayed using the syringe techique of Paynter and Hungate (1968). Otherwise, methane was assayed using a Beckman GC-2 gas chromatograph fitted with

thermal conductivity detectors and a 2 m silica gel column (0.64 cm O.D.), operated at 23° C with N_2 as carrier gas (20 ml per min).

Bacterial growth was measured by optical density (660 nm) using a Bausch and Lomb Spectronic 20 colorimeter, light path 18 mm.

Microscopy. Light microscopy was done with an Ortholux phase contrast microscope (Leitz, Wetzlar, FRG), equipped with a Wild photoautomat exposure meter. Wet mounts of cells were made directly on glass slides coated with 2% agar.

Electron micrographs of whole cells and thin sections were made with a Phillips 300 transmission electron microscope. Cells for thin sectioning were prepared by a modification of the procedure described by Zeikus and Bowen (1975). An exponential culture grown on defined medium was centrifuged, the cell-pellet resuspended, and fixed in equal volumes of 4% glutaraldehyde and sterile culture medium. After 10 h at room temperature, cells were pelleted, washed in sterile culture medium, repelleted, and mixed with 2% washed agar held at 45° C. The agar-cell suspension was cooled, cut into small cubes, and fixed for 5 h by immersion in 1% OsO_4 in 0.1 M cacodylate buffer (pH 7.1). Fixed agar cubes were washed in cacodylate buffer and dehydrated through a graded series of water-ethanol mixtures and finally propylene oxide. Cubes were infiltrated and embedded in PolyBed 812 (Polysciences, Inc., Warrington, PA, USA) embedding medium. Thin sections were stained with 2% uranyl acetate.

Whole cells from an exponential culture grown in defined medium were concentrated by centrifugation, air dried on collodion covered grids, and stained with 2% uranyl acetate.

Assay of muramic acid. The methanogenic isolate, *Bacillus subtilis* strain RUB-818 (R. Yasbin, Dept. of Microbiology Univ. of Rochester School of Medicine and Dentistry, Rochester, New York) and *Escherichia coli* strain B (ATCC 11303 American Type Culture Collection, Rockville, Maryland) were assayed for muramic acid according to the method of King and White (1977). Cells of the methanogen were grown in defined medium under an atmosphere of 80% H_2 plus 20% CO_2. *B. subtilis* and *E. coli* were grown aerobically in nutrient broth (Difco). Cells were harvested at late log growth phase.

Sediment and cell dry weight. Duplicate samples of primary sediment slurry (5 ml) and of concentrated bacterial cultures (1 ml; muramic acid assay) were dried at 100° C to constant weight. The methanogenic population was expressed as cells/g dry sediment.

Susceptibility to antibiotics. Anaerobically prepared, filter sterilized antibiotics were added to tubes of defined broth medium (4.5 ml) under $H_2:CO_2$ (4:1). Tubes were inoculated with 0.5 ml of methanogen culture and incubated, without shaking, at 37° C, for 6 days. As a control *E. coli* strain B was similarly cultured in nutrient broth (Difco). Growth was monitored by measuring absorbance at 540 nm using a Bausch and Lomb Spectronic 20 colorimeter, light path 18 mm.

Determination of osmotic pressure. Osmotic pressures of solutions were determined with an Osmette osmometer (Model 2007; Precision Systems, Inc., Sudbury, MA, USA).

Determination of DNA Base Composition. DNA was purified by the method of Marmur (1961). Cells of the methanogen were lysed by suspension in 0.05 M phosphate buffer, pH 7.0, whereas organisms employed as standards, *Micrococcus luteus* strain 4698 (ATCC 4698, American Type Culture Collection, Rockville, Maryland) and *Bacillus subtilis* strain RUB-818, were lysed by lysozyme treatment. The buoyant density of DNA was determined by cesium chloride density gradient centrifugation in a Beckman Model E centrifuge. The mol percent guanine plus cytosine base ratio was calculated by the method of Schildkraut et al. (1962).

Determination of antigenic relationship. For use as antigen in the indirect immunofluorescent assay, cells of the methanogen were centrifuged (15,000 × *g*) and resuspended in 2% (w/v) NaCl containing 4% (v/v) formalin. Details of antisera preparation and immunofluorescent techniques have been described (Conway de Macario et al. 1982a, b).

Determination of 16S ribosomal RNA oligonucleotide catalog. Procedures for determinating the ribonuclease T_1 generated oligonucleotide catalog of uniformly ^{32}P labeled 16S ribosomal RNA have been described (Fox et al. 1977a, b; Sanger et al. 1965; Uchida et al. 1974; Woese et al. 1976), and a summary of these procedures and analysis of data have been presented by Balch et al. (1979).

Results

Isolation and enumeration. Salt marsh sediment was serially diluted into anaerobic, habitat-simulating broth under an atmosphere of 80% H_2 plus 20% CO_2 and incubated at 27° C for 6 weeks. Tubes from the highest dilution (10^{-5}) showing methane production were gassed aseptically with $H_2:CO_2$ every 5–7 days for enrichment of methanogens. After 2 weeks, a broth tube showing increased methane production was selected and serially diluted into habitat-simulating agar roll tubes under an atmosphere of $H_2:CO_2$. Roll tubes were incubated at 32° C for 12 days. High dilution tubes containing methane were fed during the incubation with $H_2:CO_2$ until additional growth of some colonies was observed (5–7 days). Colonies showing growth were transferred to tubes of anaerobic habitat simulating broth and serially diluted. One-half milliliter of each dilution was inoculated into roll tubes of the agar-habitat simulating medium (4.5 ml), under an atmosphere of $H_2:CO_2$. Roll tubes were incubated for 10–14 days at 32° C. The purification procedure was repeated until only one colony type existed in an agar dilution series. Also, the isolate was judged to be pure on the basis of microscopic observation and Gram reaction.

The methanogen, designated as isolate JJ, was present at a concentration of 10^5 cells/g sediment and occurred in the highest dilution (10^{-5}) of an MPN series that produced methane. Therefore, it is a predominant member of the methanogenic population of the salt marsh sediment.

Colony and cell morphology. After 5 days incubation in defined medium at 37° C, colonies had diameters of 2–3 mm, were circular, convex, translucent, pale yellow, and had smooth surfaces and entire edges. Whole cells and colonies fluoresced under long wave UV illumination, indicating the presence of fluorescent cofactors such as F_{420}.

Cells grown to exponential phase in defined medium were pleomorphic and oval to coccoid-rod in shape (Fig. 1). Cells

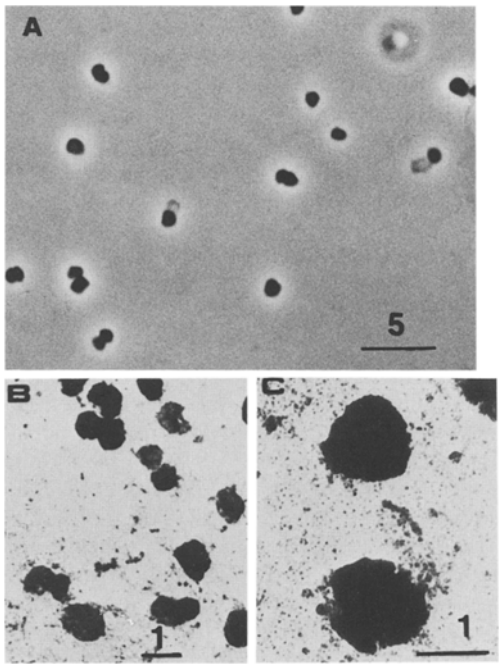

Fig. 1 A–C. Photomicrographs of methanogen JJ. (**A**) Cells viewed by phase contrast microscopy; (**B, C**) cells viewed by transmission electron microscopy, stained with uranyl acetate. Bars in **A–C** represent 5, 1 and 1 μm, respectively

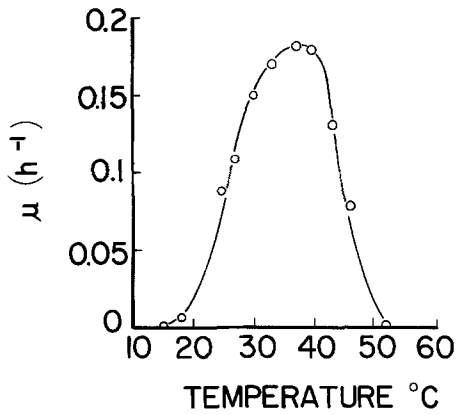

Fig. 2. Effect of temperature on the growth rate of methanogen JJ. Growth was followed under H_2:CO_2 (4:1) in bicarbonate buffered defined medium (pH 7.0) without shaking. The specific growth rate (μ) at each temperature represents the average of two experiments

averaged 1.0 – 1.3 μm in diameter and 1.5 – 1.8 μm in length. Exponentially grown cells stained Gram negative, were weakly motile and non-sporeforming.

Effect of temperature, pH, and O_2 on growth and methane production. Growth occurred between 18 and 47° C, with an optimum at 35 – 39° C (Fig. 2).

Growth and methane production occurred between pH 6.4 and 7.8, with maximal growth rate at pH 6.8 – 7.2 (Fig. 3). The upper pH limit for growth and methane production was 8.2, as determined using bicarbonate-free defined medium supplemented with 0.1 M sodium formate and Tris-HCl buffer (100 mM) under an atmosphere of N_2.

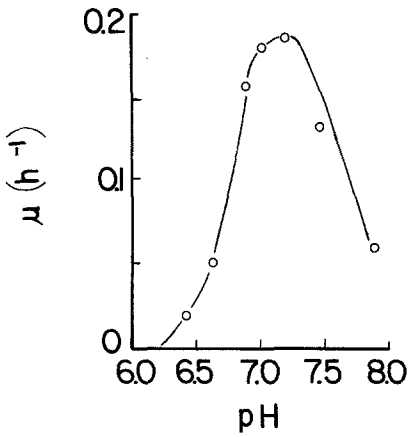

Fig. 3. Effect of pH on the growth rate of methanogen JJ. Growth was followed under H_2:CO_2 (4:1) in defined medium with various concentrations of bicarbonate (0 – 3.5%): NaCl was added to maintain a constant concentration of Na^+. Cultures were incubated at 37° C without shaking. The specific growth rate (μ) at each pH represents the average of two experiments

Various volumes of sterile deionized water, saturated with O_2 at a known temperature and atmospheric pressure, were added to duplicate habitat simulating broth cultures (5 ml) that were actively producing methane at 37° C. Cultures were reincubated and gas uptake measured with time. Addition of less than 0.77 μmol O_2 per 5 ml culture had no effect on the rate of methanogenesis. However, cultures exposed to 0.77 μmol O_2 ceased to produce methane for a period of 24 h. Thereafter, the rate of gas uptake was similar to that prior to addition of O_2. It appears that short exposure to low O_2 concentrations was inhibitory, but not lethal.

Substrate utilization and nutritional requirements. A variety of compounds (0.1 M final concentration) was added to defined growth medium and tested (in duplicate) for ability to support growth and methanogenesis. Acetate, propionate, methanol, ethanol, pyruvate and glucose were not utilized after 15 days at 37° C. Only formate or H_2 plus CO_2 served as substrates.

After initial isolation on habitat simulating medium it was found that seawater (salinity of 30 ppt) in concentrations ranging from 20 to 70% (v/v), was required for methanogenesis, and optimal methane production occurred with 40 – 70% (v/v) seawater. Sodium chloride, [1.0 – 8% (w/v)], did not replace this requirement but 1.0 – 5% (w/v) $MgCl_2 \cdot 6\,H_2O$ or $MgSO_4 \cdot 7\,H_2O$ did, indicating that a high concentration of Mg^{2+} was required. Further growth studies indicated that 15 mM magnesium was necessary for optimal growth of isolate JJ, and that no growth occurred at concentrations of 5 mM and less (Fig. 4). Calcium, at similar concentrations, did not substitute for magnesium. In defined medium, isolate JJ grew over a wide range of NaCl supplements (0 – 5% w/v). A sodium concentration of 110 mM was minimal for optimal growth.

Growth of isolate JJ was not enhanced by addition to defined medium of acetate, vitamins, amino acids, or yeast extract. However, addition of selenium (final concentration of 10 μM) stimulated the growth rate by 20%.

Characterization of cell wall. Electron micrographs of thin sections of the methanogen revealed a cell wall structure

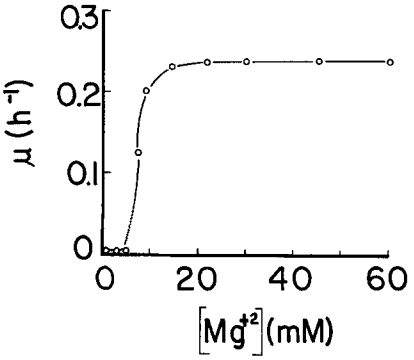

Fig. 4. Effect of magnesium concentration on the growth rate of methanogen JJ. Specific growth rates (μ) were determined, with shaking, at 37°C, after three transfers in defined medium (pH 7.0) at each magnesium concentration. Results are averages of duplicate experiments

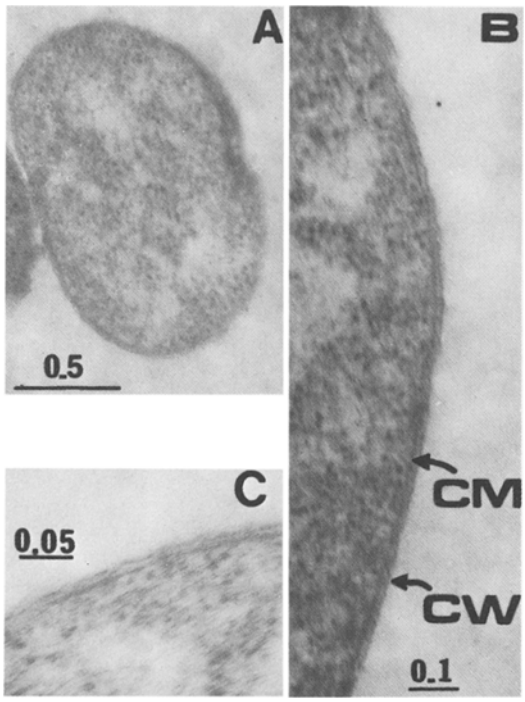

Fig. 5A–C. Electron photomicrographs of uranyl acetate stained cells of methanogen JJ. **(A)** Ultrathin section showing irregular morphology of cells; **(B, C)** ultrathin sections showing cytoplasmic membrane (*CM*) and single electron dense cell wall (*CW*). Bars in **A–C** represent 0.5, 0.1 and 0.05 μm, respectively

atypical for either Gram-positive or Gram-negative cell walls (Fig. 5). The single electron dense outer layer (approx. 10 nm) is similar to the cell wall reported for *Methanogenium cariaci*, *Methanogenium marisnigri* (Romesser et al. 1979) and *Methanococcus vannielii* (Jones et al. 1977). Attempts to isolate cell walls from the salt marsh methanogen were unsuccessful, probably because this organism lacks a rigid structural wall component (see below).

Cells of the salt marsh methanogen, *Bacillus subtilis* strain RUB-818, and *Escherichia coli* strain B were examined for the presence of muramic acid. Muramic acid was not detected in cells of the methanogen but the controls, *E. coli* and *B. subtilis*, contained approximately 2.5 mg and 3.1 mg of muramic acid per g dry weight, respectively.

Sensitivity to osmotic pressure, and susceptibility to detergents and antibiotics. Resuspension of pelleted cells of isolate JJ in distilled water or buffers of low osmolality (< 70 mOsmolal) resulted in immediate lysis. However, cells resuspended in 0.04 M $MgCl_2$ (105 mOsmolal) or 0.09 M NaCl (150 mOsmolal) or higher concentrations were stable. Thus, the organism possesses an osmotically fragile envelope.

Cells were highly susceptible to detergents and were completely disrupted in the presence of sodium dodecyl sulfate (0.01 %), Tween 20 or Tween 80 (1 %), and Triton X-100 (0.03 %).

Susceptibility of isolate JJ to several antibiotics that inhibit cell wall biosynthesis was tested, using *E. coli* as a control. Neither penicillin G (2 mg/ml) nor vancomycin (0.1 and 0.5 mg/ml) inhibited growth of the methanogen. D-cycloserine (1 mg/ml) completely inhibited growth, whereas 0.1 mg/ml caused partial inhibition (approx. 60 %) and 0.01 mg/ml had no effect. Chloramphenicol (0.1 mg/ml) completely inhibited growth, but tetracycline (10 μg/ml) had no effect. All of the above antibiotics, at each concentration tested, completely inhibited growth of *E. coli*. It has been shown that the susceptibility of certain Gram-negative bacteria to tetracycline is decreased as the concentration of magnesium in a medium is increased, probably through the formation of antibiotic-cation complexes (Chopra and Howe 1978). Since the growth medium used contained high magnesium, it is likely that this was responsible for the methanogen being insensitive to this antibiotic. Hilpert et al. (1981) reported that tetracycline inhibited growth of *M. vannielii*, but most methanogens tested were insensitive.

Characterization of DNA. DNA isolated from cells of isolate JJ had a buoyant density of 1.693 g/cm^3 and a base composition of 33 (± 1.0) mol % guanine plus cytosine.

Antigenic relationship of isolate JJ. The antigenic relationship of isolate JJ to other methanogens was determined using immunofluorescent techniques in the laboratory of M. J. Wolin. Initial analysis (R-probe) revealed that isolate JJ is antigenically related only to methanogens belonging to the genus *Methanococcus*. Further analysis (S-probe) indicated that isolate JJ is not identical to either *Methanococcus voltae* or *M. vannielii*, although a very weak reaction (lowest detectable) was observed with antiserum prepared from the latter strain and cells of isolate JJ.

Analysis of 16S rRNA catalog of isolate JJ. The 16S ribosomal RNA oligonucleotide catalog (ribonuclease T_1 generated) of isolate JJ was determined (Table 1). Comparative analysis of the catalogs of isolate JJ and other methanogens was performed to generate S_{AB} values (association coefficients) to compare the degree of sequence homology between isolate JJ and specific methanogens (Table 2). Results indicate that isolate JJ is related only to members of the genus *Methanococcus* and that it is sufficiently distinct from *M. vanielii and M. voltae* to be considered a new species.

Discussion

The antigenic relationship of isolate JJ to *Methanococcus vannielii* and *Methanococcus voltae*, and the comparative analysis of 16S ribosomal RNA catalogs of isolate JJ and

Table 1. Oligonucleotide catalog for 16S ribosomal RNA of methanogen JJ

Oligonucleotide sequence[a]

6-mers	7-mers	10-mers	Posttranscriptionally modified sequences[b]
CCCACG	CCCUACG	CACCACAACG	
CCCCAG	CUACACG	ACCACCUAUG	
CCUACG	AUACCCG	AAACUUAAAG	ĊCĊG
CCAUCG	CCUAAAG	11-mers	AψUAG
CCACUG	UAAACAG	CAUCUCACCAG	ÀÀCCUG
AUCCCG	CCCUAUG	UUUAAAUCCUG	UÀÀCAAG
AAUCCG	UAACUCG	AUUCUUUUUCG	AUṄCAACG
ACUAAG	CUCCUUG	12-mers	CACACAĊCG
AACUAG	AUCCUUG	UCACACCACCCG	
AAACUG		CUCAUUAACACG	
ACAAUG	8-mers	UUCAUAAUACUG	
AAUAAG	CAACCCUG	13-mer	
UCCCUG	UAACACCG	ACCACUUUUAUUG	
CACUUG	AUCCCAAG	14-mer	
CUCAUG	CUUAACCG	AUAAUACCCCAUAG	
ACCUUG	CUACAAUG	15-mer	
UAAUCG	UACUUCAG	UUAACAUACCCUCAG	
CAUAUG	UUUAAUUG	18-mer	
AUACUG		CAAAUCUCCUAAACCUAG	
AACUUG	9-mers		
UCCUUG	CCCACCAAG	Termini:	
CCUUUG	AAACCUCCG	5′end pAUUCCG	
UCUAUG	CCCAUCUAG		
CUAUUG	CACUCAUAG	3′end AUCACCUCC$_{OH}$	
	AAUCUCCUG		
	AUAACCUUG		
	UAAACUUUG		

[a] 16S ribosomal RNA was purified from ^{32}P labeled cells of methanogen JJ and digested with ribonuclease T1. Resulting oligonucleotides were identified by two-dimensional paper electrophoresis and their sequences determined by secondary and tertiary enzymatic procedures to generate the above catalog

[b] Posttranscriptional modifications have been described (Balch et al. 1979)

Table 2. SAB values for comparison of sequence homology of 16S ribosomal RNA of methanogen JJ and several other methanogens

Family	Organism	Organism				
		1	2	3	4	5
–	1. Methanogen JJ	–	–	–	–	–
Methanococcaceae	2. *M. vannielii*	0.63	–	–	–	–
Methanococcaceae	3. *M. voltae*	0.60	0.60[a]	–	–	–
Methanobacteriaceae	4. *M. thermoautotrophicum*	0.23	0.28[a]	0.26[a]	–	–
Methanomicrobiaceae	5. *M. mobile*	0.24	0.22[a]	0.21[a]	0.25[a]	–
Methanosarcinaceae	6. *M. barkeri* 227	0.23	0.20[a]	0.22[a]	0.30[a]	0.32[a]

[a] Data from Balch et al. (1979)

representatives of each family of methane producing bacteria, including both mesophilic species of *Methanococcus*, indicate that isolate JJ is a unique species of the genus *Methanococcus*. Although the recently described thermophile *Methanococcus thermolithotrophicus* (Huber et al. 1982) shares many nutritional and physiological similarities with isolate JJ, the considerable differences in temperature optima and temperature growth ranges are sufficient for species differentiation. This is substantiated by the 16S ribosomal RNA catalog for *M. thermolithotrophicus* (C. R. Woese, unpublished data).

Several properties of isolate JJ distinguish it from *M. vannielii* SB, *M. voltae* PS, and *M. thermolithotrophicus* DSM 2095. Microscopic observation consistently revealed that cells of isolate JJ are smaller, more irregular in shape, and less motile than cells of either *M. vannielii* or *M. voltae*. Electron micrographs of thin sections of isolate JJ, *M. vannielii* (Jones et al. 1977, and *M. thermolithotrophicus* (Huber et al. 1982) indicated that these strains possess a single electron dense outer cell wall. In contrast to *M. vannielii*, cells of isolate JJ, *M. voltae* and *M. thermolithotrophicus* are easily ruptured in solutions of low osmolality, suggesting structural differences in the cell wall of these species compared to *M. vannielii*. All methanococci lack muramic acid and growth of isolate JJ was unaffected by antibiotics that inhibit cell wall biosynthesis, suggesting that peptidoglycan is absent or an unimportant component of cell walls.

Isolate JJ does not require elevated levels of NaCl for optimal growth, as do *M. vannielii*, *M. voltae* and *M. thermolithotrophicus*. However, isolate JJ requires elevated levels of magnesium (15 mM) for optimal growth and methanogenesis, unlike *M. vannielii* or *M. voltae*. Nutritionally, isolate JJ is more similar to *M. vannielii* and *M. thermolithotrophicus* than *M. voltae*; isolate JJ, *M. vannielii* and *M. thermolithotrophicus* grow well in essentially autotrophic media. However, growth of *M. vannielii* is stimulated by yeast extract (Balch et al. 1979), by compounds containing two or three carbons and by selenium (Jones and Stadtman 1977). Neither acetate, yeast extract, trypticase, nor vitamins stimulated growth of isolate JJ, but selenium did. Recently, Whitman et al. (1982) reported that *M. voltae* requires leucine, isoleucine and acetate for growth, and that pantothenate and selenium were stimulatory. Thus, nutritional differences do exist among known strains of methanococci. In complex medium 3 of Balch et al. (1979), the minimum generation time at 37° C of isolate JJ was 2.3 h compared to 1.4 h for *M. voltae* and 8 h for *M. vannielii*. Only slight differences in DNA base compositions of isolate JJ, *M. vannielii*, *M. voltae* and *M. thermolithotrophicus* were noted (range of 31 – 33 % G + C).

Based on the above mentioned observations and characteristics, we propose that salt marsh isolate JJ is a new species of the genus *Methanococcus* and we propose the name *Methanococcus maripaludis* sp. nov.

Summary

Methanococcus maripaludis sp. nov (ma. ri. pa. lu'dis) L. *mare* "sea", L. *palus* "marsh"; M. L. gen. noun *maripaludis* "of the sea marsh."

Morphology: Pleomorphic cocci, 1.2 × 1.6 μm; occurs singly, pairs. Gram-negative, non-sporeforming, weakly motile. Extremely fragile cells, sensitive to solutions of low osmolality and 0.01 % SDS. Absence of "typical" cell wall and muramic acid. Insensitive to antibiotics inhibiting peptidoglycan synthesis.

Colony characteristics: Convex, circular, translucent, yellow in color, with a smooth surface and entire edge.

Temperature relationship: Optimum, 35 – 39° C; range 18 – 47° C.

pH relationship: Optimum, 6.8 – 7.2; range, 6.4 – 8.2.

DNA base composition: 33 mol % guanine plus cytosine.

Physiology: Obligately anaerobic; formate and $H_2 + CO_2$ serve as substrates for growth and methanogenesis.

Nutrition: Grows autotrophically in defined mineral medium with $H_2 + CO_2$. Optimal growth with 15 mM magnesium; elevated NaCl not required. Selenium stimulates growth; acetate, yeast extract, vitamins neither required nor stimulatory.

Source: Sediment from salt marsh, South Carolina, USA.

Type strain: JJ, has been deposited in the German Collection of Microorganisms (DSM) Göttingen, FRG, under the number DSM 2067.

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