Taxonomy and ecology of methanogens

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Key words: Methanogens; Archaebacteria; Taxonomy; Ecology

1. INTRODUCTION

More than nine reviews on taxonomy of methanogens have been published during the last decade [1–9], after the discovery of the unique biochemical and genetic properties of these organisms led to the concept of Archaebacteria at the end of the seventies. Moreover, important economic factors have placed these bacteria in the limelight [5], including the need to develop alternative forms of energy, xenobiotic pollution control, the enhancement of meat yields in the cattle industry, the distinction between biological and thermocatalytic petroleum generation, and the global distribution of methane in the earth's atmosphere.

2. TAXONOMY OF METHANOGENS

The methanogenic bacteria all fulfill a common physiological purpose, which is to produce methane as one of the final products of energy metabolism. Among the 68 species described up to now, 77% are hydrogenotrophs, 14% acetotrophs, and 28% methylotrophs, among which ten species are obligate methylotrophs. Only 3% of the species use H_2 to reduce methanol to methane. An as yet indeterminate number of alcoholotrophs can form

methane from CO_2 using alcohols as hydrogen donors; 2-propanol is oxidized to acetone, and 2-butanol to 2-butanone. Carbon monoxide may also be converted into methane; most hydrogenotrophic species (60%) will also use formate. Some aceticlastic species are incapable of oxidizing H_2. The aceticlastic species of the genus *Methanosarcina* are the most metabolically diverse methanogens, whereas the obligate aceticlastic *Methanosaeta (Methanothrix)* can use only acetate.

The taxonomy of the methanogenic bacteria has been extensively revised in the light of new information based on comparative studies of 16 S rRNA oligonucleotide sequences, membrane lipid composition, and antigenic fingerprinting data. The phenotypic characteristics often do not provide a sufficient means of distinguishing among taxa or determining the phylogenetic position of a taxon. Recently, Boone and Whitman [10] have proposed minimal standards for describing new taxa of methanogens, which have been approved by the Subcommittee for Taxonomy of Methanogenic Bacteria.

The characteristics of methanogens have been summarized in the recent review by Oremland [5]. Since the fundamental study by Balch et al. [1], the taxonomy of methanogens has yielded a classification including three orders, seven families and twenty genera; 68 species have been described, not all of which have yet been validated by the International List of Bacteria (Table 1).

The order Methanobacteriales contains all the methanogens with pseudomurein cell walls; C_{20} and C_{40} isopranyl glycerol ethers are abundant in

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Table 1

Characteristics of methanogenic bacteria

Organism	Morphology		Gram reac-tion	Moti-lity	Optimum growth conditions		
	Shape	Dimensions (μm)			pH	Temp. (°C)	NaCl (M)
Order Methanobacteriales							
Fam. *Methanobacteriaceae*							
Methanobacterium							
alcaliphilum	rod	0.5–0.6×2–25	–	–	8.1–9.1	37	nd
bryantii	rod	0.5–1.0×10–15	+	–	6.9–7.2	37–39	nd
espanolae	rod	0.8×3–22	+	–	5.6–6.2	35	nd
formicicum	rod	0.4–0.8×2–15	+	–	6.6–7.8	37–45	nd
ivanovii	rod	0.5–0.8×1.2	+	–	7.0–7.4	45	nd
palustre	rod	0.5×2.5–5	+	–	7.0	37	< 0.3
thermoaggregans	rod	0.4×4–8	–	–	7.0–7.5	65	0
thermoalcaliphilum	rod	0.3×3–4	–	–	7.5–8.5	58–62	nd
thermoautotrophicum	rod	0.4–0.6×3–120	+	–	7.2–7.6	65–70	nd
thermoformicicum	rod	0.3–0.6×2–120	+	–	7.0–8.0	45–60	nd
uliginosum	rod	0.2–0.6×2–4	+	–	6.0–8.5	37–40	nd
wolfei	rod	0.4×2.4–2.7	+	–	7.0–7.7	55–65	<1.7
Methanobrevibacter							
arboriphilus	coccobacillus	0.5×1.3	+	–	7.8–8.0	30–37	nd
ruminantium	coccobacillus	0.7×0.8–1.7	+	–	neutral	37–39	nd
smithii	coccobacillus	0.5–0.7×1.0–1.5	+	–	neutral	37–39	nd
Methanosphaera							
cuniculi	coccus	0.6–1.2	+	–	6.8	35–40	nd
stadtmanae	coccus	1.0	+	–	6.5–6.9	37	nd
Fam. *Methanothermaceae*							
Methanothermus							
fervidus	rod	0.3–0.4×1–3	+	+	6.5	83	nd
sociabilis	rod	0.3–0.4×3–5	+	+	6.5	88	nd
Order Methanococcales							
Fam. *Methanococcaceae*							
Methanococcus							
aeolicus	irregular coccus	nd	nd	nd	nd	nd	nd
deltae	irregular coccus	1.0–1.5	nd	–	nd	37	0.6–0.7
halophilus	irregular coccus	0.5–2.0	–	–	6.5–7.4	26–36	1.0–1.7
jannaschii	irregular coccus	1.0	nd	+	5–7	85	0.3–0.7
maripaludis	irregular coccus	1.0	nd	+	6.5–8.0	35–40	0.2–0.6
thermolithotrophicus	irregular coccus	1.0	–	+	6.5–8.0	65	0.3–0.7
vannielii	irregular coccus	1.0	nd	+	7.0–9.0	35–40	0.1
voltae	irregular coccus	1.5	nd	+	6.5–8.0	35–40	0.2–0.6
Order Methanomicrobiales							
Fam. *Methanomicrobiaceae*							
Methanomicrobium							
mobile	curved rod	0.7×1.5–2.0	–	+	6.1–6.9	40	nd
Methanolacinia							
paynteri	irregular rod	0.6×1.5–2.5	–	–	6.6–7.2	40	0.15
Methanospirillum							
hungatei	sheathed spiral	0.4×7.4–10	–	+	nd	30–37	nd
Methanogenium							
anulus	irregular coccus						
bourgense	irregular coccus	1–2	–	–	6.3–6.8	35–42	< 0.18
cariaci	irregular coccus	< 2.6	–	–	6.2–6.6	20–25	0.5

Table 1 (continued)

Organism	Morphology		Gram reaction	Motility	Optimum growth conditions		
	Shape	Dimensions (μm)			pH	Temp. (°C)	NaCl (M)
frittonii	irregular coccus	1–2.5	–	–	7.0–7.5	57	0
limiatans	irregular coccus	1.5	–	+	7.0	40	0
marisnigri	irregular coccus	<1.3	–	–	6.8–7.3	20–25	0.1
olentangyi	irregular coccus	1–1.5	–	–	nd	37	0.2
organophilum	irregular coccus	0.5–1.5	–	–	6.4–7.3	30–35	0.3
tationis	irregular coccus	3	–	–	7.0	37–40	<0.3
thermophilicum	irregular coccus	1.0–1.4	–	–	6.5–7.2	55–60	0–0.3
Fam. *Methanocorpusculaceae*							
Methanocorpusculum							
aggregans	irregular coccus	0.5–2.0	–	–	6.4–7.2	35–37	<0.18
bavaricum	irregular coccus	<1.0	–	+	7.0	37	nd
labreanum	irregular coccus	0.4–2.0	–	–	7.0	37	0–0.2
parvum	irregular coccus	0.5–1.0	–	+	6.8–7.5	37	0–0.8
sinense	irregular coccus	<1.0	–	+	7.0	30	0
Fam. *Methanoplanaceae*							
Methanoplanus							
endosymbiosus	irregular disk	0.5–1×1.6–3	–	–	6.6–7.1	32	0.25
limicola	plate	0.1–0.3×1.5–2.0	–	+	neutral	40	0.1–1.0
Fam. *Methanosarcinae*							
Methanosarcina							
acetivorans	pseudosarcina, coccoid	1.5–2.5	–	–	6.5–7.5	35–40	0.2
alcaliphilum	pseudosarcina						
barkeri	pseudosarcina	1.5–2.0	+	–	neutral	30–40	nd
frisia	pseudosarcina, coccoid	0.5–2.0	–	+	6.5–7.2	36	0.3–0.7
mazei	pseudosarcina, coccoid	1.0–3.0	+	–	7.0–7.2	30–40	nd
thermophila	pseudosarcina	1.5–2.5	+	–	6.0	50	nd
vacuolata	pseudosarcina	1–2	+	–	7.5	40	nd
Methanolobus							
Siciliae	irregular coccus	0.8–1.2	–	–	nd	37	nd
tindarius	irregular coccus	0.8–1.2	–	–	6.5	37	0.5
Vulcani	irregular coccus	0.8–1.2	–	–	nd	37	nd
Methanococcoides							
euhalobius	irregular coccus	1–2.5	–	–	6.8–7.3	28–37	1.0
methylutens	irregular coccus	1–3	–	–	neutral	30–35	0.2–0.6
Methanohalophilus							
mahii	irregular coccus	0.5–2.5	–	–	7.4–7.5	35–37	1.0–2.5
zhilinae	irregular coccus	0.8–1.5	–	–	9.2	45	0.5–1.0
Methanohalobium							
evestigatus	irregular coccus						
Halomethanococcus							
Alcaliphilum	irregular coccus						
doii	irregular coccus	3.3–1.5	–	–	6.8	35–37	1.8–3.6
Methanosaeta							
concilii	sheathed rod	0.8–1.2×0.3	–	–	7.1–7.5	35–40	nd
thermoacetophila	sheathed rod	1.0–1.2×5	–	–	6.5	60	nd
Methanothrix							
soehngenii	sheathed rod	0.8–1.2×2–3	–	–	7.4–7.8	35–40	nd
Methanopyrus	sheathed rod	0.5×8–10	+	+	6.5	98	0.25

Table 1 - Continued

Organism	Use of substrates					Proto-trophy	DNA G+C content (mol%)	Refs.
	H2 +CO2	Formate	Acetate	Methyl compounds	Alco-hols			
Order Methanobacteriales								
Fam. *Methanobacteriaceae*								
Methanobacterium								
alcaliphilum	+	−	−	−	nd	−	57	11
bryantii	+	−	−	−	+	nd	33–38	12
espanolae	+	−	−	−	−	+	33	13
formicicum	+	+	−	−	+/−	+	38–42	14
ivanovii	+	−	−	−	−	+	36.6	15
palustre	+	+	−	−	+	+	34	16
thermoaggregans	+	−	−	−	nd	+	42	17
thermoalcaliphilum	+	−	−	−	nd	+	38.8	18
thermoautotrophicum	+	−	−	−	−	+	48–52	19
thermoformicicum	+	+	−	−	−	+	43	20
uliginosum	+	−	−	−	−	nd	29–34	21
wolfei	+	−	−	−	−	+	61	22
Methanobrevibacter								
arboriphilus	+	−	−	−	−	−	28–32	23
ruminantium	+	+	−	−	nd	−	31	24
smithii	+	+	−	−	−	−	28–31	1
Methanosphaera								
cuniculi	−	−	−	−	nd	−	23	25
stadtmanae	−	−	−	−	nd	−	26	26
Fam. *Methanothermaceae*								
Methanothermus								
fervidus	+	−	−	−	nd	+	33–34	27
sociabilis	+	−	−	−	nd	+	33–34	28
Order Methanococcales								
Fam. *Methanococcaceae*								
Methanococcus								
aeolicus								29
deltae	+	+	−	−	nd	+	40.5	30
halophilus	−	−	−	+	nd	+	38	31
jannaschii	+	−	−	−	−	+	31	32
maripaludis	+	+	−	−	−	+	33	33
thermolithotrophicus	+	+	−	−	−	+	34	34
vannielii	+	+	−	−	−	+	33	35
voltae	+	+	−	−	−	−	30	1
Order Methanomicrobiales								
Fam. *Methanomicrobiaceae*								
Methanomicrobium								
mobile	+	+	−	−	nd	−	49	36
Methanolacinia								
paynteri	+	−	−	−	+	−	45	37, 38
Methanospirillum								
hungatei	+	+	−	−	+/−	+	45–49	39, 40
Methanogenium								
anulus								41
bourgense	+	+	−	−	nd	−	59	42
cariaci	+	+	−	−	−	−	50–52	43
frittonii	+	+	−	−	−	−	49.2	44
liminatans	+	+	−	−	+	+	59–60	45
marisnigri	+	+	−	−	+	−	51–62	43

Table 1 (continued)

Organism	Use of substrates					Proto-trophy	DNA G+C content (mol%)	Refs.
	H2 +CO2	Formate	Acetate	Methyl compounds	Alco-hols			
olentangyi	+	−	−	−	nd	−	54.4	30
organophilum	+	+	−	−	+	−	46.7	46
tationis	+	+	−	−	−	−	54	47
thermophilicum	+	+	−	−	+/−	−	56–60	48, 49
Fam. *Methanocorpusculaceae*								
Methanocorpusculum								
aggregans	+	+	−	−	nd	−	52	50, 51
bavaricum	+	+	−	−	+	−	47.7	52
labreanum	+	+	−	−	nd	−	50	53
parvum	+	+	−	−	+	−	48	54
sinense	+	+	−	−	−	−	52	52
Fam. *Methanoplanaceae*								
Methanoplanus								
endosymbiosus	+	+	−	−	nd	nd	39	55
limicola	+	+	−	−	−	−	48	56, 57
Fam. *Methanosarcinae*								
Methanosarcina								
acetivorans	−	−	+	+	nd	+	41	58
alcaliphilum								59
barkeri	+	−	+	+	−	+	39–51	60
frisia	+	−	−	+	nd	+	38	61, 62
mazei	+/−	−	+/−	+	nd	+	42	63
thermophila	−	−	+	+	nd	−	42	64, 65
vacuolata	+	−	+	+	nd	+	36–51	66
Methanolobus								
siciliae	−	−	−	+	−	+	41	67
tindarius	−	−	−	+	−	+	46	68
vulcani	−	−	−	+	−	+	39	67
Methanococcoides								
euhalobius	−	−	−	+	nd	−	43	69
methylutens	−	−	−	+	nd	−	42	70
Methanohalophilus								
mahii	−	−	−	+	nd	nd	49	71
zhilinae	−	−	−	+	nd	+	38	72
Methanohalobium								
evestigatus	−	−	−	+	nd	nd	nd	73
Halomethanococcus								
alcaliphilum								74
doii	−	−	−	+	nd	−	43.2	75
Methanosaeta								
concilii	−	−	+	−	nd	nd	61	76–79
thermoacetophila	−	−	+	−	nd	−	nd	79, 80
Methanothrix								
soehngenii	−	−	+	−	nd	+	52	81
Methanopyrus	+	−	−	−	nd	+	60	82

nd: not determined.

all the species tested. None of the species described are motile. The order Methanococcales contains one genus with abundant C_{20} isopranyl glycerol ethers, but C_{40} ethers are absent except in *M. jannaschii*. All of the species are irregular cocci, which are almost motile by a polar tuft of flagella

with proteinaceous cell walls. The order Methanomicrobiales shows various morphologies: small rods, spirilla, highly irregular cocci, sarcina and unusual irregular flattened plates. The cell walls are proteinaceous and the lipids include both C_{20} and C_{40} or only C_{20} isopranyl glycerol ethers.

The thermophilic methanogen, strain TAM [83] is a filamentous rod able to utilize H_2-CO_2 and formate, as well as acetate. *Methanoplasma elizabethii* [84] is an unusual species that lacks a cell wall. The taxonomic classification of these organisms has not yet been established.

3. ECOLOGY OF METHANOGENS

The distribution of methanogens in natural environments is highly dependent on their adaptation to various temperature, pH and salinity ranges. The thermophilic species, which amount to 20% of the total known methanogens, include only six genera. Most methanogens grow within a relatively narrow pH range (6.0–8.0), and two species of the genus *Methanobacterium* have been reported as alkaliphilic methanogens with an optimum pH of between 8 and 9 [11,18], and *Methanohalophilus zhilinae*, a halophilic, methylotrophic methanogen with an optimum pH of 9.2 [72].

3.1. Methanogenic interactions

In natural anaerobic habitats containing complex organic compounds, where light, sulfate, and nitrate are limited, the methanogenic bacteria cooperate with other chemo-heterotrophic bacteria in degrading organic substrates. The ultimate formation of methane and CO_2 marks the last step in a series of dissimilatory reactions by which organic compounds are completely degraded.

Methanogens can use H_2-CO_2, formate, or acetate directly produced by fermentative bacteria, or in an obligate association named syntrophy, with obligate hydrogen producing acetogenic bacteria. The latter phenomenon was termed 'interspecies hydrogen transfer' [85]. Five genera of syntrophic bacteria have been described as partnerships of methanogens, contributing to the oxidation of fatty acids, benzoic acid, or fructose. In the absence of a hydrogen scavenger, these reactions are endergonic and cannot develop. When H_2 is consumed, the reaction becomes exergonic and the syntroph can grow and oxidize the substrate.

Interspecies hydrogen transfer has also been demonstrated with defined cocultures of methanogens with non-syntrophic anaerobic bacteria and even a rumen anaerobic fungus. Other mechanisms have been proposed to account for coupling syntrophic methanogenesis: interspecies bicarbonate-formate transfer during conversion of ethanol or lactate [86], and interspecies acetate transfer during degradation of acetone [87].

Mineral terminal electron acceptors such as nitrate or sulfate inhibit methanogenesis in sediments or digesters by channeling electron flow to thermodynamically more efficient bacteria such as denitrifiers or sulfate reducers [88,89] that have higher affinity to H_2 and higher growth yields [90–92]. Methanogenesis and sulfate reduction are not always mutually exclusive and occur simultaneously, when methane is produced from methanol and/or methylated amines, substrates for which sulfate reducers show little affinity [93,94]. In ecosystems where no organic material is present, methanogenesis occurs from geochemical hydrogen evolved from hot springs, for instance. In this case methanogens act as the primary producers.

3.2. Natural habitats of methanogenic bacteria

3.2.1. Soil, aquatic environments, digestors. Rice field soils are similar to the littoral of lakes and are characterized by the presence of plants and the occurrence of oxic and anoxic zones in the sediment. The aerenchyme and intracellular space system of rice plants mediate the transport of CH_4 from the anoxic sediment into the atmosphere [95]. In the absence of plants, CH_4 is released almost exclusively by the emission of bubbles. In planted soils, up to 80% of the methane produced does not reach the atmosphere but is oxidized in the rizosphere [96]. CH_4 oxidation has also been detected in the oxic surface layer of submerged paddy soil. Methanogenesis is strongly inhibited by brackish water in these soils [96,97]. Several strains of nitrogen fixing *Methanobacterium* and

Methanosarcina have been isolated from rice soils [98].

Many workers have illustrated the importance of acetate as a methane precursor in both freshwaters and marine sediments [89,99,100], and demonstrated that H_2 is a rate limiting factor in the process of methanogenesis in sediments [90,101–103]. Landfills constitute another type of habitat from which methanogens have been isolated [104].

It has been shown that strictly anaerobic bacteria form the dominant population in digesters, and that methanogens accounted for about 10% of the total microflora [105–108]. Dolfing and Bloemen [109] have presented a rapid and reliable method for assessing the potential specific activity of methanogenic sludge. Enzyme-linked immunosorbent assay (ELISA method-110), and immunologic analysis methods [111] have been used for detecting and quantifying methanogens in digesters or mixed cultures.

3.2.2. Extreme environments. Thermophilic environments, such as hot springs, solfataras or submarine hydrothermal vents, are sites of active methanogenesis. Only a few species of hyperthermophilic methanogens have been isolated [27,32, 82].

Biogenic methane has also been detected in hypersaline environments. The patterns and rates of methane production in hypersaline algal mats may depend on a complex interaction between salinity, the use of methylated amines for osmoregulation by algae, and the formation of TMA during fermentation [112]. All the species isolated up to now are methylotrophic methanogens belonging to newly described genera [71–75]. Studies are in progress with sediments from a hypersaline lake in Senegal, containing 350 gl^{-1} salt (Ollivier, personal communication).

3.2.3. Within living organisms. Methanogens are directly involved in the digestive processes of ruminants and other animals including insects. Since the work of Hungate, the activities of methanogenic bacteria in the rumen and the cecum of herbivorous mammals have become well known. Little methane production from acetate occurs here because the animals absorb the volatile fatty acids produced during the fermentation process

through the intestinal epithelium. Thus, about 82% of the CH_4 formed in the rumen comes from H_2 reduction of CO_2, while about 18% is derived from formate [113]. However, methylotrophic methanogens are present, and participate in methanogenesis from methylamines or methanol.

Methanogens are also present in the large bowel of humans. The most prominent species of methanogens belong to the genus *Methanobrevibacter* [114], but *Methanosphaera* [115] and *Methanogenium* [115,116] strains can also be encountered. The same bacteria have been detected in the oral cavity of humans, being associated with dental plaque [117].

Methanogens have also been identified in the gut of various insects including termites, the gut microflora of which contains about 10% of methanogens [118]. The contribution of termites to atmospheric methane has given rise to some controversy during the last decade [119,120], and has been estimated at $2–5 \times 10^{12}$ g per year.

The heartwood tissues of trees can become infected with soil bacteria and develop conditions for methanogenesis at the expense of the degradation of cellulose and pectins [121]. *Methanobrevibacter arboriphilus* has been isolated from this habitat [23]. Methanogens have also been found to be endosymbionts of protozoa, removing the hydrogen produced by the protozoa via interspecies hydrogen transfer [56,122,123].

In the oceans, methane evolution results from the activities of methanogens located within the intestinal tracts of marine animals (plankton and fishes [124]), as well as in the forestomachs of baleen whales [125] where fermentation of chitin occurs in a situation analogous to that of rumen in ruminants.

3.2.4. Atmospheric methane. The global tropospheric methane concentrations average about 1.6 ppm, and a two- to threefold increase has occurred over the past 100–200 years [126], with a present increase of 2% per year. Because methane absorbs in the infrared, it plays an analogous role to that of carbon dioxide ('Greenhouse' effect). In addition, it is destroyed in the atmosphere by reacting with hydroxyl radicals, resulting in the production of carbon monoxide and hydrogen [127]. However, methane may react with chlorine

or nitrous oxide, derived from chlorofluoro-carbons and fertilizer applications respectively, and help protect the stratospheric ozone layer from being destroyed by these compounds [128]. The residence time of methane in the atmosphere is now thought to be about 8 years [129].

NOTE ADDED IN PROOF

After completion of the manuscript, publications appeared on the following topics: Description of a new species of *Methanohalophilus*: Liu, Y., Boone, D.R. and Choy, C. (1990) *Methanohalophilus oregonense* sp.nov., a methylotrophic methanogen from an alkaline, saline aquifer. Int. J. Syst. Bacteriol. 40, 111–116. Transfer of several species of genus *Methanogenium* to the new genus *Methanoculleus*: Maestrojuan, G.M., Boone, D.R., Xun, L., Mah, R.A. and Zhang, L. (1990) Transfer of *Methanogenium bourgense, Methanogenium marisnigri, Methanogenium olentangyi,* and *Methanogenium thermophilicum* to the genus *Methanoculleus* gen.nov., emendation of *Methanoculleus marisnigri* and *Methanogenium* and description of new strains of *Methanoculleus bourgense* and *Methanoculleus marisnigri.* Int. J. Syst. Bacteriol. 40, 117–122. Existence of family *Methanoplanaceae* contradicted by 16S sequencing data. Genus *Halomethanococcus* not clearly distinguished from *Methanohalophilus. Methanococcus deltae* subjective synonym of *Methanococcus maripaludis. Methanococcus halophilus* unassigned strain of *Methanohalobium*: Boone, D.R. (1990) Catalog of the Oregon Collection of methanogens. Oregon Graduate Institute of Science and Technology, Beaverton, Oregon, pp. 52.

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