

FEMSRE 00180

Taxonomy and ecology of methanogens

J.L. Garcia

Laboratoire de Microbiologie ORSTOM, Université de Provence, Marseille, France

Key words: Methanogens; Archaeobacteria; 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 Archaeobacteria 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 H₂ to reduce methanol to methane. An as yet indeterminate number of alcoholotrophs can form

methane from CO₂ 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 acetoclastic species are incapable of oxidizing H₂. The acetoclastic species of the genus *Methanosarcina* are the most metabolically diverse methanogens, whereas the obligate acetoclastic *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; C₂₀ and C₄₀ isoprenyl glycerol ethers are abundant in

Correspondence to: J.L. Garcia, Laboratoire de Microbiologie ORSTOM, Université de Provence, 3 Place Victor-Hugo, 13331 Marseille, Cedex 3, France.

Table 1

Characteristics of methanogenic bacteria

Organism	Morphology		Gram reaction	Motility	Optimum growth conditions		
	Shape	Dimensions (μm)			pH	Temp. ($^{\circ}\text{C}$)	NaCl (M)
Order Methanobacteriales							
Fam. <i>Methanobacteriaceae</i>							
<i>Methanobacterium</i>							
<i>alcaliphilum</i>	rod	0.5–0.6×2–25	–	–	8.1–9.1	37	nd
<i>bryantii</i>	rod	0.5–1.0×10–15	+	–	6.9–7.2	37–39	nd
<i>espanolae</i>	rod	0.8×3–22	+	–	5.6–6.2	35	nd
<i>formicum</i>	rod	0.4–0.8×2–15	+	–	6.6–7.8	37–45	nd
<i>ivanovii</i>	rod	0.5–0.8×1.2	+	–	7.0–7.4	45	nd
<i>palustre</i>	rod	0.5×2.5–5	+	–	7.0	37	< 0.3
<i>thermoaggregans</i>	rod	0.4×4–8	–	–	7.0–7.5	65	0
<i>thermoalcaliphilum</i>	rod	0.3×3–4	–	–	7.5–8.5	58–62	nd
<i>thermoautotrophicum</i>	rod	0.4–0.6×3–120	+	–	7.2–7.6	65–70	nd
<i>thermoformicum</i>	rod	0.3–0.6×2–120	+	–	7.0–8.0	45–60	nd
<i>uliginosum</i>	rod	0.2–0.6×2–4	+	–	6.0–8.5	37–40	nd
<i>wolfei</i>	rod	0.4×2.4–2.7	+	–	7.0–7.7	55–65	<1.7
<i>Methanobrevibacter</i>							
<i>arboriphilus</i>	coccobacillus	0.5×1.3	+	–	7.8–8.0	30–37	nd
<i>ruminantium</i>	coccobacillus	0.7×0.8–1.7	+	–	neutral	37–39	nd
<i>smithii</i>	coccobacillus	0.5–0.7×1.0–1.5	+	–	neutral	37–39	nd
<i>Methanosphaera</i>							
<i>cuniculi</i>	coccus	0.6–1.2	+	–	6.8	35–40	nd
<i>stadmanae</i>	coccus	1.0	+	–	6.5–6.9	37	nd
Fam. <i>Methanothermaceae</i>							
<i>Methanothermus</i>							
<i>fervidus</i>	rod	0.3–0.4×1–3	+	+	6.5	83	nd
<i>sociabilis</i>	rod	0.3–0.4×3–5	+	+	6.5	88	nd
Order Methanococcales							
Fam. <i>Methanococcaceae</i>							
<i>Methanococcus</i>							
<i>aeolicus</i>	irregular coccus	nd	nd	nd	nd	nd	nd
<i>deltae</i>	irregular coccus	1.0–1.5	nd	–	nd	37	0.6–0.7
<i>halophilus</i>	irregular coccus	0.5–2.0	–	–	6.5–7.4	26–36	1.0–1.7
<i>jannaschii</i>	irregular coccus	1.0	nd	+	5–7	85	0.3–0.7
<i>maripaludis</i>	irregular coccus	1.0	nd	+	6.5–8.0	35–40	0.2–0.6
<i>thermolithotrophicus</i>	irregular coccus	1.0	–	+	6.5–8.0	65	0.3–0.7
<i>vanniellii</i>	irregular coccus	1.0	nd	+	7.0–9.0	35–40	0.1
<i>voltae</i>	irregular coccus	1.5	nd	+	6.5–8.0	35–40	0.2–0.6
Order Methanomicrobiales							
Fam. <i>Methanomicrobiaceae</i>							
<i>Methanomicrobium</i>							
<i>mobile</i>	curved rod	0.7×1.5–2.0	–	+	6.1–6.9	40	nd
<i>Methanolacinia</i>							
<i>paynteri</i>	irregular rod	0.6×1.5–2.5	–	–	6.6–7.2	40	0.15
<i>Methanospirillum</i>							
<i>hungatei</i>	sheathed spiral	0.4×7.4–10	–	+	nd	30–37	nd
<i>Methanogenium</i>							
<i>anulus</i>	irregular coccus						
<i>bourgense</i>	irregular coccus	1–2	–	–	6.3–6.8	35–42	< 0.18
<i>cariaci</i>	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. ($^{\circ}\text{C}$)	NaCl (M)
<i>frittonii</i>	irregular coccus	1-2.5	-	-	7.0-7.5	57	0
<i>limiatans</i>	irregular coccus	1.5	-	+	7.0	40	0
<i>marisnigri</i>	irregular coccus	<1.3	-	-	6.8-7.3	20-25	0.1
<i>olentangyi</i>	irregular coccus	1-1.5	-	-	nd	37	0.2
<i>organophilum</i>	irregular coccus	0.5-1.5	-	-	6.4-7.3	30-35	0.3
<i>tationis</i>	irregular coccus	3	-	-	7.0	37-40	< 0.3
<i>thermophilicum</i>	irregular coccus	1.0-1.4	-	-	6.5-7.2	55-60	0-0.3
Fam. <i>Methanocorpusculaceae</i>							
<i>Methanocorpusculum</i>							
<i>aggregans</i>	irregular coccus	0.5-2.0	-	-	6.4-7.2	35-37	< 0.18
<i>bavaricum</i>	irregular coccus	<1.0	-	+	7.0	37	nd
<i>labreanum</i>	irregular coccus	0.4-2.0	-	-	7.0	37	0-0.2
<i>parvum</i>	irregular coccus	0.5-1.0	-	+	6.8-7.5	37	0-0.8
<i>sinense</i>	irregular coccus	<1.0	-	+	7.0	30	0
Fam. <i>Methanoplanaceae</i>							
<i>Methanoplanus</i>							
<i>endosymbiosus</i>	irregular disk	0.5-1×1.6-3	-	-	6.6-7.1	32	0.25
<i>limicola</i>	plate	0.1-0.3×1.5-2.0	-	+	neutral	40	0.1-1.0
Fam. <i>Methanosarcinae</i>							
<i>Methanosarcina</i>							
<i>acetivorans</i>	pseudosarcina, coccoid	1.5-2.5	-	-	6.5-7.5	35-40	0.2
<i>alcaliphilum</i>	pseudosarcina						
<i>barkeri</i>	pseudosarcina	1.5-2.0	+	-	neutral	30-40	nd
<i>frisiana</i>	pseudosarcina, coccoid	0.5-2.0	-	+	6.5-7.2	36	0.3-0.7
<i>mazei</i>	pseudosarcina, coccoid	1.0-3.0	+	-	7.0-7.2	30-40	nd
<i>thermophila</i>	pseudosarcina	1.5-2.5	+	-	6.0	50	nd
<i>vacuolata</i>	pseudosarcina	1-2	+	-	7.5	40	nd
<i>Methanolobus</i>							
<i>Siciliae</i>	irregular coccus	0.8-1.2	-	-	nd	37	nd
<i>tindarius</i>	irregular coccus	0.8-1.2	-	-	6.5	37	0.5
<i>Vulcani</i>	irregular coccus	0.8-1.2	-	-	nd	37	nd
<i>Methanococcoides</i>							
<i>euhalobius</i>	irregular coccus	1-2.5	-	-	6.8-7.3	28-37	1.0
<i>methylytens</i>	irregular coccus	1-3	-	-	neutral	30-35	0.2-0.6
<i>Methanohalophilus</i>							
<i>mahii</i>	irregular coccus	0.5-2.5	-	-	7.4-7.5	35-37	1.0-2.5
<i>zhilinae</i>	irregular coccus	0.8-1.5	-	-	9.2	45	0.5-1.0
<i>Methanohalobium</i>							
<i>evestigatus</i>	irregular coccus						
<i>Halomethanococcus</i>							
<i>Alcaliphilum doii</i>	irregular coccus						
<i>doii</i>	irregular coccus	3.3-1.5	-	-	6.8	35-37	1.8-3.6
<i>Methanosaeta</i>							
<i>concilii</i>	sheathed rod	0.8-1.2×0.3	-	-	7.1-7.5	35-40	nd
<i>thermoacetophila</i>	sheathed rod	1.0-1.2×5	-	-	6.5	60	nd
<i>Methanothrix</i>							
<i>soehngenii</i>	sheathed rod	0.8-1.2×2-3	-	-	7.4-7.8	35-40	nd
<i>Methanopyrus</i>	sheathed rod	0.5×8-10	+	+	6.5	98	0.25

Table 1 - Continued

Organism	Use of substrates					Proto- trophs	DNA G+C content (mol%)	Refs.
	H ₂ + CO ₂	Formate	Acetate	Methyl compounds	Alco- hols			
Order Methanobacteriales								
Fam. <i>Methanobacteriaceae</i>								
<i>Methanobacterium</i>								
<i>alcaliphilum</i>	+	-	-	-	nd	-	57	11
<i>bryantii</i>	+	-	-	-	+	nd	33-38	12
<i>espanolae</i>	+	-	-	-	-	+	33	13
<i>formicicum</i>	+	+	-	-	+/-	+	38-42	14
<i>ivanovii</i>	+	-	-	-	-	+	36.6	15
<i>palustre</i>	+	+	-	-	+	+	34	16
<i>thermoaggregans</i>	+	-	-	-	nd	+	42	17
<i>thermoalcaliphilum</i>	+	-	-	-	nd	+	38.8	18
<i>thermoautotrophicum</i>	+	-	-	-	-	+	48-52	19
<i>thermoformicicum</i>	+	+	-	-	-	+	43	20
<i>uliginosum</i>	+	-	-	-	-	nd	29-34	21
<i>wolfei</i>	+	-	-	-	-	+	61	22
<i>Methanobrevibacter</i>								
<i>arboriphilus</i>	+	-	-	-	-	-	28-32	23
<i>ruminantium</i>	+	+	-	-	nd	-	31	24
<i>smithii</i>	+	+	-	-	-	-	28-31	1
<i>Methanosphaera</i>								
<i>cuniculi</i>	-	-	-	-	nd	-	23	25
<i>stadimanae</i>	-	-	-	-	nd	-	26	26
Fam. <i>Methanothermaceae</i>								
<i>Methanothermus</i>								
<i>fervidus</i>	+	-	-	-	nd	+	33-34	27
<i>sociabilis</i>	+	-	-	-	nd	+	33-34	28
Order Methanococcales								
Fam. <i>Methanococcaceae</i>								
<i>Methanococcus</i>								
<i>aeolicus</i>								29
<i>deltae</i>	+	+	-	-	nd	+	40.5	30
<i>halophilus</i>	-	-	-	+	nd	+	38	31
<i>jannaschii</i>	+	-	-	-	-	+	31	32
<i>maripaludis</i>	+	+	-	-	-	+	33	33
<i>thermolithotrophicus</i>	+	+	-	-	-	+	34	34
<i>vannielii</i>	+	+	-	-	-	+	33	35
<i>voltae</i>	+	+	-	-	-	-	30	1
Order Methanomicrobiales								
Fam. <i>Methanomicrobiaceae</i>								
<i>Methanomicrobium</i>								
<i>mobile</i>	+	+	-	-	nd	-	49	36
<i>Methanolacinia</i>								
<i>paynteri</i>	+	-	-	-	+	-	45	37, 38
<i>Methanospirillum</i>								
<i>hungatei</i>	+	+	-	-	+/-	+	45-49	39, 40
<i>Methanogenium</i>								
<i>anulus</i>								41
<i>bourgense</i>	+	+	-	-	nd	-	59	42
<i>cariaci</i>	+	+	-	-	-	-	50-52	43
<i>frittonii</i>	+	+	-	-	-	-	49.2	44
<i>liminatans</i>	+	+	-	-	+	+	59-60	45
<i>marisnigri</i>	+	+	-	-	+	-	51-62	43

Table 1 (continued)

Organism	Use of substrates					Proto-trophy	DNA G+C content (mol%)	Refs.
	H ₂ +CO ₂	Formate	Acetate	Methyl compounds	Alco-hols			
<i>olentangyi</i>	+	-	-	-	nd	-	54.4	30
<i>organophilum</i>	+	+	-	-	+	-	46.7	46
<i>tationis</i>	+	+	-	-	-	-	54	47
<i>thermophilicum</i>	+	+	-	-	+/-	-	56-60	48, 49
Fam. <i>Methanocorpusculaceae</i>								
<i>Methanocorpusculum</i>								
<i>aggregans</i>	+	+	-	-	nd	-	52	50, 51
<i>bavaricum</i>	+	+	-	-	+	-	47.7	52
<i>labreanum</i>	+	+	-	-	nd	-	50	53
<i>parvum</i>	+	+	-	-	+	-	48	54
<i>sinense</i>	+	+	-	-	-	-	52	52
Fam. <i>Methanoplanaceae</i>								
<i>Methanoplanus</i>								
<i>endosymbiosus</i>	+	+	-	-	nd	nd	39	55
<i>limicola</i>	+	+	-	-	-	-	48	56, 57
Fam. <i>Methanosarcinae</i>								
<i>Methanosarcina</i>								
<i>acetivorans</i>	-	-	+	+	nd	+	41	58
<i>alcaliphilum</i>								59
<i>barkeri</i>	+	-	+	+	-	+	39-51	60
<i>frisla</i>	+	-	-	+	nd	+	38	61, 62
<i>mazei</i>	+/-	-	+/-	+	nd	+	42	63
<i>thermophila</i>	-	-	+	+	nd	-	42	64, 65
<i>vacuolata</i>	+	-	+	+	nd	+	36-51	66
<i>Methanolobus</i>								
<i>siciliae</i>	-	-	-	+	-	+	41	67
<i>tindarius</i>	-	-	-	+	-	+	46	68
<i>vulcani</i>	-	-	-	+	-	+	39	67
<i>Methanococcoides</i>								
<i>euhalobius</i>	-	-	-	+	nd	-	43	69
<i>methylutens</i>	-	-	-	+	nd	-	42	70
<i>Methanohalophilus</i>								
<i>mahii</i>	-	-	-	+	nd	nd	49	71
<i>zhilinae</i>	-	-	-	+	nd	+	38	72
<i>Methanohalobium</i>								
<i>evestigatus</i>	-	-	-	+	nd	nd	nd	73
<i>Halomethanococcus</i>								
<i>alcaliphilum</i>								74
<i>doii</i>	-	-	-	+	nd	-	43.2	75
<i>Methanosaeta</i>								
<i>concilii</i>	-	-	+	-	nd	nd	61	76-79
<i>thermoacetophila</i>	-	-	+	-	nd	-	nd	79, 80
<i>Methanotherix</i>								
<i>soehngenii</i>	-	-	+	-	nd	+	52	81
<i>Methanopyrus</i>	+	-	-	-	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 C₂₀ isopranyl

glycerol ethers, but C₄₀ 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, methylo-trophic 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 $2\text{--}5 \times 10^{12}$ 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 chlorofluorocarbons 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.

REFERENCES

- [1] Balch, W.E., Fox, G.E., Magrum, L.J., Woese, C.R. and Wolfe, R.S. (1979) Methanogens: reevaluation of a unique biological group. *Microbiol. Rev.* 43, 260–296.
- [2] Reference omitted.
- [3] Jones, W.J., Nagle, D.P., Jr. and Whitman, W.B. (1987) Methanogens and the diversity of archaeobacteria. *Microbiol. Rev.* 51, 135–177.
- [4] Mah, R.A. and Smith, M.R. (1981) The methanogenic bacteria, in *The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria*, Vol. 1 (Starr, M.P., Stolp, H., Trüper, H.G., Balows, A. and Schlegel, H.G., Eds.), pp. 948–977, Springer-Verlag, Berlin.
- [5] Oremland, R.S. (1988) Biogeochemistry of methanogenic bacteria, in *Anaerobic Bacteria* (Zehnder, A.J.B., Ed.), pp. 641–705, John Wiley & Sons, New York.
- [6] Taylor, G.T. (1982) The methanogenic bacteria. *Progr. Ind. Microbiol.* 16, 231–329.
- [7] Whitman, W.B. (1985) Methanogenic bacteria, in *Bacteria—a Treatise on Structure and Function*, Vol. VIII Archaeobacteria, pp. 3–84, Academic Press, Orlando.
- [8] Zehnder, A.J.B., Ingvorsen, K. and Marti, T. (1982) Microbiology of methane bacteria, in *Anaerobic Digestion 1981* (Hugues, D.E., Stafford, D.A., Wheatley, B.I., Baader, W., Lettinga, G., Nyns, E.J., Verstraete, W. and Wentworth, R.L., Eds.), pp. 45–68, Elsevier Biomedical Press, New York.
- [9] Boone, D.R. and Mah, R.A. (1989) Methanogenic archaeobacteria, in *Bergey's Manual of Systematic Bacteriology* (Staley, J.T., Bryant, M.P., Pfennig, N. and Holt, J.G., Eds.), Vol. 3, pp. 2173–2216, Williams & Wilkins, Baltimore.
- [10] Boone, D.R. and Whitman, W.B. (1989) Proposal of minimal standards for describing new taxa of methanogenic bacteria. *Int. J. Syst. Bacteriol.* 38, 212–219.
- [11] Worakit, S., Boone, D.R., Mah, R.A., Abdel-Samie, M.E. and El-Halwagi, M.M. (1986) *Methanobacterium alcaliphilum* sp. nov., an H₂-utilizing methanogen that grows at high pH values. *Int. J. Syst. Bacteriol.* 36, 380–382.
- [12] Boone, D.R. (1987) Replacement of the type strain of *Methanobacterium formicicum*, and reinstatement of *Methanobacterium bryantii* sp. nov., nom.rev. (ex Balch and Wolfe, 1981) with M.o.H. (DSM 863) as the type strain. *Int. J. Syst. Bacteriol.* 37, 172–173.
- [13] Patel, G.B., Sprott, G.D. and Fein, J.E. (1990) Isolation and characterization of *Methanobacterium espanolae* sp. nov., a mesophilic, moderately acidiphilic methanogen. *Int. J. Syst. Bacteriol.* 40, 12–18.
- [14] Bryant, M.P. and Boone, D.R. (1987) Isolation and characterization of *Methanobacterium formicicum* MF. *Int. J. Syst. Bacteriol.* 37, 171.
- [15] Jain, M.K., Thompson, T.E., Conway de Macario, E. and Zeikus, J.G. (1987) Speciation of *Methanobacterium* strain Ivanov as *Methanobacterium ivanovii*, sp. nov. *System. Appl. Microbiol.* 9, 77–82.
- [16] Zellner, G., Bleicher, K., Braun, E., Kneifel, H., Tindall, B.J., Conway de Macario, E. and Winter, J. (1989) Characterization of a new mesophilic, secondary alcohol-utilizing methanogen, *Methanobacterium palustre*, spec. nov. from a peat bog. *Arch. Microbiol.* 151, 1–9.
- [17] Blotevogel, K.H. and Fischer, U. (1985) Isolation and characterization of a new thermophilic and autotrophic methane producing bacterium: *Methanobacterium thermoaggregans* spec. nov. *Arch. Microbiol.* 142, 218–222.

- [18] Blotevogel, K.H., Fischer, U., Mocha, M. and Janssen, S. (1985) *Methanobacterium thermoalcaliphilum* spec. nov., a new moderately alkaliphilic and thermophilic autotrophic methanogen. Arch. Microbiol. 142, 211–217.
- [19] Zeikus, J.G. and Wolfe, R.S. (1972) *Methanobacterium thermoautotrophicus* sp. nov., an anaerobic, autotrophic, extreme thermophile. J. Bacteriol. 109, 707–713.
- [20] Zhilina, T.N. and Ilarionov, S.A. (1984) Isolation and comparative characteristics of methanogenic assimilating formate with the description of *Methanobacterium thermoformicicum* sp. nov. Mikrobiologiya 53, 785–790.
- [21] König, H. (1984) Isolation and characterization of *Methanobacterium uliginosum* sp. nov. from a marshy soil. Can. J. Microbiol. 30, 1477–1481.
- [22] Winter, J., Lerp, C., Zabel, H.P., Wildenauer, F.X., König, H. and Schindler, F. (1984) *Methanobacterium wolfei*, sp. nov., a new tungsten-requiring, thermophilic, autotrophic methanogen. Syst. Appl. Microbiol. 5, 457–466.
- [23] Zeikus, J.G. and Henning, D.L. (1975) *Methanobacterium arboriphilum* sp. nov., an obligate anaerobe isolated from wetwood of living trees. Anton. Leeuwenhoek J. Microbiol. Serol. 41, 543–552.
- [24] Smith, P.H. and Hungate, R.E. (1958) Isolation and characterization of *Methanobacterium ruminantium* n. sp. J. Bacteriol. 75, 713–718.
- [25] Biavati, B., Vasta, M. and Ferry, J.G. (1988) Isolation and characterization of *Methanospaera cuniculi* sp. nov. Appl. Environ. Microbiol. 54, 768–771.
- [26] Miller, T.L. and Wolin, M.J. (1985) *Methanospaera stadmaniae* gen. nov. sp. nov.: a species that forms methane by reducing methanol with hydrogen. Arch. Microbiol. 141, 116–122.
- [27] Stetter, K.O., Thomm, M., Winter, J., Wildgruber, G., Huber, H., Zillig, W., Janecovic, D., König, H., Palm, P. and Wunderl, S. (1981) *Methanothermus fervidus*, sp. nov., a novel extremely thermophilic methanogen isolated from an Icelandic hot spring. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C 2, 166–178.
- [28] Lauerer, G., Kristjansson, J.K., Langworthy, T.A., König, H. and Stetter, K.O. (1986) *Methanothermus sociabilis* sp. nov., a second species within the *Methanothermaceae* growing at 97°C. Syst. Appl. Microbiol. 8, 100–105.
- [29] Schmid, K., Thomm, M., Laminet, A., Lave, F.G., Kessler, C., Stetter, K.O. and Schmitt, R. (1984) Three new restriction endonucleases Mae I, Mae II, and Mae III from *Methanococcus aeolicus*. Nucl. Acid Res. 12, 2619–2628.
- [30] Corder, R.E., Hook, L.A., Larkin, J.M. and Frea, J.I. (1983) Isolation and characterization of two new methane-producing cocci: *Methanogenium olentangyi*, sp. nov., and *Methanococcus deltae*, sp. nov. Arch. Microbiol. 134, 28–32.
- [31] Zhilina, T.N. (1983) New obligate halophilic methane-producing bacterium. Mikrobiologiya, English translation 52, 290–297.
- [32] Jones, W.J., Leigh, J.A., Mayer, F., Woese, C.R. and Wolfe, R.S. (1983) *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. Arch. Microbiol. 136, 254–261.
- [33] Jones, W.J., Paynter, M.J.B. and Gupta, R. (1983) Characterization of *Methanococcus maripaludis* sp. nov., a new methanogen isolated from salt marsh sediment. Arch. Microbiol. 135, 91–97.
- [34] Huber, H., Thomm, M., König, H., Thies, G. and Stetter, K.O. (1982) *Methanococcus thermolithotrophicus*, a novel thermophilic lithotrophic methanogen. Arch. Microbiol. 132, 47–50.
- [35] Stadtman, T.C. and Barker, H.A. (1951) Studies on the methane fermentation. X. A new formate-decomposing bacterium, *Methanococcus vannielii*. J. Bacteriol. 62, 269–280.
- [36] Paynter, M.J.B. and Hungate, R.E. (1968) Characterization of *Methanomicrobium mobilis*, sp. nov., isolated from the bovine rumen. J. Bacteriol. 95, 1943–1951.
- [37] Rivard, C.J., Henson, M.V. and Smith, P.H. (1983) Isolation and characterization of *Methanomicrobium paynteri* sp. nov., a mesophilic methanogen isolated from marine sediments. Appl. Environ. Microbiol. 46, 484–490.
- [38] Zellner, G., Messner, P., Kneifel, H., Tindall, B.J., Winter, J. and Stackebrandt, E. (1989) *Methanolacinia* gen. nov., incorporating *Methanomicrobium paynteri* as *Methanolacinia paynteri* comb. nov. J. Gen. Appl. Microbiol. 35, 185–202.
- [39] Ferry, J.G., Smith, P.H. and Wolfe, R.S. (1974) *Methanospirillum*, a new genus of methanogenic bacteria, and characterization of *Methanospirillum hungatei* sp. nov. Int. J. Syst. Bacteriol. 24, 465–469.
- [40] Widdel, F. (1986) Growth of methanogenic bacteria in pure culture with 2-propanol and other alcohols as hydrogen donors. Appl. Environ. Microbiol. 51, 1056–1062.
- [41] Maestrojuan, G.M., Xun, L., Boone, D.R., Zhang, L. and Mah, R.A. (1990), in press.
- [42] Ollivier, B., Mah, R.A., Garcia, J.L. and Boone, D.R. (1986) Isolation and characterization of *Methanogenium bourgense* sp. nov. Int. J. Syst. Bacteriol. 36, 297–301.
- [43] Romesser, J.A., Wolfe, R.S., Mayer, F., Spiess, E. and Walther-Mauruschat, A. (1979) *Methanogenium*, a new genus of marine methanogenic bacteria, and characterization of *Methanogenium cariaci* sp. nov., and *Methanogenium marisnigri* sp. nov. Arch. Microbiol. 121, 147–153.
- [44] Harris, J.E., Pinn, P.A. and Davis, R.P. (1984) Isolation and characterization of a novel thermophilic, freshwater methanogen. Appl. Environ. Microbiol. 48, 1123–1128.
- [45] Zellner, G., Sleytr, U.B., Messner, P., Kneifel, H. and Winter, J. (1990) *Methanogenium liminatans* spec. nov., a new coccoid, mesophilic methanogen able to oxidize secondary alcohols. Arch. Microbiol. 153, 287–293.
- [46] Widdel, F., Rouviere, P.E. and Wolfe, R.S. (1988) Classification of secondary alcohol-utilizing methanogens including a new thermophilic isolate. Arch. Microbiol. 150, 477–481.

- [47] Zabel, H.P., König, H. and Winter, J. (1984) Isolation and characterization of a new coccoid methanogen, *Methanogenium tatii* spec. nov. from a solfataric field on Mount Tatio. Arch. Microbiol. 137, 308–315.
- [48] Rivard, C.J. and Smith, P.H. (1982) Isolation and characterization of a thermophilic marine methanogenic bacterium, *Methanogenium thermophilicum* sp. nov. Int. J. Syst. Bacteriol. 32, 430–436.
- [49] Zabel, H.P., König, H. and Winter, J. (1985) Emended description of *Methanogenium thermophilicum*. Rivard and Smith, and assignment of new isolates to this species. Syst. Appl. Microbiol. 6, 72–78.
- [50] Xun, L., Boone, D.R. and Mah, R.A. (1989) 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. Int. J. Syst. Bacteriol. 39, 109–111.
- [51] Ollivier, B., Mah, R.A., Garcia, J.L. and Robinson, R. (1985) Isolation and characterization of *Methanogenium aggregans* sp. nov. Int. J. Syst. Bacteriol. 35, 127–130.
- [52] Zellner, G., Stackebrandt, E., Messner, P., Tindall, B.J., Conway de Macario, E., Knelfel, H. and Sleytr, U.B. (1989) *Methanocorpusculaceae* fam. nov., represented by *Methanocorpusculum parvum*, *Methanocorpusculum sinense* spec. nov. and *Methanocorpusculum bavarium* spec. nov. Arch. Microbiol. 151, 381–390.
- [53] Zhao, Y., Boone, D.R., Mah, R.A., Boone, J.E. and Xun, L. (1989) Isolation and characterization of *Methanocorpusculum labreanum* sp. nov. from the LaBrea Tar Pits. Int. J. Syst. Bacteriol. 39, 10–13.
- [54] Zellner, G., Alten, C., Stackebrandt, E., Conway de Macario, E. and Winter, J. (1987) Isolation and characterization of *Methanocorpusculum parvum*, gen. nov., spec. nov., a new tungsten requiring, coccoid methanogen. Arch. Microbiol. 147, 13–20.
- [55] Van Bruggen, J.J.A., Zwart, K.B., Hermans, J.G.F., Van Hove, E.M., Stumm, C.K. and Vogels, G.D. (1986) Isolation and characterization of *Methanoplanus endosymbiosus* sp. nov., an endosymbiont of the marine sapropelic ciliate *Metopus contortus* Quennerstedt. Arch. Microbiol. 144, 367–374.
- [56] Tu, J., Prangishvili, D., Huber, H., Wildgruber, G., Zillig, W. and Stetter, K.O. (1982) Taxonomic relations between archaeobacteria including 6 novel genera examined by cross hybridization of DNAs and 16S rRNAs. J. Mol. Evol. 18, 109–114.
- [57] Wildgruber, G., Thomm, M., König, H., Ober, K., Ricchiuto, T. and Stetter, K.O. (1982) *Methanoplanus limicola*, a plate shaped methanogen representing a novel family, the *Methanoplanaceae*. Arch. Microbiol. 132, 31–36.
- [58] Sowers, K.R., Baron, S.F. and Ferry, J.G. (1984) *Methanosarcina acetivorans* sp. nov., an acetotrophic methane-producing bacterium isolated from marine sediments. Appl. Environ. Microbiol. 47, 971–978.
- [59] Nakatsugawa, N. and Horikoshi, J.P. (1989) Alkaliphilic, methanogenic bacteria (*Methanosarcina alkaliphilum*) and fermentation method for the fast production of methane. Research Development Corporation of Japan, No. 33134, EP.
- [60] Bryant, M.P. and Boone, D.R. (1987) Emended description of strain MS^T (DSM 800^T), the type strain of *Methanosarcina barkeri*. Int. J. Syst. Bacteriol. 37, 169–170.
- [61] Blotevogel, K.H., Fischer, U. and Lüpkes, K.H. (1986) *Methanococcus frisius* sp. nov., a new methylotrophic marine methanogen. Can. J. Microbiol. 32, 127–131.
- [62] Blotevogel, K.H. and Fischer, U. (1989) Transfer of *Methanococcus frisius* to the genus *Methanosarcina* as *Methanosarcina frisiana* comb. nov. Int. J. Syst. Bacteriol. 39, 91–92.
- [63] Mah, R.A. and Kuhn, D.A. (1984) Transfer of the type species of the genus *Methanococcus* to the genus *Methanosarcina*, naming it *Methanosarcina mazei* (Barker 1936) comb. nov. et emend. and conservation of the genus *Methanococcus* (Approved lists 1980) with *Methanococcus vannielii* (Approved lists 1980) as the type species. Int. J. Syst. Bacteriol. 34, 263–265.
- [64] Zinder, S.H. and Mah, R.A. (1979) Isolation and characterization of a thermophilic strain of *Methanosarcina* unable to use H₂-CO₂ for methanogenesis. Appl. Environ. Microbiol. 38, 996–1008.
- [65] Zinder, S.H., Sowers, K.R. and Ferry, J.G. (1985) *Methanosarcina thermophila* sp. nov., a thermophilic, acetotrophic methane-producing bacterium. Int. J. Syst. Bacteriol. 35, 522–523.
- [66] Zhilina, T.N. and Zavarzin, G.A. (1987) *Methanosarcina vacuolata* - vacuolated species of methanosarcinae. Int. J. Syst. Bacteriol. 37, 281–283.
- [67] Stetter, K.O. (1989) Genus *Methanobolus*, in Bergey's Manual of Systematic Bacteriology (Staley, J.T., Bryant, M.P., Pfennig, N. and Holt, J.G., Eds.), Vol. 3, pp. 2205–2207, Williams & Wilkins, Baltimore.
- [68] König, H. and Stetter, K.O. (1982) Isolation and characterization of *Methanobolus tindarius* sp. nov., a coccoid methanogen growing only on methanol and methylamines. Zentralbl. Bakteriologie. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C 3, 478–480.
- [69] Obratsova, A.Y., Shipin, O.V., Bezrukova, L.V. and Belyaev, S.S. (1987) Properties of the coccoid methylotrophic methanogen, *Methanococcoides euhalobius* sp. nov. Microbiology, English translation 56, 523–527.
- [70] Sowers, K.R. and Ferry, J.G. (1983) Isolation and characterization of a methylotrophic marine methanogen, *Methanococcoides methylutens* gen. nov., sp. nov. Appl. Environ. Microbiol. 45, 684–690.
- [71] Paterek, J.R. and Smith, P.H. (1988) *Methanohalophilus mahii* gen. nov. sp. nov., a methylotrophic halophilic methanogen. Int. J. Syst. Bacteriol. 38, 122–123.
- [72] Mathrani, I.M., Boone, D.R., Mah, R.A., Fox, G.E. and Lau, P.P. (1988) *Methanohalophilus zhilinae* sp. nov., an alkaliphilic, halophilic, methylotrophic methanogen. Int. J. Syst. Bacteriol. 38, 139–142.

- [73] Zhilina, T.N. and Zavarzin, G.A. (1987) *Methanohalobium evestigatus*, n. gen., n. sp., the extremely halophilic methanogenic Archaeobacterium. Dokl. Akad. Nauk. SSSR 293, 464-468.
- [74] Nakasugawa, N. and Horikoshi, J.P. (1989) Extremely halophilic methanogenic archaeobacteria and process for the production of methane. Research Development Corporation of Japan, No. 313900, EP.
- [75] Yu, I.K. and Kawamura, F. (1987) *Halomethanococcus doii* gen. nov., sp. nov.: an obligately halophilic methanogenic bacterium from solar salt ponds. J. Gen. Appl. Microbiol. 33, 303-310.
- [76] Touzel, J.P., Prensier, G., Roustan, J.L., Thomas, I., Dubourguier, H.C. and Albagnac, G. (1988) Description of a new strain of *Methanotherix soehngenii* and rejection of *Methanotherix concilii* as a synonym of *Methanotherix soehngenii*. Int. J. Syst. Bacteriol. 38, 30-36.
- [77] Macario, A.J.L. and Conway de Macario, E. (1987) Antigenic distinctiveness, heterogeneity, and relationships of *Methanotherix* spp. J. Bacteriol. 169, 4099-4103.
- [78] Patel, G.B. (1984) Characterization and nutritional properties of *Methanotherix concilii* sp. nov., a mesophilic, acetoclastic methanogen. Can. J. Microbiol. 30, 1383-1396.
- [79] Patel, G.B. and Sprott, G.D. (1990) *Methanosaeta concilii* gen. nov., sp. nov. ("*Methanotherix concilii*") and *Methanosaeta thermoacetophila* nom. rev., comb. nov. Int. J. Syst. Bacteriol. 40, 79-82.
- [80] Nozhevnikova, A.N. and Chudina, V.I. (1985) Morphology of the thermophilic acetate methane bacterium *Methanotherix thermoacetophila* sp. nov. Microbiology 51, 534-541.
- [81] Huser, B.A., Wuhrmann, K. and Zehnder, A.J.B. (1982) *Methanotherix soehngenii* gen. nov. sp. nov., a new acetotrophic non-hydrogen-oxidizing methane bacterium. Arch. Microbiol. 132, 1-9.
- [82] Huber, R., Kurr, M., Jannasch, H.W. and Stetter, K.O. (1989) A novel group of abyssal methanogenic archaeobacteria (*Methanopyrus*) growing at 110°C. Nature 342, 833.
- [83] Ahring, B.K. and Westermann, P. (1985) Methanogenesis from acetate: physiology of a thermophilic, acetate-utilizing methanogenic bacterium. FEMS Microbiol. Lett. 28, 15-19.
- [84] Rose, C.S. and Pirt, S.J. (1981) Conversion of glucose and fatty acids to methane: roles of two mycoplasmal agents. J. Bacteriol. 147, 248-254.
- [85] Ianotti, E.L., Kafkewitz, P., Wolin, M.J. and Bryant, M.P. (1973) Glucose fermentation products of *Ruminococcus albus* grown in continuous culture with *Vibrio succinogenes*: changes caused by interspecies transfer of H₂. J. Bacteriol. 114, 1231-1240.
- [86] Thiel, J.H. and Zeikus, J.G. (1988) Control of interspecies electron flow during anaerobic digestion: significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. Appl. Environ. Microbiol. 54, 20-29.
- [87] Platen, H. and Schink, B. (1987) Methanogenic degradation of acetone by an enrichment culture. Arch. Microbiol. 149, 136-141.
- [88] Balderston, W.L. and Payne, W.J. (1976) Inhibition of methanogenesis in salt marsh sediments and whole-cell suspensions of methanogenic bacteria by nitrogen oxides. Appl. Environ. Microbiol. 32, 264-269.
- [89] Ward, D.M. and Winfrey, M.R. (1985) Interactions between methanogenic and sulfate-reducing bacteria in sediments. Adv. Aquat. Microbiol. 3, 141-179.
- [90] Winfrey, M.R. and Zeikus, J.G. (1977) Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. Appl. Environ. Microbiol. 33, 275-281.
- [91] Abram, J.W. and Nedwell, D.B. (1978) Inhibition of methanogenesis by sulfate reducing bacteria competing for transferred hydrogen. Arch. Microbiol. 117, 89-92.
- [92] Cord-Ruwisch, R., Seitz, H.J. and Conrad, R. (1988) The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on the redox potential of the terminal electron acceptor. Arch. Microbiol. 149, 350-357.
- [93] Oremland, R.S. and Polcin, S. (1982) Methanogenesis and sulfate-reduction: competitive and noncompetitive substrate in estuarine sediments. Appl. Environ. Microbiol. 44, 1270-1276.
- [94] King, G.M. (1984) Metabolism of trimethylamine, choline, and glycine betaine by sulfate-reducing and methanogenic bacteria in marine sediments. Appl. Environ. Microbiol. 48, 719-725.
- [95] Raimbault, M., Rinaudo, G., Garcia, J.L. and Boureau, M. (1977) A device to study metabolic gases in the rice rhizosphere. Soil Biol. Biochem. 9, 193-196.
- [96] Holzapfel-Pschorn, A., Conrad, R. and Seiler, W. (1985) Production, oxidation and emission of methane in rice paddies. FEMS Microbiol. Ecol. 31, 343-351.
- [97] Garcia, J.L., Raimbault, M., Jacq, V., Rinaudo, G. and Roger, P. (1974) Activités microbiennes dans les sols de rizières du Sénégal: relations avec les propriétés physico-chimiques et influence de la rhizosphère. Rev. Ecol. Biol. Sol. 11, 169-185.
- [98] Rajagopal, B.S., Belay, N. and Daniels, L. (1988) Isolation and characterization of methanogenic bacteria from rice paddies. FEMS Microbiol. Ecol. 53, 153-158.
- [99] Cappenberg, T.E. (1974) Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a fresh-water lake. I. Field observation. Anton. Leeuwenhoek J. Microbiol. Serol. 40, 285-295.
- [100] Cappenberg, T.E. and Prins, R.A. (1974) Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a fresh-water lake. III. Experiments with ¹⁴C-labelled substrates. Anton. Leeuwenhoek J. Microbiol. Serol. 40, 457-469.
- [101] Oremland, R.S. and Taylor, B.F. (1978) Sulfate reduction and methanogenesis in marine sediments. Geochim. Cosmochim. Acta 42, 209-214.
- [102] Strayer, R.F. and Tiedje, J.M. (1978) Kinetic parameters

- of the conversion of methane precursors to methane in hypereutrophic lake sediment. *Appl. Environ. Microbiol.* 36, 330-340.
- [103] Conrad, R., Phelps, T.J. and Zeikus, J.G. (1985) Gas metabolism evidence in support of the juxtaposition of hydrogen-producing and methanogenic bacteria in sewage sludge and lake sediments. *Appl. Environ. Microbiol.* 50, 595-601.
- [104] Fielding, E.R., Archer, D.B., Conway de Macario, E. and Macario, A.J.L. (1988) Isolation and characterization of methanogenic bacteria from landfills. *Appl. Environ. Microbiol.* 54, 835-836.
- [105] Siebert, M.L., Toerien, D.F. and Hattingh, W.H.J. (1967) Estimation of methane-producing bacterial count by the most probable number (MPN) technique. *Water Res.* 1, 13-19.
- [106] Hobson, P.N. and Shaw, B.G. (1973) The bacterial population of piggery-waste anaerobic digesters. *Water Res.* 8, 507-516.
- [107] Ianotti, E.L., Fischer, J.R. and Sievers, D.M. (1978) Medium for the enumerations and isolation of bacteria from a swine waste digester. *Appl. Environ. Microbiol.* 36, 555-566.
- [108] Labat, M. and Garcia, J.L. (1986) Study on the development of methanogenic microflora during anaerobic digestion of sugar beet pulp. *Appl. Microbiol. Biotechnol.* 25, 163-168.
- [109] Dolfig, J. and Bloemen, W.G.B.M. (1985) Activity measurements as a tool to characterize the microbial composition of methanogenic environments. *J. Microbiol. Methods* 4, 1-12.
- [110] Archer, D.B. (1984) Detection and quantitation of methanogens by enzyme-linked immunosorbent assay. *Appl. Environ. Microbiol.* 48, 797-801.
- [111] Macario, A.J.L. and Conway de Macario, E. (1988) Quantitative immunologic analysis of the methanogenic flora of digestors reveals a considerable diversity. *Appl. Environ. Microbiol.* 54, 79-86.
- [112] King, G.M. (1988) Methanogenesis from methylated amines in a hypersaline algal mat. *Appl. Environ. Microbiol.* 54, 130-136.
- [113] Hungate, R.E., Smith, W., Bauchop, T., Yu, I. and Rabinowitz, J.C. (1970) Formate as an intermediate in the bovine rumen fermentation. *J. Bacteriol.* 102, 389-397.
- [114] Miller, T.L., Wolin, M.J., Hongxue, Z. and Bryant, M.P. (1986) Characteristics of methanogens isolated from bovine rumen. *Appl. Environ. Microbiol.* 51, 201-202.
- [115] Miller, T.L. and Wolin, M.J. (1986) Methanogens in human and animal intestinal tracts. *System. Appl. Microbiol.* 7, 223-229.
- [116] Miller, T.L., Wolin, M.J. and Kusel, E.A. (1986) Isolation and characterization of methanogens from animal feces. *System. Appl. Microbiol.* 8, 234-238.
- [117] Belay, N., Johnson, R., Rajagopal, B.S., Conway de Macario, E. and Daniels, L. (1988) Methanogenic bacteria from human dental plaque. *Appl. Environ. Microbiol.* 54, 600-603.
- [118] Brauman, A. (1989) Etude du métabolisme bactérien de termites supérieurs à régimes alimentaires différenciés. Mise en évidence d'une nouvelle voie de dégradation du benzoate et du 3-hydroxybenzoate. Thèse Université d'Aix-Marseille II.
- [119] Zimmerman, P.R., Greenberg, J.P., Wandiga, S.O. and Crutzen, P.J. (1982) Termite: a potentially large source of atmosphere methane, carbon dioxide, and molecular hydrogen. *Science* 218, 563-565.
- [120] Rasmussen, R.A. and Khalil, M. (1983) Global production of methane by termites. *Nature (Lond.)* 301, 700-702.
- [121] Schink, B., Ward, J.C. and Zeikus, J.G. (1981) Microbiology of wetwood: importance of pectin degradation and *Clostridium* species in living trees. *Appl. Environ. Microbiol.* 42, 526-532.
- [122] Van Bruggen, J.J.A., Stumm, C.K., Zwart, K.B. and Vogels, G.D. (1985) Endosymbiotic methanogenic bacteria of the sapropelic amoeba *Mastigella*. *FEMS Microbiol. Ecol.* 31, 187-192.
- [123] Lee, M.J., Schreurs, P.J., Messer, A.C. and Zinder, S.H. (1987) Association of methanogenic bacteria with flagellated protozoa from a termite hindgut. *Curr. Microbiol.* 15, 337-341.
- [124] Oremland, R.S. (1979) Methanogenic activity in plankton samples and fish intestines: a mechanism for in situ methanogenesis in oceanic surface waters. *Limnol. Oceanogr.* 24, 1136-1141.
- [125] Herwig, R.P., Staley, J.T., Nerini, M.K. and Braham, H.W. (1984) Baleen whales: preliminary evidence for forestomach microbial fermentation. *Appl. Environ. Microbiol.* 47, 421-423.
- [126] Khalil, M.A.K. and Rasmussen, R.A. (1982) Secular trends of atmospheric methane (CH₄). *Chemosphere* 11, 877-883.
- [127] Levy, H. (1973) Tropospheric budgets for methane, carbon monoxide, and related species. *J. Geophys. Res.* 78, 5325-5332.
- [128] Owens, A.J., Steed, J.M., Filkin, D.L., Miller, C. and Jesson, J.P. (1982) The potential effects of increased methane on atmospheric ozone. *Geophys. Res. Lett.* 9, 1105-1108.
- [129] Khalil, M.A.K. and Rasmussen, R.A. (1983) Sources, sinks and cycles of atmospheric methane. *J. Geophys. Res.* 88, 5131-5144.