Part 7

The Microbes

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42 Taxonomy of Methanogens

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Abstract: Methanogens are strictly anaerobic, methane-producing Archaea. They all belong to the phylum *Euryarchaeota*. Although methanogens share a set of physiological characteristics, they are phylogenetically very diverse. The current taxonomy classifies methanogens into five well-established orders: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, and *Methanopyrales*. This taxonomy is supported by 16S rRNA gene sequences as well as a number of physiological properties, e.g., substrates for methanogenesis, nutritional requirements, morphologies, and structures of cell envelopes. The 16S rRNA gene sequence analysis of the strain SANAE, a representative of the clone lineage Rice Cluster I, suggests that it represents a novel order of methanogens, *Methanocellales*. Methanogens are abundant in a wide variety of anaerobic environments where they catalyze the terminal step in the anaerobic food chain by converting methanogenic substrates into methane. The complexity of methanogenesis pathways suggests an ancient monophyletic origin of methanogens, a hypothesis, which is supported by phylogenetic analyses based upon DNA sequences.

1 Introduction

Methanogens are microorganisms that produce methane as the end-product of their anaerobic respiration. All methanogens share three common features. (1) They are obligate methane producers, obtaining all or most of their energy for growth from producing large quantities of methane. (2) They are archaea, belonging to the phylum *Euryarchaeota*. (3) They are strict anaerobes, limiting their growth to anaerobic environments.

Methanogens can only utilize a restricted number of substrates for methane production or methanogenesis. The substrates are limited to three major types: CO_2 + H_2 or a few other electron donors such as formate, methyl-group-containing compounds, and acetate. Methanogens using these three types of substrates are classified as hydrogenotrophs, methylotrophs, and acetotrophs, respectively. Most organic substances, for instance, carbohydrates, proteins, and long-chain fatty acids and alcohols, are not substrates for methanogenesis. Exceptions are that some hydrogenotrophs can also use secondary alcohols, such as 2-propanol, 2-butanol, and cyclopentanol, as electron donors. A small number can use ethanol (Bleicher et al., 1989; Frimmer and Widdel, 1989; Widdel, 1986; Widdel et al., 1988). However, even these organic compounds, which can obviously be assimilated, are only incompletely oxidized to ketones (secondary alcohols) and acetate (ethanol), and methane is derived from CO_2 reduction.

Methanogenesis is a complex process that requires a number of unique enzyme complexes and unusual coenzymes (reviewed by Hedderich and Whitman, 2006). Although the methanogenesis pathways of the three nutritional groups start differently, the final steps leading to methane are common in virtually all methanogens. The bioenergetics of methanogenesis employs both proton and sodium gradients generated by primary pumps for ATP synthesis. Due to the complexity of methanogenesis, all common methanogens are expected to originate from an ancient ancestor.

2 Taxonomy of Methanogens

Although methanogens are united by a few common features, they are phylogenetically diverse. The taxonomy of methanogens that has been developed in the last 3 decades has aimed to reflect the phylogenetic diversity of methanogens and be consistent with the taxonomy

of other prokaryotes (Balch et al., 1979; Boone et al., 1993; Whitman et al., 2001). An overview of the current taxonomy of methanogens is given in *Table 1*. Organisms from different orders have less than 82% 16S rRNA sequence similarity. Organisms with less than 88–93% and less than 93–95% 16S rRNA sequence similarity are separated into different families and genera, respectively. Organisms are distinguished as separate species if their DNA reassociation is less than 70%, the change in the melting temperature of their hybrid DNA is greater than 5°C, and substantial phenotypic differences exist (Stackebrandt et al., 2002; Wayne et al., 1987). When 16S rRNA data are available, organisms with a similarity of less than 98% are considered as separate species. However, sequence similarity of greater than 98% is not considered as sufficient evidence that two organisms belong to the same species.

Methanogens are currently classified into five orders: *Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales*, and *Methanopyrales* (Whitman et al., 2001, 2006). This taxonomy is supported by comparative 16S rRNA gene sequence analyses as well as distinctive phenotypic properties, such as different cell envelope structure, lipid composition, and substrate range. Some representative characteristics are listed in *Table 2* and further described in following chapters.

A novel order of methanogen, *Methanocellales*, was proposed recently (Sakai et al., 2008). This order is currently represented by a single strain, *Methanocella paludicola* strain SANAE, which was originally isolated from rice paddy soil (Sakai et al., 2007). Based on comparative 16S rRNA gene sequence analysis, strain SANAE is affiliated with Rice Cluster I (RC-I), a clone lineage within the radiation of *Methanosarcinales* and *Methanomicrobiales* (review in Conrad et al., 2006). The 16S rRNA gene sequence similarities between strain SANAE and members of *Methanosarcinales* range from 80% to 82.8%, and those between the strain and members of *Methanomicrobiales* range from 77.5% to 82.4% (Sakai et al., 2008). This novel strain utilizes $H_2 + CO_2$ or formate as methanogenesis substrates. The cells are nonmotile and rod shaped. The growth temperature ranges from 25°C to 40°C, with an optimum of 35–37°C. The pH for growth ranges from 6.5 to 7.8, with an optimum of 7.0. The salinity for growth ranges from 0% to 0.1% (w/v) NaCl. It is physiologically distinguished from members of *Methanosarcinales*, which can use acetate and methylated compounds for methanogenesis. However, the phenotypic distinction between strain SANAE and members of *Methanomicrobiales* needs further investigation.

3 Phylogeny of Methanogens

All modern methanogens share the same set of homologous enzymes and cofactors required for methanogenesis, suggesting an ancient monophyletic origin of methanogens. In the phylogenetic tree based on 16S rRNA gene sequences, methanogens are separated into two major groups (*Fig. 1*). The Class I methanogens include *Methanobacteriales*, *Methanococcales*, and *Methanopyrales*, and the Class II methanogens include *Methanomicrobiales* and *Methanosarcinales* (Bapteste et al., 2005). Non-methanogenic lineages, *Archaeoglobales*, *Halobacteriales*, and *Thermoplasmatales*, are interspersed in the tree. Three hypotheses are proposed to explain this branching of methanogens.

1. Methanogens and these non-methanogen lineages shared a common ancestor, and genes required for methanogenesis were lost in these non-methanogens. This hypothesis is supported by the presence of a few genes encoding methanogenesis enzymes in the genome of *Archaeoglobus fulgidus* but is challenged by aerobic growth in both the *Halobacteriales*

Table 1

Taxonomy of methanogens

Order	Family	Genus	Species[a]
Methanobacteriales	*Methanobacteriaceae*	*Methanobacterium*	*M. aarhusense, M. alcaliphilum, M. beijingense, M. bryantii, M. congolense, M. espanolae,* ***M. formicicum****, M. ivanovii, M. oryzae, M. palustre, M. subterraneum, M. uliginosum*
		Methanobrevibacter	*M. acididurans, M. arboriphilus, M. curvatus, M. cuticularis, M. filiformis, M. gottschalkii, M. millerae, M. olleyae, M. oralis,* ***M. ruminantium****, M. smithii, M. thaueri, M. woesei, M. wolinii*
		Methanosphaera	*M. cuniculi,* ***M. stadtmanae***
		Methanothermobacter	*M. defluvii, M. marburgensis,* ***M. thermoautotrophicus****, M. thermoflexus, M. thermophilus, M. wolfeii*
	Methanothermaceae	*Methanothermus*	***M. fervidus****, M. sociabilis*
Methanococcales	*Methanococcaceae*	*Methanococcus*	*M. aeolicus, M. maripaludis,* ***M. vannielii****, M. voltae*
		Methanothermococcus	*M. okinawensis,* ***M. thermolithotrophicus***
	Methanocaldococcaceae	*Methanocaldococcus*	*M. fervens, M. indicus, M. infernus,* ***M. jannaschii****, M. vulcanius*
		Methanotorris	*M. formicicus,* ***M. igneus***
Methanomicrobiales	*Methanomicrobiaceae*	*Methanoculleus*	***M. bourgensis****, M. chikugoensis, M. marisnigri, M. palmolei, M. submarinus, M. thermophilus*
		Methanofollis	*M. aquaemaris, M. formosanus, M. liminatans,* ***M. tationis***
		Methanogenium	***M. cariaci,*** *M. frigidum, M. marinum, M. organophilum*
		Methanolacinia	***M. paynteri***
		Methanomicrobium	***M. mobile***
		Methanoplanus	*M. endosymbiosus,* ***M. limicola****, M. petrolearius*
	Methanospirillaceae	*Methanospirillum*	***M. hungatei***

	Methanocorpusculaceae	*Methanocorpusculum*	*M. bavaricum, M. labreanum,* ***M. parvum****, M. sinense*
		Methanocalculus[b]	*M. chunghsingensis,* ***M. halotolerans****, M. pumilus, M. taiwanensis*
	Unclassified	*Methanolinea*	*M. tarda*
		Candidatus Methanoregula	M. boonei
		Candidatus Methanosphaerula	M. palustris
Methanosarcinales	*Methanosarcinaceae*	*Methanosarcina*	*M. acetivorans, M. baltica,* ***M. barkeri****, M. lacustris, M. mazei, M. semesiae, M. siciliae, M. thermophila, M. vacuolata*
		Methanococcoides	*M. alaskense, M. burtonii,* ***M. methylutens***
		Methanohalobium	*M. evestigatum*
		Methanohalophilus	*M. halophilus,* ***M. mahii****, M. portucalensis*
		Methanolobus	*M. bombayensis, M. oregonensis, M. taylorii,* ***M. tindarius****, M. vulcani*
		Methanomethylovorans	***M. hollandica****, M. thermophila*
		Methanimicrococcus[b]	***M. blatticola***
		Methanosalsum	***M. zhilinae***
	Methanosaetaceae	*Methanosaeta*	***M. concilii,*** *M. harundinacea, M. thermophila*
	Methermicoccaceae	*Methermicoccus*	***M. shengliensis***
Methanopyrales	*Methanopyraceae*	*Methanopyrus*	***M. kandleri***
Methanocellales	*Methanocellaceae*	*Methanocella*	***M. paludicola***

[a]Type species of the genera are in bold
[b]Placement in higher taxon is tentative

Table 2

Some characteristics of the methanogen orders

Order	Shape	Methanogenesis substrates[a]	Motility	Cell wall	Cellular lipids[b]	
					Core lipids	Polar lipids
Methanobacteriales	Rods, cocci	$H_2 + CO_2$ (formate, CO, methanol, secondary alcohols)	−[c]	Pseudomurein, protein	Caldarchaeol, archaeol, hydroxyarchaeol	Glucose, *N*-acetylglucosamine, *myo*-inositol, ethanolamine, serine
Methanococcales	Cocci	$H_2 + CO_2$, formate	+	Protein	Archaeol, caldarchaeol, hydroxyarchaeol, macrocyclic archaeol	Glucose, *N*-acetylglucosamine, serine, ethanolamine
Methanomicrobiales	Cocci, rods, spirals, sheathed rods	$H_2 + CO_2$, formate (secondary alcohols)	+/−	Protein, glycoprotein	Archaeol, caldarchaeol	Glucose, galactose, aminopentanetetrol, glycerol
Methanosarcinales	Pseudosarcina, cocci, sheathed rods	Methanol, methylamine, acetate ($H_2 + CO_2$)	−	Protein, glycoprotein	Archaeol, hydroxyarchaeol, caldarchaeol	Glucose, galactose, mannose, *myo*-inositol, ethanolamine, serine, glycerol
Methanopyrales	Rods	$H_2 + CO_2$	+	Pseudomurein	Archaeol	nd
Methanocellales	Rods	$H_2 + CO_2$, formate	−	nd	nd	nd

nd, not determined

[a]Major substrates utilized for methanogenesis. Parentheses means utilized sometimes

[b]Compounds can be contained in cellular lipids, depending on the species

[c]Except the genus *Methanothermus*

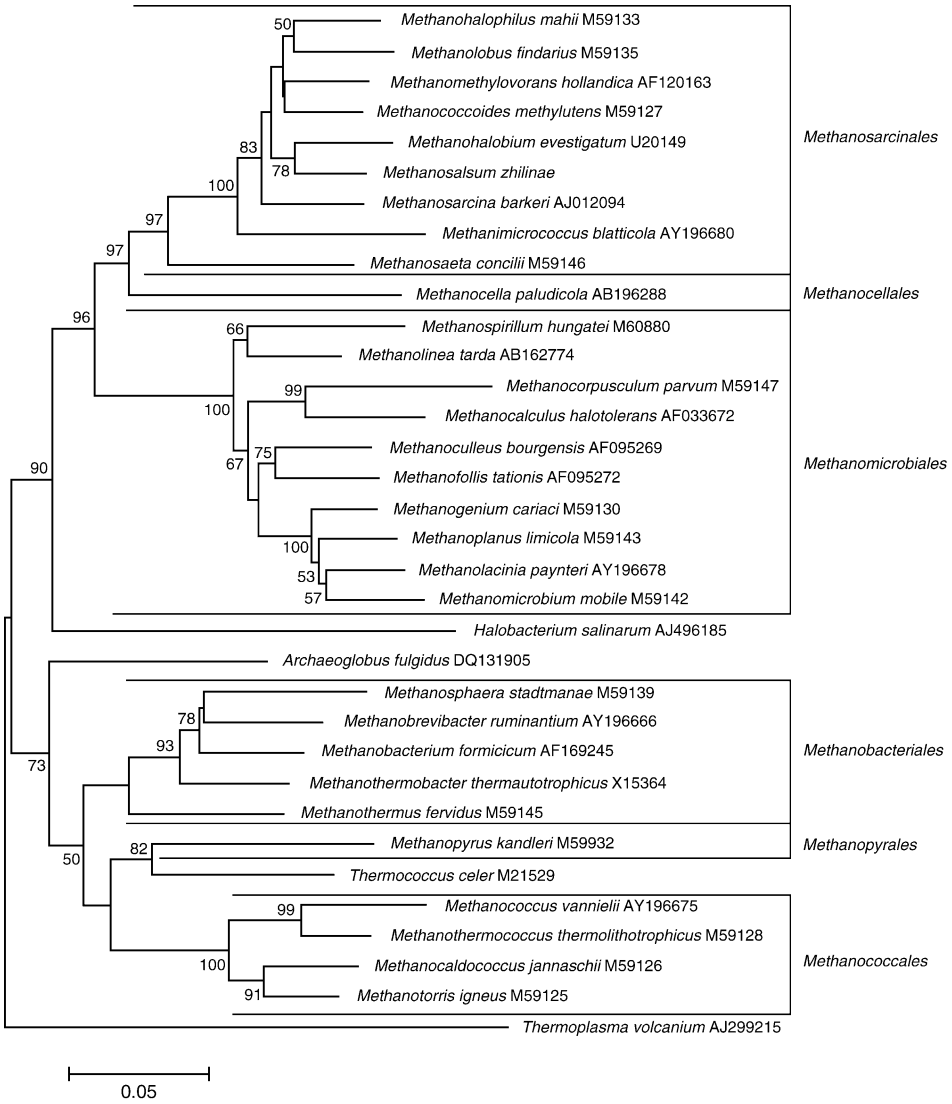

Figure 1

Phylogenetic tree for the methanogenic archaea and other euryarchaeotes based upon 16S rRNA sequences. The alignment was manually edited to include 1,251 positions. The tree was constructed with the neighbor-joining algorithm in MEGA4. Bootstrap analysis was performed with 1,000 replicates, and values greater than 50% are labeled on the nodes. The scale bar is 0.05 expected nucleotide substitutions per site. The 16S rRNA sequence of *Methanosalsum zhilinae* was according to Mathrani et al. (1988). The GenBank accession numbers for the other sequences are given following their names.

and *Thermoplasmatales*. This hypothesis also suggests that the common ancestor of *Euryarchaeota* was a methanogen (Gribaldo and Brochier-Armanet, 2006).

2. Methanogenesis in various branches was acquired by horizontal gene transfer (HGT). However, the core genes required for methanogenesis are not linked on the genomes of methanogens, thus the simultaneous acquisition via lateral transfer is unlikely, and the transfer of single genes would not confer a selective advantage (Gribaldo and Brochier-Armanet, 2006).
3. The phylogeny based on 16S rRNA gene is misleading, and methanogens and *Archaeoglobus* shared a common ancestor exclusive of all other archaea. This hypothesis is supported by a recent phylogenomics analysis showing that ten proteins are exclusively shared in methanogens and *A. fulgidus* (Gao and Gupta, 2007), while no proteins are exclusively shared in methanogens and any of the *Halobacteriales* or *Thermoplasmatales* (Gao and Gupta, 2007). Therefore, methanogens and *Archaeoglobus* appear to have a closer relationship within the *Euryarchaeota*, but this leaves open the question of the apparently paradoxical phylogenetic position of both the *Halobacteriales* and *Thermplasmatales*. Therefore, more genomic sequences of archaea are needed to prove this hypothesis.

4 Ecology of Methanogens

Methanogens are abundant in a wide variety of anaerobic habitats such as marine sediments, freshwater sediments, flooded soils, human and animal gastrointestinal tracts, anaerobic digestors, landfills, and geothermal systems (Liu and Whitman, 2008). In some natural habitats, methanogens are also present in micro-oxic environments. For example, members of *Methanobrevibacter* have been isolated from large dental caries and subgingival plaque in the human mouth and gut periphery in termites. They are also somewhat oxygen tolerant, probably due to the presence of catalase activity and the protection given by O_2-utilizing microbes (Brusa et al., 1987; Belay et al., 1988; Leadbetter and Breznak, 1996). RC-I methanogens are prevalent in rice rhizosphere, which is transiently oxic, and a reconstituted RC-I genome encodes a unique set of antioxidant enzymes, which may explain an aerotolerant life style (Erkel et al., 2006).

In methanogenic habitats, electron acceptors such as O_2, NO_3^-, Fe^{3+}, and SO_4^{2-} are limiting. When electron acceptors other than CO_2 are present, methanogens are outcompeted by the bacteria that utilize them. This phenomenon occurs mainly because the reduction of these compounds is thermodynamically more favorable than CO_2 reduction to methane. However, because CO_2 is generated during fermentations, it is seldom limiting in anaerobic environments. Besides methanogens, homoacetogens are another group of anaerobes that can reduce CO_2 for energy production. However, acetogenesis with H_2 is thermodynamically less favorable than methanogenesis. Therefore, homoacetogens do not compete well with methanogens in many habitats. However, homoacetogens outcompete methanogens in some environments, such as the hindgut of certain termites and cockroaches. Possible explanations may be their metabolic versatility as well as lower sensitivity to O_2.

5 Research Needs

Recent culture-independent studies have revealed the presence of novel phylogenetic groups of methanogens. Their isolation and characterization will shed new insight into these organisms.

For instance, investigations of rumen methanogens have found a novel lineage containing at least two families. The 16S rRNA gene sequences of this group have similarities closest to, but less than 80%, with those of *Methanosarcinales* (Nicholson et al., 2007). The Rice Cluster I is abundant in rice paddy soils, but only one strain has been isolated so far. Discovery and isolation of new strains will certainly add to our knowledge of the diversity of methanogens.

Methanogens have fewer easily determined physiological characteristics than most bacteria. Comparative 16S rRNA gene sequence analyses are indispensable for determination of taxonomic levels higher than species. However, it is frequently insufficient for taxonomy of methanogens at species and subspecies levels. For instance, some isolates of *Methanobrevibacter* have >98% 16S rRNA gene sequence similarities but exhibit less than 50% DNA relatedness, suggesting that they belong to different species (Lin and Miller, 1998; Keswani and Whitman, 2001). The discovery of novel molecular markers is desirable. The methyl-coenzyme M reductase alpha-subunit (*mcrA*) gene has been applied as a phylogenetic marker for methanogens in addition to 16S rRNA genes (Springer et al., 1995) and as a target for the detection of methanogens in a wide range of environments (Ohkuma et al., 1995; Lueders et al., 2001; Luton et al., 2002; Earl et al., 2003; Kemnitz et al., 2004). Phylogenomic analyses based upon whole-genome sequences may lead to improvement of the taxonomy and better view of phylogenetic relationships. A more complete database of methanogen genome sequences is required for this purpose.

References

Balch WE, Fox GE, Magrum LJ, Woese CR, Wolfe RS (1979) Methanogens: reevaluation of a unique biological group. Microbiol Mol Biol Rev 43: 260–296.

Bapteste E, Brochier C, Boucher Y (2005) Higher-level classification of the Archaea: evolution of methanogenesis and methanogens. Archaea 1: 353–363.

Belay N, Johnson R, Rajagopal BS, de Macario EC, Daniels L (1988) Methanogenic bacteria from human dental plaque. Appl Environ Microbiol 54: 600–603.

Bleicher K, Zellner G, Winter J (1989) Growth of methanogens on cyclopentanol/CO_2 and specificity of alcohol dehydrogenase. FEMS Microbiol Lett 59: 307–312.

Boone DR, Whitman WB, Rouviere P (1993) Diversity and taxonomy of methanogens. In Methanogenesis: Ecology, Physiology, Biochemistry and Genetics. JG Ferry (ed.). New York: Chapman & Hall, pp. 35–80.

Brusa T, Conca R, Ferrara A, Ferrari A, Pecchioni A (1987) The presence of methanobacteria in human subgingival plaque. J Clin Periodontol 14: 470–471.

Conrad R, Erkel C, Liesack W (2006) Rice Cluster I methanogens, an important group of Archaea producing greenhouse gas in soil. Curr Opin Biotechnol 17: 262–267.

Earl J, Hall G, Pickup RW, Ritchie DA, Edwards C (2003) Analysis of methanogen diversity in a hypereutrophic lake using PCR-RFLP analysis of *mcr* sequences. Microb Ecol 46: 270–278.

Erkel C, Kube M, Reinhardt R, Liesack W (2006) Genome of Rice Cluster I archaea – the key methane producers in the rice rhizosphere. Science 313: 370–372.

Frimmer U, Widdel F (1989) Oxidation of ethanol by methanogenic bacteria. Arch Microbiol 152: 479–483.

Gao B, Gupta R (2007) Phylogenomic analysis of proteins that are distinctive of Archaea and its main subgroups and the origin of methanogenesis. BMC Genomics 8: 86.

Gribaldo S, Brochier-Armanet C (2006) The origin and evolution of Archaea: a state of the art. Phil Trans R Soc B 361: 1007–1022.

Hedderich R, Whitman WB (2006) Physiology and biochemistry of the methane-producing Archaea. In The Prokaryotes, M Dworkin, S Falkow, E Rosenberg, K-H Schleifer, E Stackebrandt (eds.). 3rd edn. New York: Springer-Verlag.

Kemnitz D, Chin K-J, Bodelier P, Ralf Conrad (2004) Community analysis of methanogenic archaea within a riparian flooding gradient. Environ Microbiol 6: 449–461.

Keswani J, Whitman W (2001) Relationship of 16S rRNA sequence similarity to DNA hybridization in prokaryotes. Int J Syst Evol Microbiol 51: 667–678.

Leadbetter JR, Breznak JA (1996) Physiological ecology of *Methanobrevibacter cuticularis* sp. nov. and *Methanobrevibacter curvatus* sp. nov., isolated from the hindgut of the termite *Reticulitermes* flavipes. Appl Environ Microbiol 62: 3620–3631.

Lin C, Miller TL (1998) Phylogenetic analysis of *Methanobrevibacter* isolated from feces of humans and other animals. Arch Microbiol 169: 397–403.

Liu Y, Whitman WB (2008) Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. Ann N Y Acad Sci 1125: 171–189.

Lueders T, Chin K-J, Conrad R, Friedrich M (2001) Molecular analyses of methyl-coenzyme M reductase alpha-subunit (*mcrA*) genes in rice field soil and enrichment cultures reveal the methanogenic phenotype of a novel archaeal lineage. Environ Microbiol 3: 194–204.

Luton PE, Wayne JM, Sharp RJ, Riley PW (2002) The *mcrA* gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. Microbiology 148: 3521–3530.

Mathrani IM, Boone DR, Mah RA, Fox GE, Lau PP (1988) *Methanohalophilus zhilinae* sp. nov., an alkaliphilic, halophilic, methylotrophic methanogen. Int J Syst Bacteriol 38: 139–142.

Nicholson M, Evans P, Joblin K (2007) Analysis of methanogen diversity in the rumen using temporal temperature gradient gel electrophoresis: identification of uncultured methanogens. Microb Ecol 54: 141–150.

Ohkuma M, Noda S, Horikoshi K, Kudo T (1995) Phylogeny of symbiotic methanogens in the gut of the termite *Reticulitermes speratus.* FEMS Microbiol Lett 134: 45–50.

Sakai S, Imachi H, Sekiguchi Y, Ohashi A, Harada H, Kamagata Y (2007) Isolation of key methanogens for global methane emission from rice paddy fields: a novel isolate affiliated with the clone cluster Rice Cluster I. Appl Environ Microbiol 73: 4326–4331.

Sakai S, Imachi H, Hanada S, Ohashi A, Harada H, Kamagata Y (2008) *Methanocella paludicola* gen. nov., sp. nov., a methane-producing archaeon, the first isolate of the lineage 'Rice Cluster I', and proposal of the new archaeal order *Methanocellales* ord. nov. Int J Syst Evol Microbiol 58: 929–936.

Springer E, Sachs MS, Woese CR, Boone DR (1995) Partial gene sequences for the A subunit of methyl-coenzyme M reductase (*mcr*I) as a phylogenetic tool for the family *Methanosarcinaceae.* Int J Syst Bacteriol 45: 554–559.

Stackebrandt E, Frederiksen W, Garrity GM, Grimont PAD, Kampfer P, Maiden MCJ, Nesme X, Rossello-Mora R, Swings J, Truper HG, Vauterin L, Ward AC, Whitman WB (2002) Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. Int J Syst Evol Microbiol 52: 1043–1047.

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37: 463–464.

Whitman W, Bowen T, Boone D (2006) The methanogenic bacteria. In The Prokaryotes, 3rd edn. M Dworkin, S Falkow, E Rosenberg, K-H Schleifer, E Stackebrandt (eds.) New York: Springer-Verlag, pp. 165–207.

Whitman WB, Boone DR, Koga Y, Keswani J (2001) Taxonomy of methanogenic archaea. In Bergey's Mannual of Systematic Bacteriology, 2nd edn. DR Boone, RW Castenholtz, GM Garrity (eds.). New York: Springer-Verlag.

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.

Widdel F, Rouvière PE, Wolfe RS (1988) Classification of secondary alcohol-utilizing methanogens including a new thermophilic isolate. Arch Microbiol 150: 477–481.