Diversity and Taxonomy of Methanogens

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Zhe Lyu and Yuchen Liu

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Z. Lyu
Department of Microbiology, University of Georgia, Athens, GA, USA
e-mail: zhelyu@uga.edu

Y. Liu (✉)
Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, USA
e-mail: yuchenliu@lsu.edu

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Abstract

Methanogens are strictly anaerobic, methane-producing archaea. All characterized members belong to the phylum *Euryarchaeota*, but methanogenesis pathway is also predicted to be present in the newly proposed phyla *Bathyarchaeota* and *Verstraetearchaeota*. This indicates that the diversity of methanogens may be larger than previously excepted. Although methanogens share a set of physiological characteristics, they are phylogenetically very diverse. The current taxonomy classifies methanogens into seven well established orders: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, *Methanopyrales*, *Methanocellales*, and *Methanomassiliicoccales*. 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. 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 to methane. The complexity of methanogenesis pathways suggests an ancient monophyletic origin of methanogens, a hypothesis that 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. (i) They are obligate methane producers, obtaining all or most of their energy for growth from producing large quantities of methane. (ii) They are archaea, belonging to the phylum *Euryarchaeota* and possibly other archaeal phyla too. (iii) They are obligate anaerobes, limiting their growth to anaerobic environments.

Then known 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 (Widdel 1986; Widdel et al. 1988; Bleicher et al. 1989; Frimmer and Widdel 1989). Athough these organic compounds can obviously be assimilated, they 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 in 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 modern methanogens perhaps originate from a common ancient ancestor.

2 Taxonomy and Phylogeny 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 three 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. 1993b; Whitman et al. 2001b). 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 (Wayne et al. 1987; Stackebrandt et al. 2002). 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 a sufficient evidence that two organisms belong to the same species.

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 seven orders (Fig. 1). Non-methanogenic lineages such as *Archaeoglobales* and *Thermoplasmatales*, are interspersed in the tree. Phylogenomic studies using more gene markers including ribosomal proteins and/or methanogenesis proteins further classified methanogens collectively into three classes (Bapteste et al. 2005; Anderson et al. 2009). The Class I methanogens include *Methanobacteriales*, *Methanococcales*, and *Methanopyrales*, the Class II methanogens include *Methanomicrobiales*, and the Class III methanogens include *Methanosarcinales*. However, when *Methanocellales* was included in phylogenomic analyses, the boundaries between the Classes II and III could not be fully resolved, suggesting that they could also belong to a single class (Lyu and Lu 2017). Although the seventh order *Methanomassiliicoccales* is distantly related to all three methanogen classes, its close affiliation to the Class Thermoplasmata could not warrant an immediate establishment of a fourth methanogen class.

Four hypotheses are proposed to explain the 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* and *Thermoplasmatales*. This hypothesis also suggests that the common ancestor of *Euryarchaeota* was a methanogen (Gribaldo and Brochier-Armanet 2006). However, this view is now challenged by the possible

presence of methanogens outside *Euryarchaeota* as shown by metagenomic surveys (Evans et al. 2015; Vanwonterghem et al. 2016). (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

Table 1 Taxonomy of methanogens (Modified from Liu (2010e))

Order	Family	Genus	Species[b]
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*, *M. aggregans*, *M. arcticum*, *M. ferruginis*, *M. flexile*, *M. kanagiense*, *M. lacus*, *M. movens*, *M. movilense*, *M. paludis*, *M. petrolearium*, *M. veterum*
		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*, *M. boviskoreani*
		Methanosphaera	*M. cuniculi*, ***M. stadtmanae***
		Methanothermobacter	*M. defluvii*, *M. marburgensis*, ***M. thermoautotrophicus***, *M. thermoflexus*, *M. thermophilus*, *M. wolfeii*, *M. crinale*, *M. tenebrarum*
	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*, *M. villosus*, *M. bathoardescens*
		Methanotorris	*M. formicicus*, ***M. igneus***

(*continued*)

Table 1 (continued)

Order	Family	Genus	Species[b]
Methanomicrobiales	*Methanomicrobiaceae*	*Methanoculleus*	***M. bourgensis***, *M. chikugoensis*, *M. marisnigri*, *M. palmolei*, *M. submarinus*, *M. thermophiles*, *M. horonobensis*, *M. hydrogenitrophicus*, *M. receptaculi*, *M. sediminis*, *M. taiwanensis*
		Methanofollis	*M. aquaemaris*, *M. formosanus*, *M. liminatans*, ***M. tationis***, *M. ethanolicus*
		Methanogenium	***M. cariaci***, *M. frigidum*, *M. marinum*, *M. organophilum*
		Methanolacinia	***M. paynteri***, *M. petrolearius*
		Methanomicrobium	***M. mobile***
		Methanoplanus	*M. endosymbiosus*, ***M. limicola***
	Methanospirillaceae	*Methanospirillum*	***M. hungatei***, *M. lacunae*, *M. psychrodurum*, *M. stamsii*
	Methanocorpusculaceae	*Methanocorpusculum*	*M. bavaricum*, *M. labreanum*, ***M. parvum***, *M. sinense*
	Methanoregulaceae	*Methanolinea*	*M. tarda*, *M. mesophila*
		Methanoregula	*M. boonei*
		Methanosphaerula	*M. palustris*
	Unassigned	*Methanocalculus*[a]	*M. chunghsingensis*, ***M. halotolerans***, *M. pumilus*, *M. taiwanensis*, *M. natronophilus*, *M. alkaliphilus*
Methanosarcinales	*Methanosarcinaceae*	*Methanosarcina*	*M. acetivorans*, *M. baltica*, ***M. barkeri***, *M. lacustris*, *M. mazei*, *M. semesiae*, *M. siciliae*, *M. thermophila*, *M. vacuolata*, *M. horonobensis*, *M. soligelidi*, *M. splelaei*, *M. subterranea*
		Methanococcoides	*M. alaskense*, *M. burtonii*, ***M. methylutens***, *M. vulcani*
		Methanohalobium	***M. evestigatum***
		Methanohalophilus	*M. halophilus*, ***M. mahii***, *M. portucalensis*, *M. levihalophilus*

(*continued*)

Table 1 (continued)

Order	Family	Genus	Species[b]
		Methanolobus	*M. bombayensis*, *M. oregonensis*, *M. taylorii*, ***M. tindarius***, *M. vulcani*, *M. chelungpuianus*, *M. profundi*, *M. zinderi*
		Methanomethylovorans	***M. hollandica***, *M. thermophile*, *M. uponensis*
		Methanimicrococcus[a]	***M. blatticola***
		Methanosalsum	***M. zhilinae***, *M. natronophilum*
	Methanosaetaceae	*Methanosaeta*	***M. concilii***, *M. harundinacea*, *M. thermophila*
	Methermicoccaceae	*Methermicoccus*	***M. shengliensis***
Methanopyrales	*Methanopyraceae*	*Methanopyrus*	***M. kandleri***
Methanocellales	*Methanocellaceae*	*Methanocella*	***M. paludicola***, *M. avoryzae*, *M. conradii*
Methanomassiliicoccales	*Methanomassiliicoccaceae*	*Methanomassiliicoccus*	***M. luminyensis***

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

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 phylogenomics analyses showing that 10 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*. However, the presence of methanogens in the *Thermoplasmata* suggests otherwise. (4) The last archaeal common ancestor was a methanogen, and the methanogenesis pathway was inherited, modified or lost in various lineages throughout evolution. This view is supported by (i) recent metagenomics surveys that indicate possible presence of methanogens in at least two other archaeal phyla besides the *Euryarchaeota* (Evans et al. 2015; Vanwonterghem et al. 2016), and (ii) the root of the archaeal tree based on phylogenomic analyses was placed between *Euryarchaeota* and the rest of archaeal phyla (Petitjean et al. 2015).

Methanogens are currently classified into seven orders: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, *Methanomassiliicoccales*, *Methanocellales* and *Methanopyrales* (Whitman et al. 2001b, 2006; Sakai et al. 2008; Iino et al. 2013). This taxonomy is supported by comparative 16S rRNA gene sequence and phylogenomic analyses as well as distinctive phenotypic properties, such as different cell envelope structures, lipid compositions, and substrate ranges. Some representative characteristics are listed in Table 2 and further described in following subsections.

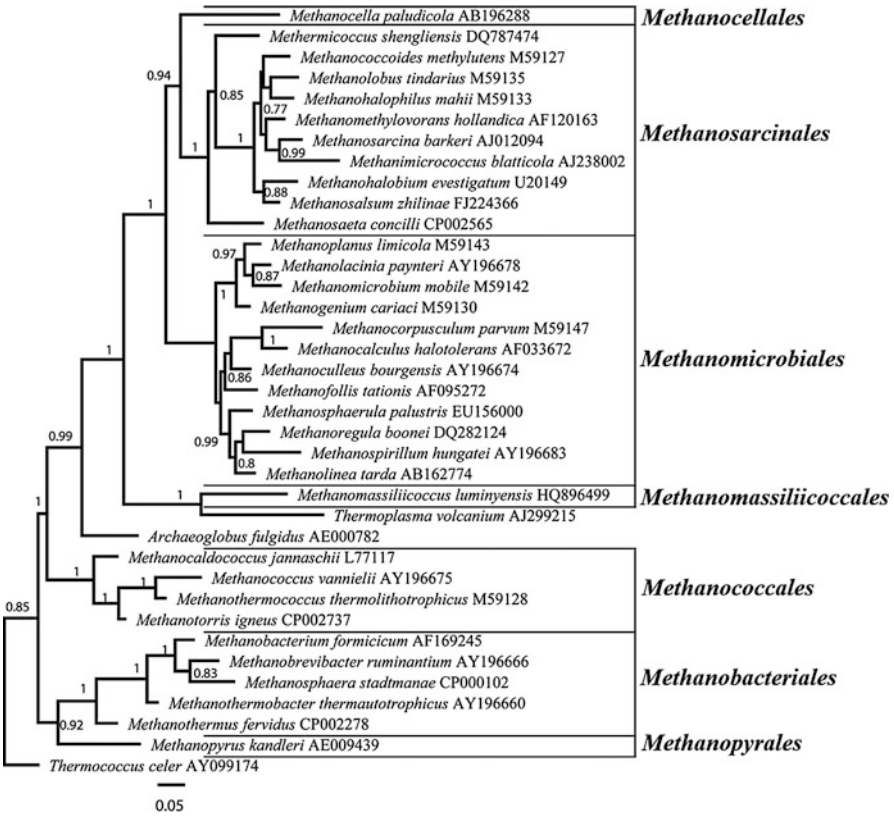

Fig. 1 Maximum-likelihood tree based on nearly full length 16S rRNA gene sequences from type species of 34 methanogen genera. The tree was built by FastTree 2.1.5 using *Thermococcus celer* as an outgroup. Bootstrap values >0.77 are indicated at nodes and were based on 1000 replicates (Price 2010). There were a total of 1555 positions in the final dataset, which were aligned in the RDP 11 database. The scale bar represents substitutions per position. The GenBank accesion numbers are indicated following the species name

2.1 Methanobacteriales

Methanobacteriales are currently classified into two families and five genera based upon 16S rRNA sequences, DNA reassociation levels, and phenotypic characteristics. The two families *Methanobacteriaceae* and *Methanothermaceae* are distinguished by 16S rRNA sequence similarities below 89% and differences in cell wall structure and growth temperatures. The family *Methanobacteriaceae* contains three mesophilic genera – *Methanobacterium*, *Methanobrevibacter*, and *Methanosphaera* – and one thermophilic genus *Methanothermobacter*. Members of the *Methanobacteriaceae* possess pseudomurein as a major component of the cellular envelope. The family *Methanothermaceae* is represented by one hyperthermophilic genus, *Methanothermus*. Members of the *Methanothermaceae* possess a protein surface layer in addition to the pseudomurein layer.

Table 2 Some characteristics of the methanogen orders (Modified from Liu (2010e))

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, ($H_2 + CO_2$, methoxylated aromatic compounds)	−	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
Methanomassiliicoccales	Cocci	H_2 + methanol, methylamine	−	nd	nd	nd

Abbreviation: *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*

The placement of the hyperthermophilic *Methanothermus* into a separate family from other *Methanobacteriales* genera is justified by the deep branching of the phylogeny of its 16S rRNA gene (Schuchmann and Muller 2014). The 16S rRNA gene sequence similarities within the *Methanothermus* species are much higher (98%) than the similarities between *Methanothermus* and other members of the *Methanobacteriales* (83–89%). This classification is further confirmed by DNA reassociation. For instance, the DNA relatedness between *Methanothermus* isolates and *Methanothermobacter thermoautotrophicus* strain IM is 2–8% (Lauerer et al. 1986). Phenotypically, the genus *Methanothermus* is distinguished from other *Methanobacteriales* by their high temperature optima (80–88 °C), double-layered cell wall, and motility by bipolar polytrichous flagellation.

Methanobacteriaceae is a diverse family, including mesophilic and thermophilic species. The phylogeny of the 16S rRNA gene indicates that the thermophilic species are divergent from mesophilic members at the genus level. The 16S rRNA sequence similarities within the thermophilic genus *Methanothermobacter* are above 98%, while the similarities between thermophilic and mesophilic members of *Methanobacteriaceae* are generally below 93% (Wasserfallen et al. 2000). The DNA relatedness between *Methanothermobacter* species are 22–47%, confirming that they are genetically distant and should be assigned to separate species (Boone et al. 2001a).

The separation of mesophilic members of *Methanobacteriales* into three genera is supported by both genetic and phenotypic analyses. Species of *Methanobacterium* are usually autotrophs, while species of *Methanobrevibacter* and *Methanosphaera* are commonly mixotrophic or heterotrophic. Species of *Methanosphaera* use only H_2 and methanol as substrates for methanogenesis, while all species of *Methanobrevibacter* and *Methanobacterium* can use H_2 and CO_2.

Members of the order *Methanobacteriales* use a limited range of substrates for methanogenesis. Most of them reduce CO_2 to CH_4 with H_2. Some *Methanobacterium* species can also reduce methanol with H_2, which are the exclusive substrates for the genus *Methanosphaera*. There is one *Methanobacterium* species that can also reduce methylamine with H_2. Some *Methanobacteriales* members can also use formate, CO, or secondary alcohols as electron donors. Some species can grow autotrophically using CO_2 as the sole carbon source, and some species are mixotrophs or heterotrophs, which may require acetate, amino acids, peptones, yeast extract, vitamins, and/or rumen fluid for growth. Ammonium is a major nitrogen source. Sulfide can serve as the sole sulfur source, and some species can reduce elemental sulfur to sulfide. Cells are generally rod-shaped with a length of 0.6–25 μm, often forming chains or filaments up to 40 μm in length. Cells typically stain Gram positive, but the wall does not contain muramic acid. Pesudomurein is the predominant polymer in the cell wall. Members of the genus *Methanothermus* have double-layered cell wall, consisting of an inner pseudomurein layer and an outer S-layer composed of protein. The cellular lipids contain caldarchaeol, archaeol, and, in some species, hydoxyarchaeol as core lipids. The polar lipids can contain glucose, *N*-acetylglucosamine, *myo*-inositol, ethanolamine, and serine, depending on the species. Most species are nonmotile. However, *Methanobacterium movens* and members of the genus *Methanothermus* are motile via one or two polar flagella

and peritrichous flagella, respectively. The optimum growth temperatures of members of the *Methanobacteriales* vary from 20 °C to 88 °C. The genus *Methanothermus* can grow at temperatures up to 97 °C, while multiple *Methanobacterium* species can grow at as low as 10 °C and one species can even grow at 0 °C. The pH optima of *Methanobacteriales* members vary from 5.5 to 9.

Descriptive properties of the *Methanobacteriales* are summarized in Tables 3, 4, 5, 6, and 7. Further information can be found in Bonin and Boone (2006) and Boone et al. (2001a). Our current knowledge on the diversity of the *Methanobacteriales* is largely incomplete. As an example, investigations of 16S rRNA gene from clone libraries recognized a large number of uncultured *Methanobrevibacter*, especially from the rumen and termite gut (Dighe et al. 2004; Wright et al. 2004). Moreover, the cloned sequences from termite gut formed separate lineages from cultured *Methanobrevibacter* (Dighe et al. 2004). The correlation between ecological habitat and 16S rRNA based phylogeny need more ecological surveys to unravel.

2.2 Methanococcales

The order *Methanococcales* is composed of two families, *Methanocaldococcaceae* and *Methanococcaceae*, which are distinguished by 16S rRNA sequence similarities below 93% and differences in growth temperatures. The *Methanocaldococcaceae* are all hyperthermophilic, while the *Methanococcaceae* are extremely thermophilic and mesophilic. Members of this order are all capable of forming methane by CO_2 reduction with H_2. Many species can use formate as an alternative electron donor. Most species can grow autotrophically.

Phylogenetic analyses with DNA sequences reveal a high diversity of the *Methanococcales*. The sequence similarities of the 16S rRNA genes between hyperthermophilic and mesophilic methanococci are generally below 90%. For instance, the 16S rRNA gene sequence similarity between the mesophile *Methanococcus voltae* and the hyperthermophile *Methanocaldococcus infernus* is about 85%, which is comparable to the similarity between *Escherichia* and *Pseudomonas*. In addition, the mesophilic methanococci possess 91–96% (average 94%) 16S rRNA gene sequence similarities and 5–30% DNA reassociation values, suggesting that they are related only at the genus level (Keswani et al. 1996).

The *Methanococcales* are currently divided into two families and four genera, according to their growth temperatures. The family *Methanocaldococcaceae* includes two hyperthermophilic genera, *Methanocaldococcus* and *Methanotorris*. The family *Methanococcaceae* includes the mesophilic genus *Methanococcus* and the extremely thermophilic genus *Methanothermococcus*. This taxonomy generally agrees with the phylogeny of the 16S rRNA genes (Liu 2010b), in which the lineages formed by the deepest bifurcation represent the two methanococcal families. However, some ambiguity remains. For instance, 16S rRNA gene sequences indicate that *Methanococcus aeolicus* forms a deep branch of the mesophilic methanococci and is more closely related to the thermophile *Methanothermococcus okinawensis* (95% sequence similarity) than to the other *Methanococcus* (91–93% sequence similarity). In addition, *Methanothermococcus okinawensis* also has low sequence similarity to

Table 3 Descriptive characteristics of the species of the genus *Methanobacterium* (Modified from Liu (2010a))

Species	Type strain	Source[a]	Cell width (μm)	Cell length (μm)	Methanogenesis substrates[b]	Required organic compounds	Temperature range (optimum) (°C)	pH range (optimum)	NaCl range (%, w/v)	Doubling time[c] (h)	GC content (mol%)	References
aarhusense	H2-LR	Marine sediment	0.7	5–18	$H_2 + CO_2$	None	>5–<48 (45)	5–9 (7.5–8)	0.6–5.4	nd	34.9 (LC)	(Shlimon et al. 2004)
aggregans	E09F.3	Anaerobic digester	0.2–0.5	2–2.5	$H_2 + CO_2$, formate	None	25–45 (40)	nd (6.5–7.0)	0–0.3	5–6	39.1 (LC)	(Kern et al. 2015)
alcaliphilum	WeN4	Alkaline lake	0.5–0.6	2–25	$H_2 + CO_2$	TP or YE	25–45 (37)	7.0–9.9 (8.1–9.1)	nd	nd	57 (BD)	(Worakit et al. 1986)
arcticum	M2	Permafrost sediments	0.45–0.5	3–6	$H_2 + CO_2$, formate	None	15–45 (37)	5.5–8.5 (6.8–7.2)	0–1.8	nd	38.1 (T_m)	(Shcherbakova et al. 2011)
beijingense	8-2	Anaerobic digestor	0.4–0.5	3–5	$H_2 + CO_2$, formate	YE	25–50 (37)	6.5–8.0 (7.2)	0–3	14	38.9 (T_m)	(Ma et al. 2005)
bryantii	M.o.H.	Anaerobic digestor	0.5–1.0	10–15	$H_2 + CO_2$, (2-propanol, 2-butanol, cyclopentanol)	None	nd (37–39)	nd (6.9–7.2)	nd	nd	33–38 (Bd)	(Boone 1987)
congolense	C	Anaerobic digestor	0.4–0.5	2–10	$H_2 + CO_2$, (2-propanol, 2-butanol, cyclopentanol)	None	25–50 (37–42)	5.9–8.2 (7.2)	nd	7.5	39.5 (LC)	(Cuzin et al. 2001)
espanolae	GP9	Sludge of a bleach-craft mill	0.8	3–22	$H_2 + CO_2$, (2-propanol, 2-butanol)	nd[d]	15–50 (35)	4.6–7.0 (5.6–6.2)	nd	10	34 (T_m)	(Patel et al. 1990)
ferruginis	Mic6c05	Corroded pipe sediment	nd	nd	$H_2 + CO_2$, (2-propanol), (isobutanol), (cyclopentanol)	None	20–45 (40)	5.5–9.0 (6.0–7.5)	0–7	18.5	37.6 (LC)	(Mori and Harayama 2011)
flexile	GH	Lake sediments	0.3–0.5	2–5	$H_2 + CO_2$, formate	YT	10–50 (35–38)	6.5–9.5 (7.0–7.5)	0–0.6	21.7	36.4 (T_m)-	(Zhu et al. 2011)
formicicum	MF	Sewage sludge	0.4–0.8	2–15	$H_2 + CO_2$, formate	None	25–50 (37–45)	6.6–7.8 (7–7.5)	nd	13	41–42 (Bd)	(Boone 1987)

(*continued*)

Table 3 (continued)

Species	Type strain	Source[a]	Cell width (μm)	Cell length (μm)	Methanogenesis substrates[b]	Required organic compounds	Temperature range (optimum) (°C)	pH range (optimum)	NaCl range (%, w/v)	Doubling time[c] (h)	GC content (mol%)	References
ivanovii	Ivanov	Rock core	0.5–0.8	1–15	$H_2 + CO_2$	None	>10–<55 (37–45)	6.5–8.2 (7.0–7.4)	0–1	16–18	36.6 (T_m)	(Belyaev et al. 1983)
kanagiense	169	Rice field	0.35–0.5	1.6–5.0	$H_2 + CO_2$	None	15–45 (40)	6.5–9.6 (7.5–8.5)	0–7	21	39.3 (LC)	(Kitamura et al. 2011)
lacus	17A1	Lake sediments	0.2–0.4	2–15	$H_2 + CO_2$, methanol + H_2	None	14–41 (30)	5.0–8.5 (6.5)	0–2.3	22	37.0 (LC)	(Borrel et al. 2012b)
movens	TS − 2	Lake sediments	0.3–0.5	2–5	$H_2 + CO_2$	YT	10–50 (35–38)	6.0–9.0 (7.2–7.5)	0–1.8	25.7	39.1 (T_m)	(Zhu et al. 2011)
movilense	MC-20	Lake sediments	0.6–0.7	3.5–4.0	$H_2 + CO_2$, formate, 2-propanol, 2-butanol	None	0–44 (33)	6.2–9.9 (7.4)	0.1–3.5	79.2	33.0	(Schirmack et al. 2014)
oryzae	FPi	Rice field	0.3–0.4	3–10	$H_2 + CO_2$, formate	None	20–42 (40)	6.0–8.5 (7.0)	0–2.5	nd	31 (LC)	(Joulian et al. 2000)
paludis	SWAN1	Minerotrophic fen	0.6	1.5–2.8	$H_2 + CO_2$	None	16–40 (32–37)	4.8–6.6 (5.4–5.7)	0–1.5	35	35.7 (G_s)	(Cadillo-Quiroz et al. 2014)

palustre	F	Peat bog	0.5	2.5–5	$H_2 + CO_2$, formate, 2-propanol, (2-butanol)	None	20–45 (33–37)	nd (7.0)	0–1.8	18	34 (T_m)	(Zellner et al. 1988)
	FG694aF	Fault gouge	0.5–0.7	1.7–0.24	$H_2 + CO_2$, formate	None	20–45 (37)	5.7–8.3 (5.7–6.8)	0–3.2	8.6	nd	(Wu and Lai 2011)
petrolearium	Mic5c12	Crude oil sludge	nd	nd	$H_2 + CO_2$	YE, ac	20–40 (35)	5.5–9.0 (6.5)	0–7	39.5	38.3 (LC)	(Mori and Harayama 2011)
subterraneum	A8p	Deep granitic groundwater	0.1–0.15	0.6–1.2	$H_2 + CO_2$, formate	None	3.6–45 (20–40)	6.5–9.2 (7.8–8.8)	0–8	2.5	54.5 (T_m)	(Kotelnikova et al. 1998)
uliginosum	P2St	Marshy soil	0.2–0.6	2–4	$H_2 + CO_2$	None	15–45 (37–40)	6.0–8.5 (6.0–7.5)	nd	11	29.4 (T_m)	(Koenig 1984)
veterum	MK4	Permafrost	0.4–0.45	2.0–8.0	$H_2 + CO_2$, methanol + H_2, methylamine + H_2	None	10–46 (28)	5.2–9.4 (7.2–7.4)	0–1.8	26.7	33.8 (T_m)	(Krivushin et al. 2010)

Abbreviations: *nd* not determined, *TP* trypticase peptones, *YE* yeast extract, *ac* acetate, *LC* liquid chromatography, *BD* buoyant density method, T_m melting point method, G_s genome sequencing method

[a]Environment from which the type strain was isolated

[b]Substrates in parentheses are oxidized, but do not result in growth

[c]Doubling time of the type strain under optimal growth conditions of temperature, pH, and NaCl

[d]Cells grew in vitamin-free medium containing acetate

Table 4 Descriptive characteristics of the species of the genus *Methanobrevibacter* (Modified from Liu (2010a))

Species	Type strain	Source[a]	Cell width (μm)	Cell length (μm)	Methanogenesis substrates	Required organic compounds	Temperature range (optimum) (°C)	pH range (optimum)	NaCl range (%, w/v)	Doubling time[b] (h)	GC content (mol%)	References
acididurans	ATM	Acidogenic digestor	0.3–0.5	0.3–0.5	$H_2 + CO_2$	RF, ac, AAs	25–37 (35)	5.0–7.5 (6.0)	nd	~16	nd	(Savant et al. 2002)
arboriphilus	DH1	Decaying cottonwood tissue	0.5	1.2–1.4	$H_2 + CO_2$, (formate)[c]	B-vit	25–45 (30–37)	6.0–8.6 (7.5–8)	0–0.6	13	25.5–31.6 (Bd or T_m)	(Zeikus and Henning 1975)
boviskoreani	JH1	Bovine rumen	0.6	1.5–1.8	$H_2 + CO_2$, formate	YE, CoM, FA	35–45 (37–40)	5.5–8.0 (6.5–7.0)	0.6–3.0	nd	28 (LC)	(Lee et al. 2013)
curvatus	RFM-2	Termite hindgut	0.34	1.6	$H_2 + CO_2$	RF	10–<37 (30)	6.5–8.5 (7.1–7.2)	nd	40	nd	(Leadbetter and Breznak 1996)
cuticularis	RFM-1	Termite hindgut	0.4	1.2	$H_2 + CO_2$, (formate)[d]	None	10–<42 (37)	6.5–8.5 (7.7)	nd	35	nd	(Leadbetter and Breznak 1996)
filiformis	RFM-3	Termite hindgut	0.23–0.28	4	$H_2 + CO_2$	YE	10–33.5 (30)	6.0–7.5 (7.0–7.2)	nd	37	nd	(Leadbetter et al. 1998)
gottschalkii	HO	Horse faeces	0.7	0.9	$H_2 + CO_2$	ac or YE or TP	27–41 (37)	5.0–10.0 (7)	nd	nd	29 (T_m)	(Miller and Lin 2002)
millerae	ZA-10	Bovine rumen	nd	nd	$H_2 + CO_2$, formate	Ac, YE or TP	33–43 (36–42)	5.5–10.0 (7.0–8.0)	up to 2.6	nd	31–32 (T_m)	(Rea et al. 2007)
olleyae	KM1H5-1P	Ovine rumen	nd	nd	$H_2 + CO_2$, formate	ac	28–42 (36–40)	6.0–10.0 (7.5)	up to 2.6	nd	27–29 (T_m)	(Rea et al. 2007)

oralis	ZR	Human subgingival plaque	0.4–0.5	0.7–1.2	$H_2 + CO_2$	Fecal extract	25–39 (35–38)	6.2–8.0 (6.9–7.4)	0.06–0.6	15	28 (T_m)	(Ferrari et al. 1994)
ruminantium	M1	Bovine rumen	0.7	0.8–1.7	$H_2 + CO_2$, (formate)[d]	ac, B-vit, CoM, 2-MBA, AAs	33–42 (37–39)	5.5–7.7 (6–7)	nd	nd	30.6 (Bd)	(Smith and Hungate 1958)
smithii	PS	Sewage sludge	0.6–0.7	~1	$H_2 + CO_2$, (formate)[d]	ac, B-vit	26–46 (34–46)	5.0–8.5 (5.5–7.0)	nd	nd	30–31 (T_m or Bd)	(Balch et al. 1979)
thaueri	CW	Cow faeces	0.5	0.6–1.2	$H_2 + CO_2$	ac or YE or TP	nd (37)	nd (7)	nd	nd	38 (T_m)	(Miller and Lin 2002)
woesei	GS	Goose faeces	0.6	1	$H_2 + CO_2$, (formate)[d]	ac or YE or TP	nd (37)	nd (7)	nd	nd	31 (T_m)	(Miller and Lin 2002)
wolinii	SH	Sheep faeces	0.6	1.0–1.4	$H_2 + CO_2$	ac or YE or TP	nd (37)	nd (7)	nd	nd	33 (T_m)	(Miller and Lin 2002)

Abbreviations: *nd* not determined, *RF* rumen fluid, *ac* acetate, *AAs* amino acids, *B-vit* B vitamins, *TP* trypticase peptones, *YE* yeast extract, *CoM* 2-mercaptoethanesulfonic acid (conenzyme M), *FA* fatty acids, *2-MBA* 2-methylbutyric acid, *BD* buoyant density method, T_m melting point method

[a]Environment from which the type strain was isolated

[b]Doubling time of the type strain under optimal growth conditions of temperature, pH, and NaCl

[c]Formate is used by some, but not all strains

[d]Growth on formate is poor

Table 5 "Descriptive characteristics of the species of the genus *Methanosphaera*" (Originally published in Liu (2010a), published with kind permission of)

Species	Type strain	Source[a]	Cell width (μm)	Cell length (μm)	Methanogenesis substrates	Required organic compounds	Temperature range (optimum) (°C)	pH range (optimum)	NaCl range (%, w/v)	GC content (mol%)	References
cuniculi	1R7	Rabbit rectum	0.6–1.2	0.6–1.2	H_2 + methanol	ac	>25–<45 (35–40)	nd (6.8)	nd	23 (T_m)	(Biavati et al. 1988)
stadtmanae	MCB3	Human feces	~1	~1	H_2 + methanol	Thiamine, ac, Ile, Leu	30–40 (36–40)	nd (6.5–6.9)	nd	25.8 (T_m)	(Miller and Wolin 1985)

Abbreviations: *nd* not determined, *ac* acetate, *Ile* isoleucine, *Leu* leucine, T_m melting point method
[a]Environment from which the type strain was isolated

Table 6 Descriptive characteristics of the species of the genus *Methanothermobacter* (Modified from Liu (2010a))

Species	Type strain	Source[a]	Cell width (μm)	Cell length (μm)	Methanogenesis substrates	Required organic compounds	Temperature range (optimum) (°C)	pH range (optimum)	NaCl range (%, w/v)	Doubling time[b] (h)	GC content (mol%)	References
crinale	Tm2	Oil sands	0.3	2.2–5.9	$H_2 + CO_2$	ac	45–80 (65)	6.9–8.0 (6.9)	0–4	nd	41.1 (LC)	(Cheng et al. 2011)
defluvii	ADZ	Anaerobic digestor	0.4	3–6	$H_2 + CO_2$, formate	CoM	45–65 (60)	6.0–7.5 (7.0)	0.08–2	1.5	62.2 (T_m)	(Kotelnikova et al. 1993)
marburgenesis	Marburg	Sewage sludge	0.4–0.6	3–6	$H_2 + CO_2$	None	45–70 (65)	5.0–8.0 (6.8–7.4)	0.01–3.5	1.6–2.5	47.6 (T_m)	(Wasserfallen et al. 2000)
tenebrarum	RMAS	Natural gas field	0.5	3.5–10.5	$H_2 + CO_2$	CA, TP, YE, vit	45–80 (70)	5.8–8.7 (6.9–7.7)	0.01–2	12	41.5 (LC)	(Nakamura et al. 2013)
thermoautotrophicus	ΔH	Sewage sludge	0.35–0.6	3–7	$H_2 + CO_2$, (formate)[c]	None	40–75 (65–70)	6.0–8.8 (7.2–7.6)	0.01–3.5	3	49 (T_m)	(Zeikus and Wolee 1972, Schönheit et al. 1980)
thermoplexus	IDZ	Anaerobic digestor	0.4	7–20	$H_2 + CO_2$, formate	CoM	45–70 (55)	7.5–8.5 (7.9–8.2)	0.1–3	3.5	55 (T_m)	(Kotelnikova et al. 1993)
thermophilus	M	Sludge of methane tank	0.36	1.4–6.5	$H_2 + CO_2$	CoM	47–75 (57)	6.5–8.5 (7.5)	0–0.6	2–3	44.7 (T_m)	(Laurinavichyus et al. 1988)
wolfeii	DSM2970	Sewage sludge and river sediment	0.4–0.6	2.5–6	$H_2 + CO_2$, formate	None	37–74 (55–65)	6.0–8.2 (7.0–7.5)	nd (up to 1)	3.5–4	61 (T_m)	(Winter et al. 1984)

Abbreviations: *nd* not determined, *CoM* 2-mercaptoethanesulfonic acid (conenzyme M), *ac* acetate, *CA* casamino acids, *TP* tryptone, *YE* yeast extract, *vit* vitamins, T_m melting point method

[a]Environment from which the type strain was isolated

[b]Doubling time of the type strain under optimal growth conditions of temperature, pH, and NaCl

[c]Formate is used by some, but not all strains

Table 7 "Descriptive characteristics of the species of the genus *Methanothermus*" (Originally published in Liu (2010a), published with kind permission of)

Species	Type strain	Source[a]	Cell width (μm)	Cell length (μm)	Methanogenesis substrates	Required organic compounds	Temperature range (optimum) (°C)	pH range (optimum)	NaCl range (%, w/v)	Doubling time[b] (h)	GC content (mol%)	References
fervidus	V24S	Icelandic hot spring	0.3–0.4	1–3	$H_2 + CO_2$	None	67–97 (80–85)	nd (6.5)	nd	3	33 (T_m)	(Stetter et al. 1981)
sociabilis	Kf1-F1	Icelandic hot spring	0.3–0.4	1–3	$H_2 + CO_2$	None	55–97 (88)	5.5–7.5 (6.5)	nd	3	33 (T_m)	(Lauerer et al. 1986)

Abbreviations: *nd* not determined, T_m melting point method

[a]Environment from which the type strain was isolated

[b]Doubling time of the type strain under optimal growth conditions of temperature, pH, and NaCl

the other thermophile *Methanothermococcus thermolithotrophicus* (95% sequence similarity). Therefore, the phylogenetic analysis implies that *Methanococcus aeolicus* and *Methanothermococcus okinawensis* could be classified into two novel genera. Nevertheless, phylogeny of additional genes and phenotypic differences other than growth temperature should be examined to justify reclassification.

DNA relatedness and cellular protein patterns are often determined for the phylogenetic and taxonomic analyses of methanococci. They are especially useful to distinguish relationships at the species and subspecies levels, at which levels the 16S rRNA gene sequence analysis is frequently incongruent. For instance, two heterotrophic *Methanococcus voltae* strains A2 and A3 exhibit 37% DNA relatedness to the type train PS (Keswani et al. 1996). Similarly, four autotrophic *Methanococcus maripaludis* strains C5, C6, C7, and C8 exhibit 54–69% DNA relatedness to the type strain JJ (Keswani et al. 1996). Moreover, differences in cellular protein patterns between these strains are also readily recognized. Therefore, classification of these strains into separate species is suggested based on their genetic diversities. However, because distinguishable phenotypic properties are few, these strains are not currently considered as novel species.

Autotrophy and thermophily are represented in both methanococcal families, suggesting that the mesophilic methanococci may have evolved from an autotrophic thermophile (Keswani et al. 1996). The heterotrophy of *Methanococcus voltae* is possibly a recently acquired characteristic. This hypothesis is consistent with the presence of enzymes required for autotrophic CO_2 fixation in *M. voltae* (Shieh et al. 1988).

Members of the *Methanococcales* or the methanococci are coccoid methanogens isolated from marine environments. They share a set of phenotypic characteristics. They all use H_2 or formate to reduce CO_2 for methanogenesis. Acetate, methyl-containing compounds, and alcohols are not used as substrates for methanogenesis. Most of them can grow autotrophically with CO_2 as the sole carbon source. Sulfide is a sufficient sulfur source for all methanococci, and elemental sulfur is reduced to sulfide with slight inhibition of growth in most strains. Ammonium is a sufficient nitrogen source for all methanococci, and nitrogen gas, nitrate, and alanine are used as a nitrogen source by some species. They all require sea salts for optimal growth. Cells are irregular cocci, 1–3 μm in diameter during balanced growth. Most of them are motile by means of polar tuft(s) of flagella. Cells strain Gram negative. They are susceptible to lysis by 0.01% (w/v) SDS and hypotonic solutions. Cell envelopes are composed of a protein cell wall or S-layer. Glycoproteins and cell wall carbohydrates are not abundant. The cellular lipids contain archaeol, caldarchaeol, hydroxyarchaeol, and macrocyclic archaeol, depending upon the species. The polar lipids can contain glucose, *N*-acetylglucosamine, serine, and ethanolamine. The optimal growth temperatures of methanococci are diverse, ranging from 35 °C to 88 °C. They are among the fastest growing methanogens at either mesophilic or thermophilic temperatures, with generation times of about 2 h at 37 °C and less than 30 min at 85 °C.

Descriptive properties of the methanococci are summarized in Tables 8 and 9. Further information can be found in Whitman et al. (2001a), and Whitman and Jeanthon (2006). Creation of new families and genera may be necessary with addition of new isolates and identification of new phenotypic and genetic markers. The *Methanotorris* may represent a new family because they have only 92–93% 16S

Table 8 Descriptive characteristics of the species of the genera *Methanocaldococcus* and *Methanotorris* (Modified from Liu (2010b))

			Methanocaldococcus					*Methanotorris*	
Character	*jannaschii*	*infernus*	*fervens*	*indicus*	*villosus*	*bathoardescens*	*vulcanius*	*igneus*	*formicicus*
Type strain	JAL-1	ME	AG86	SL 43	KIN24-T80	JH146	M7	Kol 5	Mc-S-70
Cell diameter (μm)	1.5	1–3	1–2	1–3	1–2	1–2	1–3	1–2	0.8–1.5
Flagella[a]	2 tufts	3 tufts	nd	1 tuft	1 tuft[d]	1 tuft	3 tufts	±	±
Substrates for methanogenesis	$H_2 + CO_2$	$H_2 + CO_2$	$H_2 + CO_2$	$H_2 + CO_2$	$H_2 + CO_2$	$H_2 + CO_2$	$H_2 + CO_2$	$H_2 + CO_2$	$H_2 + CO_2$, formate
Autotrophy	+	+	+	+	+	+	+	+	+
Yeast extract stimulates growth	−	+	+	+	+	−	+	−	−
Selenium simulates growth	+	+	+	+	+	nd	+	−	−
Nitrogen source	NH_3	NH_3, NO_3^-	NH_3, NO_3^-	NH_3, NO_3^-	NH_3, nd	NH_3	NH_3, NO_3^-	NH_3	NH_3, N_2, NO_3^-
Sulfur source	S^{2-}, S^0	S^{2-}, S^0	S^{2-}, S^0	S^{2-}, S^0	S^{2-}, nd	nd	S^{2-}, S^0	S^{2-}, S^0	S^{2-}
Temperature range (°C)	50–91	55–91	48–92	50–86	55–90	58–90	49–89	45–91	55–83
Temperature optimum (°C)	85	85	85	85	80	82	80	88	75
pH range	5.2–7.0	5.25–7.0	5.5–7.6	5.5–6.7	5.5–7.0	4.5–9.0	5.2–7.0	5.0–7.5	6.0–8.5
pH optimum	6.0	6.5	6.5	6.5	6.5	7.0	6.5	5.7	6.7

NaCl range (%, w/v)	1.0–5.0	0.8–3.5	0.5–5.0	1.5–5.0	0.5–5.5	1.6–7.4	0.6–5.6	0.9–5.4	0.4–6.0
NaCl optimum (%, w/v)	3.0	2.0	3.0	3.0	2.5	2.9	2.5	1.8	2.4
GC content (mol%)	31 (Bd)	33 (T_m)	33 (T_m)	31 (LC)	30 (*Gs*)	30.8 (*Gs*)	31 (T_m)	31 (T_m)	33 (LC)
Doubling time (min)[b]	26	35–40	20–30	25–30	45	20	45	30	30
Source[c]	Deep sea hydrothermal vent	Deep sea hydrothermal vent	Deep sea hydrothermal vent	Deep sea hydrothermal vent	Shallow submarine hydrothermal system	Deep sea hydrothermal fluid	Deep sea hydrothermal vent	Shallow marine hydrothermal vent	Deep sea black smoker chimney
References	(Jones et al. 1983a)	(Jeanthon et al. 1998)	(Jeanthon et al. 1999; Zhao et al. 1988)	(L'Haridon et al. 2003)	(Bellack et al. 2011)	(Ver Eecke et al. 2013; Stewart et al. 2015)	(Jeanthon et al. 1999)	(Burggraf et al. 1990)	(Takai et al. 2004)

Abbreviations: *nd* not determined, *Bd* buoyant density method, T_m melting point method, *LC* liquid chromatography, G_s genome sequencing

[a]Number of flagellar tufts. ±, non-motile, but flagella-like structures are observed by electron microscopy

[b]Doubling time of the type strain under optimal growth conditions of temperature, pH, and NaCl

[c]Environment from which the type strain was isolated

[d]tuft is only formed in some cases, which may mediate cell-cell contact. Otherwise, 50 polarly inserted flagella are observed

Table 9 "Descriptive characteristics of the species of the genera *Methanococcus* and *Methanothermococcus*" (Originally published in Liu (2010b), published with kind permission of © Springer Science+Business Media New York, 2003. All rights reserved)

	Methanococcus				*Methanothermococcus*	
Character	*vannielii*	*voltae*	*maripaludis*	*aeolicus*	*thermolithotrophicus*	*okinawensis*
Type strain	SB	PS	JJ	Nankai-3	SN1	IH1
Cell diameter (μm)	1.3	1.3–1.7	0.9–1.3	1.5–2.0	1.5	1.0–1.5
Flagella[a]	2 tufts	Multiple tufts	1 tuft	nd	1 tuft	1 tuft
Substrates for methanogenesis	$H_2 + CO_2$, formate	$H_2 + CO_2$, formate	$H_2 + CO_2$, formate	$H_2 + CO_2$, formate	$H_2 + CO_2$, formate	$H_2 + CO_2$, formate
Autotrophy	+	–[d]	+	+	+	+
Acetate stimulates growth	–	+	+	–	–	–
Amino acids stimulate growth	–	+	+	–	–	–
Selenium simulate growth	+	+	+	+	nd	+
Nitrogen source	NH_3, purines	NH_3,	NH_3, N_2, alanine	NH_3, N_2	NH_3, N_2, NO_3^-	NH_3
Sulfur source	S^{2-}, S^0	S^{2-}, S^0	S^{2-}, S^0, $(S_2O_3^{2-})$[e]	S^{2-}, S^0	S^{2-}, S^0, $S_2O_3^{2-}$, SO_3^{2-}, SO_4^{2-}	S^{2-}
Temperature range (°C)	<20–45	<20–45	<20–45	<20–55	17–70	40–75

Temperature optimum (°C)	35–40	35–40	35–40	46	60–65	60–65
pH range	6.5–8.0	6.5–8.0	6.5–8.0	5.5–7.5	4.9–9.8	4.5–8.5
pH optimum	7–8	6.0–7.0	6.8–7.2	7.0	5.1–7.5	6–7
NaCl range (%, w/v)	0.3–5	0.6–6	0.3–5	0.3–6	0.6–9.4	1.2–9.6
NaCl optimum (%, w/v)	0.6–2	1–2	0.6–2	1–2	2–4	2.5–5.0[f]
GC content (mol%)[a]	33	30	33	32	34	33.5
Doubling time (h)[b]	8	3	2	1.3	~1	0.5
Source[c]	Marine sediments	Marine sediments	Salt marsh sediments	Marine sediments	Coastal geothermally heated sea sediments	Deep sea hydrothermal vent
References	(Stadtman and Barker 1951)	(Balch et al. 1979; Whitman et al. 1982)	(Jones et al. 1983b)	(Kendall et al. 2006)	(Huber et al. 1982)	(Takai et al. 2002)

Abbreviations: *nd* not determined
[a]The G+C content of the DNA determined by liquid chromatography
[b]Doubling time of the type strain under optimal growth conditions of temperature, pH, and NaCl
[c]Environment from which the type strain was isolated
[d]Acetate and the amino acids leucine and isoleucine are required for growth
[e]Thiosulfate is used by some strains

rRNA similarities with the *Methanocaldococcus*. These two groups are also distinguished by the presence of hydroxyarchaeol and the absence of caldarchaeol in the *Methanotorris*. *Methanococcus aeolicus* and *Methanothermococcus okinawensis* may represent two new genera because they form a lineage separate from other *Methanococcaceae* in the 16S rRNA phylogenetic tree.

2.3 Methanomicrobiales

The order *Methanomicrobiales* is composed of four families, *Methanomicrobiaceae*, *Methanocorpusculaceae*, *Methanospirillaceae*, and *Methanoregulaceae*, which are distinguished by 16S rRNA sequence similarities below 89%. The *Methanospirillaceae* is further distinguished from the other two families by its unique morphology of curved rod-shape and exterior sheath. All members of this order are capable to produce methane by CO_2 reduction with H_2. Formate and secondary alcohols are used as alternative electron donors in many species.

Because the members of *Methanomicrobiales* share many phenotypic characteristics, it is difficult to divide them based solely on their physiological properties. Both of the families *Methanomicrobiaceae* and *Methanocorpusculaceae* contain coccoid organisms, and nearly all members require organic carbon sources for growth (except *Methanofollis aquaemaris*). Therefore, they are difficult to distinguish except by molecular phylogenetic analyses. The family *Methanospirillaceae* is distinguished from the other three families by its unique morphology of curved rod-shape and capability of autotrophic growth. The family *Methanoregulaceae* is unique by having members that grow in acidic conditions.

The family *Methanomicrobiaceae* is divided into six genera. The 16S rRNA gene sequence similarities between different genera are 87–95%, suggesting that they are sufficiently distinctive at genus level. The 16S rRNA gene sequence similarities between different species within a genus are above 95.4%. Both *Methanomicrobium* and *Methanolacinia* are represented by a single species. Cells of both genera are rod-shaped, but they can be differentiated by some other physiological characters. In addition to H_2, *Methanolacinia paynteri* can use secondary alcohols to reduce CO_2. In contrast, *Methanomicrobium mobile* can only use H_2 or formate as electron donors for methanogenesis. *Methanolacinia paynteri* is a marine organism, while *Methanomicrobium mobile* was isolated from bovine rumen. Cells of *Methanoculleus*, *Methanofollis*, and *Methanogenium* are irregular cocci. These three genera are difficult to differentiate by phenotypic characteristics. *Methanoplanus* differs from the other genera by its plate or disc cell shape.

The family *Methanospirillaceae* is represented by a single species, *Methanospirillum hungatei.* Cells have a unique spiral shape that is not found in other methanogens. Cell walls consist of an inner protein S-layer and a rigid paracrystalline outer sheath conferring the α-helical spiral shape of the cells (Sprott and McKellar 1980; Sprott et al. 1983). Cells usually grow as single cells or short filaments within their sheath. The cellular lipid of *M. hungatei* contains two unusual phosphoglycolipids, which are derivatives of the dibiphytanyl diglycerol tetraether. One of the free hydroxyls of this tetraether is esterified with glycerophosphoric acid, and the other is linked to a disaccharide (Kushwaha et al. 1981).

The family *Methanocorpusculaceae* is represented by the genus *Methanocorpusculum*. Cells are irregular cocci with diameters generally <1 μm. All species can use formate in addition to H_2 as electron donor for methanogenesis. For some species, secondary alcohols are alternative electron donors. Acetate and either yeast extract, peptones, or rumen fluid are required as carbon sources. The habitats of *Methanocorpusculum* are usually anaerobic digesters or freshwater sediments. They have not been found in marine environments.

The family *Methanoregulaceae* is divided into three genera (Sakai et al. 2012). The 16S rRNA gene sequence similarities between different genera are 93–96%, suggesting that they are sufficiently distinctive at genus level. Both *Methanolinea* (Imachi et al. 2008; Sakai et al. 2012) and *Methanoregula* (Brauer et al. 2006; Wang et al. 2009) are represented by two species, while *Methanosphaerula* is represented by one (Cadillo-Quiroz et al. 2009). *Methanolinea* is morphologically distinct from other *Methanomicrobiales* by forming rod-shaped, multicellular filaments within a sheath-like structure. *Methanoregula* and *Methanosphaerula* are distinguished from others by their acidophilic growth.

The assignment of *Methanocalculus* into a novel family is tentative. The 16S rRNA sequence similarities between all known *Methanocalculus* species are >98%, but those between *Methanocalculus* and other methanogens are <91%. Different species of *Methanocalculus* exhibited <10–51% DNA relatedness. The closest neighbor of *Methanocalculus* in the phylogenic tree based on 16S rRNA gene is *Methanocorpusculum*. All members of *Methanocalculus* are irregular cocci, can only use H_2 and CO_2 or formate for methanogenesis, and require acetate for growth.

All members of the order *Methanomicrobiales* produce methane using CO_2 as the electron acceptor and H_2 as the electron donor. Most species use formate and many species also use secondary alcohols as alternative electron donors, while two unique species can also grow on primary alcohols. They cannot use acetate and methyl-group containing compounds for methanogenesis. Most species are mixotrophic and require acetate as a carbon source; some species also require additional organic growth factors. Their morphologies are diverse, including cocci, rods, and sheathed rods. Most cells have single-layered protein cell walls, but cells of *Methanospirillum hungatei* are surrounded by an external sheath. Peptidoglycan and pseudomurein are absent. The cellular lipids contain archaeol and caldarchaeol as core lipids. Hydroxyarchaeol is absent. Glucose, galactose, aminopentanetetrols, and glycerol are common polar lipids; and aminopentanetetrols are unique to this order of organisms. Motility varies between species. Most species are mesophilic, with the exceptions of two psychrophilic species (*Methanogenium marinum* and *Methanogenium frigidum*) and one thermophilic species (*Methanoculleus thermophilicus*). Most species grow best near neutral pH. Exceptions are *Methanoregula boonei* and *Methanosphaerula palustris*, which have an optimal pH of 5.1~5.7 and were isolated from acidic peat bog; and *Methanocalculus alkaliphilus* and *Methanocalculus natronophilus*, which grow best at pH of 9.5 and were isolated from soda lake sediments. Many species are marine organisms and grow optimally with 0.1–1 M of NaCl. Descriptive properties of the *Methanomicrobiales* are summarized in Table 10. Further information can be found in Boone et al. (2001b) and Garcia et al. (2006).

Table 10 Descriptive characteristics of the species of the order *Methanomicrobiales* (Modified from Liu (2010c))

Organism	Type strain	Source[a]	Dimensions (μm)	Flagella	Methano-genesis substrates[b]
Methanoculleus					
bourgensis	MS2	Anaerobic digestor	Ø 1–2	None	$H_2 + CO_2$, formate, (2-propanol, 2-butanol)
chikugoensis	MG62	Paddy field soil	Ø 1–2	Flagellated[d]	$H_2 + CO_2$, formate, 2-propanol, 2-butanol, cyclopentanol
horonobensis	T10	Deep subsurface groundwater	Ø 0.7–1.6	Flagellated[d]	$H_2 + CO_2$, formate
hydrogenitrophicus	HC	Wetland soil	Ø 0.8–2	None	$H_2 + CO_2$
marisnigri	JR1	Black sea sediments	Ø <1.3	Peritrichous[d]	$H_2 + CO_2$, formate, 2-propanol, 2-butanol
palmolei	INSLUZ	Anaerobic digestor	Ø1.25–2	Flagellated[d]	$H_2 + CO_2$, formate, 2-propanol, 2-butanol, cyclopentanol
receptaculi	ZC-2	Oil field	Ø 0.8–1.7	None	$H_2 + CO_2$, formate
sediminis	S3Fa	Deep marine sediments	Ø 0.5–1.0	None	$H_2 + CO_2$, formate
submarinus	Nankai-1	Deep marine sediments	Ø 0.8–2.0	Flagellated[d]	$H_2 + CO_2$, formate
taiwanensis	CYW4	Deep marine sediments	Ø 0.6–1.5	None	$H_2 + CO_2$, formate
thermophilus	CR-1	Nuclear power plant sediment	Ø 0.6–1.8	Single[e]	$H_2 + CO_2$, formate
Methanofollis					
aquaemaris	N2F9704	Marine–water fish pond	Ø 1.2–2.0	None	$H_2 + CO_2$, formate
ethanolicus	HASU	Lotus field	Ø 2.0–3.0	nd	$H_2 + CO_2$, formate, ethanol, 1-propanol, 1-butanol
formosanus	ML15	Marine–water fish pond	Ø 1.5–2.0	None	$H_2 + CO_2$, formate
liminatans	GKZPZ	Wastewater reactor	Ø 1.25–2.0	Flagellated[f]	$H_2 + CO_2$, formate, 2-propanol, 2-butanol, cyclopentanol

Required organic compounds	Temperature range (optimum) (°C)	pH range (optimum)	NaCl optimum (%, w/v)	Doubling time[c] (h)	GC content (mol%)	References
ac	37–45 (35–40)	5.5–8.0 (6.7)	0.2–1	18	59 (Bd)	(Ollivier et al. 1986)
ac, YE/TP	15–40 (25–30)	6.7–8.0 (6.7–7.2)	0.6	46	62.2 (LC)	(Dianou et al. 2001)
None	25–45 (37–42)	5.8–8.2 (6.7–6.8)	0.6–1.2	6.3–6.9	62.9 (LC)	(Shimizu et al. 2013)
None	18–45 (37)	5.0–8.5 (6.6)	1.2	22.4	60.2 (T_m)	(Tian et al. 2010)
TP	10–45 (20–25)	5.8–7.6 (6.2–6.6)	0.6–1.1	10	61 (Bd)	(Romesser et al. 1979)
ac	22–50 (40)	6.5–8.0 (6.9–7.5)	nd	13.5	59.5 (LC)	(Zellner et al. 1998)
ac	<30–65 (50–55)	6.5–8.5 (7.5–7.8)	1.2	8.3	55.2 (T_m)	(Cheng et al. 2008)
ac	20–50 (37)	5.6–7.5 (7.1)	1.0	15.1	62.3 (G_s)	(Chen et al. 2015)
ac	>10–<55 (45)	5.0–8.7 (6.0–7.5)	0.6–2.3	~6.8	nd	(Mikucki et al. 2003)
None	20–42 (37)	6.5–8.1 (8.1)	0.5	6.7	61.0 (LC)	(Weng et al. 2015)
ac, TP, vit	37–65 (55–60)	6.2–7.8 (6.5–7.2)	1.2	2.5	55–60 (T_m)	(Rivard and Smith 1982)
None	20–43 (37)	6.3–8.0 (6.5)	0.5	13	59.1 (T_m)	(Lai and Chen 2001)
ac[g]	15–40 (37)	6.5–7.5 (7.0)	0	72[i]	60.9 (LC)	(Imachi et al. 2009)
YE, TP	20–42 (40)	5.6–7.3 (6.6–7.0)	3	36	58.4 (T_m)	(Wu et al. 2005)
ac	≥15–44 (40)	nd (7)	0–3.5	7.5	60 (T_m)	(Zellner et al. 1990)

(*continued*)

Table 10 (continued)

Organism	Type strain	Source[a]	Dimensions (μm)	Flagella	Methanogenesis substrates[b]
tationis	Chile 9	Solfataric pool mud	Ø 1.5–3	Peritrichous[f]	$H_2 + CO_2$, formate
Methanogenium					
cariaci	JR1	Marine sediments	Ø <2.6	Pertrichous	$H_2 + CO_2$, formate
frigidum	Ace-2	Anoxic Ace Lake water	Ø 1.5–2.5	None	$H_2 + CO_2$, formate
marinum	AK-1	Marine sediments	Ø 1–1.2	Flagellated[d]	$H_2 + CO_2$, formate
organophilum	CV	Marine mud	Ø 0.5–1.5	None	$H_2 + CO_2$, formate, ethanol, 1-propanol, [1-butanool], 2-propanol, 2-butanol,
Methanolacinia					
paynteri	G2000	Marine sediment	0.6 × 1.5–2.5	Flagellated[d]	$H_2 + CO_2$, 2-propanol, 2-butanol
petrolearius	SEBR4847	Offshore oil field	Ø 1–3	None	$H_2 + CO_2$, formate, 2-propanol
Methanomicrobium					
mobile	BP	Bovine rumen	0.7 × 1.5–2.0	Single	$H_2 + CO_2$, formate
Methanoplanus					
endosymbiosus	MC1	Marine ciliate	0.5–1 × 1.6–3.4	peritrichous	$H_2 + CO_2$, formate
limicola	M3	Swamp	0.1–0.3 × 1.5–2.8	Polar tuft	$H_2 + CO_2$, formate
Methanospirillum					
hungatei	JF-1	Sewage sludge	0.4–0.5 × 7.4–10 (often 15– > 100)	Polar tufts	$H_2 + CO_2$, formate
lacunae	Ki8-1	Puddly soil	0.5–0.6 × 11–25 (often 8–26)	Single or tufted	$H_2 + CO_2$, formate
psychrodurum	X-18	Wetland soil	0.4–0.5 × 11–62	None	$H_2 + CO_2$, formate
stamsii	Pt1	Anaerobic digestor	04–0.5 × 7–25 (sometimes 15– > 100)	tufted5	$H_2 + CO_2$, [formate]
Methanocorpusculum					
bavaricum	SZSXXZ	Sediment of wastewater treatment pond	Ø <1	Flagellated	$H_2 + CO_2$, formate, 2-propanol, 2-butanol

Required organic compounds	Temperature range (optimum) (°C)	pH range (optimum)	NaCl optimum (%, w/v)	Doubling time[c] (h)	GC content (mol%)	References
ac, YE, TP, tung	≥15–44 (40–44)	6.3–8.8 (7)	0.8–1.2	12	54 (T_m)	(Zabel et al. 1984)
ac, YE	10–32 (20–25)	nd (6.8–7.3)	2.7	11	52 (Bd)	(Romesser et al. 1979)
ac	−12[h]–18 (15)	6.5–7.9 (7.5–7.9)	2–3.5	69.6	nd	(Franzmann et al. 1997)
ac	5–25 (25)	5.5–7.5 (6.0)	1.5–7.3	42	nd	(Chong et al. 2002)
ac, PABA, biotin, tung, vit-B_{12}	nd-39 (30–35)	nd (6.4–7.3)	2.0	6	46.7 (T_m)	(Widdel et al. 1988)
ac	20–45 (40)	6.6–7.3 (7.0)	0.88	4.8	44.9 (Bd)	(Rivard et al. 1983)
ac	28–43 (35–40)	5.3–8.2 (7.0)	1–3	10	50 (LC)	(Ollivier et al. 1997, Göker et al. 2014)
Complex	35–45 (40)	5.9–7.7 (6.1–6.9)	nd	nd	48.8 (Bd)	(Paynter and Hungate 1968)
p-Cresol, tung	16–36 (32)	6.1–8.0 (6.8–7.3)	1.5	7	38.7 (T_m)	(Bruggen et al. 1986)
ac	17–41 (40)	nd (6.5–7.5)	1	7	47.5 (T_m)	(Wildgruber et al. 1982)
(ac)	45 (15–50)	6.5–10.0 (7.5–8.5)	0	20.7	45 (Bd)	(Ferry et al. 1974, Iino et al. 2010)
ac/YE	15–37 (30)	6.0–9.5 (7.2–7.5)	0	32.3	45.3 (LC)	(Iino et al. 2010)
YE	15–35 (30)	6.5–8.0 (7.0)	0–0.6	10.7	44.4 (LC)	(Iino et al. 2010)
None	5–37 (20–30)	6.0–10 (7.0–7.5)	0	39.8	40.0 (T_m)	(Parshina et al. 2014)
RF	15–45 (37)	nd (7.0)	nd	~5	51 (LC)	(Zellner et al. 1989)

(*continued*)

Table 10 (continued)

Organism	Type strain	Source[a]	Dimensions (μm)	Flagella	Methanogenesis substrates[b]
labreanum	Z	Lake sediments	Ø 0.4–2.0	None	$H_2 + CO_2$, formate
parvum	XII	Anaerobic digestor	Ø <1	Single	$H_2 + CO_2$, formate, 2-propanol, 2-butanol
sinense	China Z		Ø <1	Flagellated	$H_2 + CO_2$, formate
Methanocalculus					
alkaliphilus	AMF2	Hypersaline soda lake sediments	Ø 1.5–2.5	Peritrichous	$H_2 + CO_2$, formate
chunghsingensis	K1F9705b	Marine water fishpond	Ø 0.7–1.8	Flagellated[e]	$H_2 + CO_2$, formate
halotolerans	SEBR 4845	Oilfield	Ø 0.8–1.0	Peritrichous	$H_2 + CO_2$, formate
natronophilus	Z-7105	Soda lake sediments	Ø 0.2–1.2	Peritrichous	$H_2 + CO_2$, formate
pumilus	MHT-1	Waste disposal site	Ø 0.8–1.0	None	$H_2 + CO_2$, formate
taiwanensis	P2F9704a	Estuary	Ø 0.9–1.4	None	$H_2 + CO_2$, formate
Methanolinea					
mesophila	TNR	Rice field soil	0.3 × 2.0–6.5	nd	$H_2 + CO_2$, formate
tarda	NOBI-1	Sewage sludge	0.7–1.0 × 2.0	None	$H_2 + CO_2$, formate
Methanoregula					
boonei	6A8	Acid peat bog	0.2–0.3 × 0.8–3.0	Flagella-like filaments	$H_2 + CO_2$
Methanosphaerula					
palustris	E1-9c	Minerotrophic fen peatland	Ø 0.5–0.8	Multiple	$H_2 + CO_2$, formate

Abbreviations: *nd* not determined, *RF* rumen fluid, *ac* acetate, *(ac)* acetate required or stimulatory depending on the strain, *PABA* *p*-aminobenzoate, *vit* vitamins, *tung* tungsten, *TP* trypticase peptones, *YE* yeast extract, *CoM* 2-mercaptoethanesulfonic acid (conenzyme M), *Bd* buoyant density method, T_m melting point method, *LC* liquid chromatography, G_s genome sequencing

[a]Environment from which the type strain was isolated

[b]Parentheses mean utilized by some strains, but not all strains; brackets indicate very poor growth and methane production

[c]Doubling time of the type strain under optimal growth conditions of temperature, pH, and NaCl

[d]Nonmotile, although flagella are detected by electron microscopy

[e]Present in some strains

[f]Some strains are non-motile

[g]Acetate is not required for growth on ethanol

[h]The minimum growth temperature is predicted by applying the Ratkowsky model to temperature growth data

[i]Calculated from cultures that grow on ethanol

Required organic compounds	Temperature range (optimum) (°C)	pH range (optimum)	NaCl optimum (%, w/v)	Doubling time[c] (h)	GC content (mol%)	References
YE/TP	<45 (37)	6.5–7.5 (7.0)	0–1.5	~10	50 (Bd)	(Zhao et al. 1989)
ac, YE, tung	15–45 (37)	nd (6.8–7.5)	0–4.7	8	48.5 (T_m)	(Zellner et al. 1987, 1989)
RF	15–45 (30)	nd (7.0)	0	~20	50.0 (LC)	(Zellner et al. 1989)
ac	nd–41 (35)	8–10.2 (9.5)	3.5	nd	51.1 (T_m)	(Sorokin et al. 2015)
ac	20–45 (37)	5.8–7.7 (7.2)	0.5–1.0	7	50.3–50.8 (T_m)	(Lai et al. 2004)
ac	25–45 (38)	7.0–8.4 (7.6)	5	12	55 (LC)	(Ollivier et al. 1998)
ac	15–45 (35)	8.0–10.2 (9.0–9.5)	8.0–11.1	nd	50.2 (T_m)	(Zhilina et al. 2013)
ac	24–45 (35)	5.5–9.0 (6.5–7.5)	1	12	51.9 (LC)	(Mori et al. 2000)
ac	25–42 (37)	5.6–8.3 (6.7)	0.5	7.1	nd	(Lai et al. 2002)
ac	20–40 (37)	6.5–7.4 (7.0)	0	28.8	56.4 (LC)	(Sakai et al. 2012)
ac, YE	35–55 (50)	6.7–8.0 (7.0)	0	98	nd	(Imachi et al. 2008)
ac, YE, coM, vit	10–40 (35–37)	4.5–5.5 (5.1)	<0.1	40.8	54.5 (G_s)	(Brauer et al. 2006, 2011)
ac, CoM, vit	14–35 (30)	4.8–6.4 (5.7)	<0.2	30	58.9 (G_s)	(Cadillo-Quiroz et al. 2008, 2009)

2.4 Methanosarcinales

The order *Methanosarcinales* is divided into three families, *Methanosarcinaceae*, *Methanosaetaceae* and *Methermicoccus* based on phenotypic properties and 16S rRNA gene sequence analysis (Cheng et al. 2007). The three families are distinguished by 16S rRNA sequence similarities below 91% and differences in substrates for methanogenesis, lipid components, and cell wall structures. The *Methanosarcinaceae* are all capable of producing methane from methyl group containing compounds, and some can use acetate or H_2/CO_2. The cells can form aggregates within an outer layer composed of heteropolysaccharide. The *Methanosaetaceae* can only produce methane by splitting acetate. The cells can form chains within a proteinaceous sheath. The family *Methermicoccus* is represented by only one species, which is a thermophilic, methylotrophic methanogen isolated from an oilfield (Cheng et al. 2007).

The family *Methanosarcinaceae* currently comprises eight genera, *Methanococcoides*, *Methanohalobium*, *Methanohalophilus*, *Methanolobus*, *Methanomethylovorans*, *Methanosalsum*, *Methanimicrococcus* and *Methanosarcina*. The genus *Methanosarcina* can be differentiated from other genera by the unique morphology of pseudosarcinae or large cysts, which are formed by aggregation of cells within a common outer layer. The outer layer is composed of heteropolysaccharide, consisting mainly of galactosamine, glucose, mannose, and galacturonic acid. Some *Methanosarcina* species can also be distinguished from other genera of *Methanosarcinaceae* by their ability to split acetate for methanogenesis. The genus *Methanohalobium* is represented by a single species, *M. evestigatum*, which is an extreme halophile that requires 4 M of NaCl for optimal growth. The genus *Methanosalsum* is represented by *M. zhilinae* and *M. natronophilum*, which are moderate halophiles and alkaliphiles. The genus *Methanohalophilus* comprises moderate halophilic and halotolerant species, which grow best with 1–2 M of NaCl. The genera *Methanococcoides* and *Methanolobus* are difficult to differentiate by phenotypic properties, as they all use methylated compounds for methanogenesis; they require phylogenetic analysis for taxonomy. The genus *Methanimicrococcus* is represented by a single spcies *Methanimicrococcus blatticola*, which is a dominant methylotrophic methanogen in the cockroach hindgut (Sprenger et al. 2000). It has 83.4–89.8% 16S rRNA gene sequence similarities with other species of *Methanosarcinales*, suggesting that it could potentially represent a new family. This is further supported by the fact that it cannot disproportionate methyl-group containing compounds, a feature shared by all other *Methanosarcinaceae* spp. Instead, methanol and methylated amines must be reduced with H_2 for methanogenesis. This obligately hydrogenotrophic and methylotrophic mode of growth is shared with *Methanosphaera* and *Methanomassiliicoccus*, which belongs to the *Methanobacteriales* and *Methanomassiliicoccales*, respectively.

Members of the family *Methanosaetaceae* use acetate as the sole energy source. Acetate and CO_2 serve as carbon sources. Cells form filament-like structures within the sheath, which is composed predominantly with proteins and contains carbohydrates.

Methanogens from only two genera, *Methanosarcina* and *Methanosaeta*, can use acetate as a substrate for methanogenesis. However, they metabolize acetate differently. *Methanosarcina* is a relative generalist that prefers methanol and methylamine to acetate, and many species also utilize H_2. *Methanosaeta* is a specialist that uses only acetate. *Methanosaeta* is a superior acetate utilizer in that it can use acetate at concentrations as low as 5–20 μM, while *Methanosarcina* requires a minimum concentration of about 1 mM (Jetten et al. 1992). The difference of acetate affinity is probably due to different systems for acetate activation. Moreover, based upon their genome sequences, these two genera probably have different modes of electron transfer and energy conservation, even though the methanogenesis pathways are likely to be similar (Smith and Ingram-Smith 2007).

The family *Methermicoccus* is represented by *Methermicoccus shengliensis*. Its closest neighbor in the 16S rRNA phylogenetic tree is *Methanosaeta* (< 90.7% sequence similarities). It is morphologically differentiated from *Methanosaeta* by its coccoid-shape and formation of large cysts. Moreover, *M. shengliensis* uses methanol and methylated amines, but not acetate, for methanogenesis.

Members of the order *Methanosarcinales* have the widest substrate range among methanogens. All members can produce methane by disproportionating methyl-group containing compounds (methanol, methylamines, methylethanolamines, betaine, or methyl sulfides) or by splitting acetate. Some mesophilic *Methanosarcia* species can reduce CO_2 with H_2, but formate, secondary alcohols, and ethanol are not used as electron donors. Recently, it has been shown that *Methermicoccus* spp. are surprisingly capable of growth and methane production using methoxylated aromatic compounds (MACs) such as methoxy-benzoate (Mayumi et al. 2016). Ammonium and sulfide serve as the major nitrogen and sulfur sources, respectively. Their cellular morphologies are diverse, including cocci, pseudosarcinae, and sheathed rods. Most cells have protein cell walls, and some cells are surrounded by a sheath or acidic heteropolysaccharide. Most strains are nonmotile. The cellular lipids contain archaeol, hydroxyarchaeol, and caldarchaeol. Polar lipids can contain glucose, galactose, mannose, *myo*-inositol, ethanolamine, serine, and glycerol, depending upon the species. Most species of *Methanosarcinales* are mesophilic. Four species are moderately thermophilic (*Methanosarcina thermophila*, *Methanomethylovorans thermophila*, *Methanosaeta thermophila*, and *Methermicoccus shengliensis*), and six species are psychrotolerant (*Methanococcoides alaskense*, *Methanococcoides burtonii*, *Methanosarcina lacustris*, *Methanosarcina soligelidi*, *Methanosarcina splelaei*, and *Methanosarcina baltica*). Most species grow best at near neutral pH, except for three species that are alkaliphilic (*Methanolobus oregonensis*, *Methanolobus taylorii*, *Methanosalsum natronophilum*, and *Methanosalsum zhilinae*). Many species were isolated from marine environments and require a salinity near that of seawater for optimal growth. Some species are halophilic or halotolerant. Descriptive properties of members of the *Methanosarcinales* are summarized in Table 11. Further information can be found in Boone et al. (2001c) and Kendall and Boone (2006).

Table 11 Descriptive characteristics of the species of the order *Methanosarcinales* (Modified from Liu (2010d))

Organism	Type strain	Source[a]	Dimensions (μm)	Flagella	Methano-genesis substrates[b]
Methanococcoides					
alaskense	AK-5	Marine sediments	1.5–2.0	Flagellated[d]	(Methanol), TMA
burtonii	DSM 6242	Hypolimnion of ice lake	0.8–1.8	Monotrichous	Methanol, $MeNH_2$
methylutens	TMA-10	Submarine canyon sediments	1.0	None	Methanol, $MeNH_2$
vulcani	SLH33	Marine sediments	0.6–1.7	Single to four	Methanol, $MeNH_2$, TMA, DMA, betaine, choline, DMEA
Methanohalobium					
evestigatum	Z-7303	Saline lagoon sediments	0.2–2	None	$MeNH_2$
Methanohalophilus					
euhalobius	283	Mineralized stratal waters of oil deposits	1.0–2.5	None	Methanol, $MeNH_2$
halophilus	Z-7982	Salinarium sediments	0.5–2.0	None	(Methanol), $MeNH_2$
levihalophilus	GTA13	Palaeo-seawater	0.7–1.0	None	TMA, DMA
mahii	SLP	Salt lake sediments	1.0	None	Methanol, $MeNH_2$
portucalensis	FDF-1	Salinarium sediments	0.6–2.0	None	Methanol, $MeNH_2$
Methanolobus					
bombayensis	B-1	Marine sediments	1.0–1.5	None	Methanol, $MeNH_2$, DMS
chelungpuianus	St54 5 Mb	Deep fault sandstone	0.5–0.7	None	Methanol, TMA
oregonensis	WAL1	Alkaline, saline aquifer	1.0–1.5	None	Methanol, $MeNH_2$, DMS
profundi	MobM	Deep sediments of a natural gas field	0.9–1.2	Multiple	methanol, $MeNH_2$, DMA, TMA
taylorii	GS-16	Estuarine sediments	0.5–1.0	None	Methanol, $MeNH_2$, DMS
tindarius	Tindari3	Marine sediments	0.8–1.25	Monotrichous	Methanol, $MeNH_2$
vulcani	PL-12/M	Marine sediments	0.8–1.25	None	Methanol, $MeNH_2$
zinderi	SD1	Saline coal seam	1.0–2.0	None	Methanol, $MeNH_2$, DMA, TMA

Organic growth factors	Temperature range (optimum) (°C)	pH range (optimum)	NaCl range (optimum) (M)	Doubling time[c] (h)	GC content (mol%)	References
None	−2.3–30.6 (23.6)	6.3–7.5 (7.5)	0.1–0.8 (0.3–0.4)	~85	39.5–41.9 (T_m)	(Singh et al. 2005)
None	−2.54[f]–29.5 (23.4)	6.8–8.2 (7.7)	0.2–0.5 (0.2)	24	39.6 (T_m)	(Franzmann et al. 1992)
Biotin	15–35 (30–35)	6.0–8.0 (7.0–7.5)	0.1–1.0 (0.4)	5.2	42 (T_m)	(Sowers and Ferry 1983)
None	nd–35 (30)	6–7.8 (7.0)	0.08–1.02 (0.5)	21	43.4 (LC)	(L'Haridon et al. 2014)
Vit	25–60 (50)	6.0–8.3 (7.0–7.5)	1.7–5.1 (4.3)	nd	37 (T_m)	(Zhilina and Zavarzin 1987a)
Biotin	15–50 (28–37)	5.8–8.0 (6.8–7.3)	0.16–2.3 (1.0)	nd	43.0	(Davidova et al. 1997)
None	18–42 (26–36)	6.3–7.4 (6.5–7.4)	0.3–2.6 (1.2–1.5)	nd	41–44 (T_m)	(Wilharm et al. 1991)
Vit	20–40 (35)	6.2–8.3 (7.0–7.5)	0.2–1.3 (0.35–0.4)	18	43.7	(Katayama et al. 2014)
Biotin, thiamine	10–45 (35)	6.8–8.2 (7.5)	0.4–3.5 (2.0)	nd	48.5 (Bd)	(Paterek and Smith 1988)
Biotin	>25–45 (40)	6.2–8.2 (7.2)	0.5–3.5 (2)	~7	43–44 (Bd)	(Boone et al. 1993a)
None	20–42 (37)	6.2–8.2 (7.2)	0.3–2 (0.5)	4.4	39.2 (LC)	(Kadam et al. 1994)
None	24–45 (37)	6.8–7.4 (7.0)	0–0.678 (0–0.08)	7.6	48.3 (LC)	(Wu and Lai 2011)
Biotin, thiamine	25–42 (35)	8.2–9.2 (8.6)	0.1–1.6 (0.35)	7	40.9 (LC)	(Liu et al. 1990)
None	9–37 (30)	6.1–7.8 (6.5)	0.1–1.0 (0.35)	5	42.4 (LC)	(Wu and Lai 2011)
Biotin	5–42 (37)	5.5–9.2 (8)	0.2–1.2 (0.5)	nd	40.8 (LC)	(Oremland and Boone 1994)
None	10–45 (25)	5.5–8.0 (6.5)	0.06–1.27 (0.5)	nd	40 (T_m)	(Konig and Stetter 1982)
Biotin	13–45 (40)	6.0–7.5 (7.2)	0.1–1.2 (0.5)	5.3	39 (Bd)	(Kadam and Boone 1995)
None	25–50 (45–50)	6.0–9.0 (7.0–8.0)	0.05–1.8 (0.2–0.6)	~9.9	42 (T_m)	(Doerfert et al. 2009)

(*continued*)

Table 11 (continued)

Organism	Type strain	Source[a]	Dimensions (μm)	Flagella	Methanogenesis substrates[b]
Methanomethylovorans					
hollandica	DMS1	Freshwater sediments	1–1.5	None	Methanol, $MeNH_2$, MT, DMS
thermophila	L2FAW	UASB reactor	0.7–1.5	None	Methanol, $MeNH_2$
uponensis	EK1	Wetland sediment	0.9–1.1	nd	Methanol, $MeNH_2$, DMA, TMA, DMS, MT
Methanosalsum					
natronophilum	AME2	Hypersaline soda lake sediments	0.7–2	None	Methanol, TMA, DMS
zhilinae	WeN5	Alkaline, saline lake sediments	0.75–1.5	Mono/ ditrichous	Methanol, $MeNH_2$, DMS
Methanosarcina					
acetivorans	C2A	Marine sediments	1.7–2.1	None	ac, methanol, $MeNH_2$, CO
baltica	GS1-A	Marine sediments	1.5–3.0	Monotrichous	ac, methanol, $MeNH_2$
barkeri	MS	Sewage sludge	1.5–2.0	None	$H_2 + CO_2$, ac, methanol, $MeNH_2$, CO
horonobensis	HB-1	Deep subsurface groundwater	1.4–2.9	None	Methanol, DMA, TMA, DMS, ac
lacustris	ZS	Lake sediments	1.5–3.5	None	$H_2 + CO_2$, methanol, $MeNH_2$
mazei	S-6	Sewage sludge	1.0–3.0	None	($H_2 + CO_2$), (ac), methanol, $MeNH_2$
semesiae	MD1	Mangrove sediment	0.8–2.1	nd	Methanol, $MeNH_2$, MT, DMS
siciliae	T4/M	Marine canyon sedimetns	3.4	nd	Methanol, $MeNH_2$, DMS
soligelidi	SMA-21	Permafrost-affected soil	1.3–2.5	nd	$H_2 + CO_2$, methanol, ac
splelaei	MC-15	Sulphurous subsurface lake	2.0–4.0	nd	$H_2 + CO_2$, methanol, ac, methanol, $MeNH_2$, DMA, TMA
subterranea	HC-2	Subsurface groundwater	0.9–1.4	None	Methanol, $MeNH_2$, DMA, TMA, DMS
thermophila	TM-1	Anaerobic digestor	100[e]	None	ac, methanol, $MeNH_2$, CO

Organic growth factors	Temperature range (optimum) (°C)	pH range (optimum)	NaCl range (optimum) (M)	Doubling time[c] (h)	GC content (mol%)	References
Vit	12–40 (34–37)	6.0–8.0 (6.5–7.0)	0–0.3 (0–0.04)	11.6	34.4 (T_m)	(Lomans et al. 1999)
None	42–58 (50)	5–7.5 (6.5)	<0.3 (0–0.1)	14	37.6 (T_m)	(Jiang et al. 2005)
None	25–40 (37)	5.5–7.5 (6.0–6.5)	0–0.1 (0)	11.6	39.2 (T_m)	(Cha et al. 2013)
None	nd–43 (37)	8.2–10.2 (9.5)	0.5–3.5 (1.5)	nd	44.8 (T_m)	(Sorokin et al. 2015)
None	20–50 (45)	8.0–10 (9.2)	0.2–2.1 (0.4–0.7)	6	39.5 (T_m)	(Mathrani et al. 1988)
None	15–48 (35–40)	5.4–8.5 (6.5–7.0)	0.1–1.0 (0.2)	5.2	41 (T_m)	(Sowers et al. 1984)
None	4–27 (25)	4–8.5 (6.5–7.5)	0.1–0.7 (0.3–0.4)	84	nd	(von Klein et al. 2002)
None	25–50 (30–40)	5.5–7.5 (7.0)	0.1–0.7 (<0.2)	nd	39–44 (Bd)	(Bryant and Boone 1987)
None	20–42 (37)	6.0–7.75 (7.0–7.25)	0–0.35 (0.1)	5.0	41.4 (LC)	(Shimizu et al. 2011)
YE	1–35 (25)	4.5–8.5 (7.0)	nd (nd)	49	43.4 (T_m)	(Simankova et al. 2001)
None	25–45 (35–42)	5.5–8.0 (6.8–7.2)	0.1–0.7 (0.2–0.4)	7	42 (Bd)	(Mah and Kuhn 1984)
nd	18–39 (30–35)	6.2–8.3 (6.5–7.5)	>0– < 1.5 (0.2–0.6)	3.9	nd	(Lyimo et al. 2000)
None	15–42 (40)	5.0–7.8 (6.5–6.8)	0.2–0.8 (0.4–0.6)	7	41–43	(Elberson and Sowers 1997)
None	0–54 (28)	4.8–9.9 (7.8)	0.02–0.6 (0.02)	122.4	40.9 (LC)	(Wagner et al. 2013)
None	0–54 (33)	4.0–10.0 (6.5)	0.02–0.6 (0.05)	122.4	39.0 (LC)	(Ganzert et al. 2014)
None	10–40 (35)	5.9–7.4 (6.6–6.8)	0–0.6 (0.1–0.2)	8.9	41.5 (LC)	(Shimizu et al. 2015)
PABA	<35–55 (50)	5.5–8.0 (6.0–7.0)	0–1.2 (0.6)	5.3	42 (Bd)	(Zinder et al. 1985)

(*continued*)

Table 11 (continued)

Organism	Type strain	Source[a]	Dimensions (μm)	Flagella	Methanogenesis substrates[b]
vacuolata	Z-761	Methanogenic digestor	1.0–2.0	None	$H_2 + CO_2$, ac, methanol, $MeNH_2$
Methanosaeta					
concilii	GP6	Sewage sludge	0.8–1.3 × 2.0–7.0	None	ac
harundinacea	8Ac	UASB reactor	0.8–1.0 × 3.0–5.0	None	ac
thermophila	P_T	Thermophilic anaerobic digestor	0.8–1.3 × 2.0–6.0	None	ac
Methanimicrococcus					
blatticola	PA	Cockroach hindgut	0.8	nd	Methanol, $MeNH_2$, H_2
Methermicoccus					
shengliensis	ZC-1	Oilfield	0.7–1.0	Flagellated	Methanol, $MeNH_2$, MACs

Abbreviations: *nd* not determined, *ac* acetate, *$MeNH_2$* methylamines, *DMS* dimethylsulfide, *MT* methanethiol, *TMA* trimethylamine, *DMA* dimethylamine, *DMEA N,N*-dimethylethanolamine, *MACs* methoxylated aromatic compounds, *vit* vitamins, *TP* trypticase peptone, *YE* yeast extract, *CoM* 2-mercaptoethanesulfonic acid (conenzyme M), *PAPA p*-aminobenzoate, *Bd* buoyant density method, T_m melting point method, *LC* liquid chromatography

[a]Environment from which the type strain was isolated

[b]Parentheses means utilized by some strains, but not all strains

[c]Doubling time of the type strain under optimal growth conditions of temperature, pH, and NaCl

[d]Flagellated in some strains, but not all strains

[e]Irregular aggregates composed of coccoid cells

[f]The minimum growth temperature is predicted by applying the Ratkowsky model to temperature growth data

Organic growth factors	Temperature range (optimum) (°C)	pH range (optimum)	NaCl range (optimum) (M)	Doubling time[c] (h)	GC content (mol%)	References
None	18–42 (37–40)	6.0–8.0 (7.5)	0.1–0.5 (0.1)	nd	36.3 (T_m)	(Zhilina and Zavarzin 1987b)
Vit	>10– ≤ 45 (35–40)	≥6.6– < 7.8 (7.1–7.5)	nd (nd)	65	49.0 (T_m)	(Patel and Sprott 1990)
YE/TP	25–45 (34–37)	6.5–9.0 (7.2–7.6)	nd (nd)	28	55.7 (T_m)	(Ma et al. 2006)
None	>30– ≤ 70 (55–60)	>5.5– ≤ 8.4 (6.5–6.7)	nd (nd)	35.8	52.7–54.3 (LC)	(Kamagata and Mikami 1991)
ac, CoM, YE, tryptic soy broth, vit	20–40 (39)	6.8–8.2 (7.2–7.7)	0–0.3 (<0.1)	3.1	nd	(Sprenger et al. 2000)
YE/TP	50–70 (65)	5.5–8.0 (6.0–6.5)	0.2–1.1 (0.3–0.5)	5	56 (T_m)	(Cheng et al. 2007; Mayumi et al. 2016)

2.5 Methanopyrales

The order of *Methanopyrales* is represented by only one species, *Methanopyrus kandleri*. It is hyperthermophilic and produces methane by CO_2 reduction with H_2. Genomic sequence analysis of *M. kandleri* suggests that it is closely related to *Methanobacteriales* and *Methanococcales* but possesses unusual features.

The phylogenetic position of *M. kandleri* is ambiguous. The phylogenic analyses based on 16S rRNA gene (Burggraf et al. 1991), elongation factor 1α (Rivera and Lake 1996), and transcription factors (Brochier et al. 2004) suggested that *M. kandleri* is distantly related to other methanogens and represent a separate lineage emerging at the base of the euryarchaeal phylum. On the other hand, phylogenetic analyses based on methyl coenzyme M reductase (MCR) operons (Nolling et al. 1996), translation factors (Brochier et al. 2004), and whole genome sequences (Slesarev et al. 2002; Gao and Gupta 2007) suggested that *M. kandleri* is more closely related to other methanogens and grouped with *Methanobacteriales* and *Methanococcales*. Indeed, *M. kandleri* encodes the core of proteins shared uniquely by methanogens such as proteins evolved in the methanogenesis pathway, and it closely resembles other methanogens in terms of local gene order. Therefore, *M. kandleri* very likely belongs to the monophyletic methanogen group and not a deep-branch close to the root of archaea. The deep branching in 16S rRNA phylogenetic tree is probably due to a very high GC content of *M. kandleri*, a characteristic shared by hyperthermophiles outside the methanogen group.

The genome of *M. kandleri* displays several unusual features (Slesarev et al. 2002; Brochier et al. 2004). The RNA polymerase subunit H is replaced by a homologous protein from a distantly related archael lineage. The transcription factor S (TFS) is missing. The diversity of predicted signal transduction systems and DNA-binding proteins are underrepresented. The histone protein is formed by a fusion of two monomers into a single peptide with two tandemly repeated histone folds. *M. kandleri* possesses a unique topoisomerase, Topo V, which is related to eukaryotic topoisomerase I (Slesarev et al. 1994). These unusual features suggest a high level of gene loss, gene capture, and gene fusion in this archaeon.

Methanopyrus kandleri is the only methanogen known so far that catalyzes methanogenesis at temperatures higher than 100 °C. It reduces CO_2 with H_2 for methanogenesis. It is an obligate chemolithoautotroph that uses CO_2 as the sole carbon source. Ammonium and sulfide are the nitrogen and sulfur sources, respectively. The cells are rod-shaped and stain Gram positive. The cell wall is double layered. The inner layer is composed of a new type of pseudomurein, containing ornithine and lysine. The outer layer is detergent-sensitive, indicating a protein composition. The core lipid is composed of an unsaturated terpenoid lipid, which is considered the most primitive lipid in the evolution of membranes (Hafenbradl et al. 1993). The cells are motile via flagella arranged as polar tufts. They grow at temperatures ranging from 84 °C to 110 °C, with an optimum of 98 °C. The range of pH for growth is 5.5–7, with an optimum of 6.5. The optimal NaCl concentration for growth is 2.0% (w/v). The GC content of its DNA is 60 mol%. *M. kandleri* was

isolated from hydrothermally heated deep-sea sediments and from a shallow marine hydrothermal system (Kurr et al. 1991).

2.6 Methanocellales

The order *Methanocellales* is represented by one family and genus, *Methanocellaceae* and *Methanocella*, respectively. Three species have been described, and they are distinguished by 16S rRNA sequence similarities below 92% and differences in growth temperatures, substrates for methanogenesis, possession of a flagellum, doubling time and NaCl range. The low 16S rRNA sequence similarities suggest potential separation into more genera, which is supported by comparative genomic studies (Sakai et al. 2011; Lyu and Lu 2015). The *Methanocella* are all capable of producing methane from H_2/CO_2, but acetate is required for growth. Formate can also be used as an alternative substrate by two species.

Members of *Methanocellales* are isolated from rice soils. They do not appear to grow autotrophically due to the requirement of acetate for growth. Sulfide and ammonium is a sufficient sulfur and nitrogen source, respectively. Cells are typically rods, but coccoid cells are also seen during late stage of growth. Cells can form a unique lens-shaped colony. Cell envelopes are composed of an S-layer as determined in *Methanocella avoryzae*. Cell envelopes have not been determined in *Methanocella paludicola* and *Methanocella conradii*, but they are resistant to lysis by 2.0% and 0.1% of SDS, respectively. A flagellum is also present in both *M. avoryzae* and *M. conradii*, but not in *M. paludicola*. Cellular lipids have not been determined. They all grow optimally in the absence of NaCl and at neutral pH. The optimal growth temperatures range from 37 °C to 55 °C. Descriptive properties of the *Methanocellales* are summarized in Table 12. Further information can be found in Sakai et al. (2008, 2010), and Lü and Lu (2012b).

2.7 Methanomassiliicoccales

The order *Methanomassiliicoccales* is represented by one family and genus, *Methanomassiliicoccaceae* and *Methanomassiliicoccus*, respectively (Dridi et al. 2012; Iino et al. 2013). Although a few enrichment cultures are available, only one species *Methanomassiliicoccus luminyensis* has been described (Borrel et al. 2012a, 2013; Dridi et al. 2012; Iino et al. 2013). This species was isolated from human faeces, and it reduces methanol with H_2 to produce methane. However, genomic, transcriptomic and *in vivo* studies suggest that members of *Methanomassiliicoccales* also reduce tri-, di- and monomethylamine with H_2 (Poulsen et al. 2013; Borrel et al. 2014; Brugere et al. 2014). Cells are non-motile cocci and lysed in 0.1% (w/v) SDS. It grows optimally at 1% of NaCl, 37 °C and at pH 7.6. Descriptive properties of the *Methanomassiliicoccales* are summarized in Table 13. Further information can be found in Dridi et al. (2012) and Brugere et al. (2014).

Table 12 Descriptive characteristics of the species of the genus *Methanocella*

	Methanocella		
Character	*paludicola*	*avoryzae*	*conradii*
Type strain	SANAE	MRE50	HZ254
Cell width (μm)	0.3–0.6	0.4–0.7	0.2–0.3
Cell length (μm)	1.8–2.4	1.3–2.8	1.4–2.8
Flagellum	None	Single	Single
Substrates for methanogenesis	$H_2 + CO_2$, formate	$H_2 + CO_2$, formate	$H_2 + CO_2$
Acetate requirement	+	+	+
Yeast extract stimulates growth	+	+	+
Nitrogen source	NH_3	NH_3[a]	NH_3[a]
Sulfur source	S^{2-}[b]	S^{2-}	S^{2-}[b]
Temperature range (°C)	25–40	37–55	37–60
Temperature optimum (°C)	35–37	45	55
pH range	6.5–7.8	6.0–7.8	6.4–7.2
pH optimum	7.0	7.0	6.8
NaCl range (%, w/v)	0–0.1	0–2	0–0.5
NaCl optimum (%, w/v)	0	0–0.2	0–0.1
GC content (mol%)[c]	54.9 (G_s)	54.6 (G_s)	52.7 (G_s)
Doubling time (h)	100.8	8.0	6.4–7.2
Source	Rice soil	Rice soil	Rice soil
References	(Sakai et al. 2008)	(Sakai et al. 2010)	(Lü and Lu 2012b)

[a]May use N_2 according to genomic predictions (Lyu and Lu 2015)
[b]May use SO_4^{2-} according to genomic predictions (Erkel et al. 2006; Sakai et al. 2011)
[c]G_s genome sequencing

2.8 Potential Novel Taxa

Through metagenomics guided discovery, a few potential novel taxa of methanogens have been proposed recently. That includes a euryarchaeon, *Candidatus* 'Methanofastidiosa', and members of the archaeal phyla Bathyarchaeota (previously known as the Miscellaneous Crenarchaeota Group) and Verstraetearchaeota previously represented by the Terrestrial Miscellaneous Crenarchaeota Group or TMCG) (Evans et al. 2015; Nobu et al. 2016; Vanwonterghem et al. 2016). They are all predicted to reduce different methylated compounds with H_2 for methanogenesis, but members of Bathyarchaeota and Verstraetearchaeota may also use complex substrates such as lactate. Pure cultures are still needed to further confirm these findings, which would likely not only lead to proposals of novel methanogen classes but establishment of methanogen taxa outside the Euryarchaeota.

Table 13 Descriptive characteristics of *Methanomassiliicoccus luminyensis*

Character	*M. luminyensis*
Type strain	B10
Cell diameter (μm)	0.7–1.0
Flagellum	None
Substrates for methanogenesis	H_2 + methanol/TMA[a]/DMA[b]/$MeNH_2$[c]
Acetate requirement	−
Yeast extract requirement	+
Temperature range (°C)	25–45
Temperature optimum (°C)	37
pH range	7.2–8.4
pH optimum	7.6
NaCl range (%, w/v)	0.1–1.5
NaCl optimum (%, w/v)	1
GC content (mol%)	59.9 (G_s)[d]
Doubling time	nd[e]
Source	Human faeces
References	(Dridi et al. 2012; Brugere et al. 2014)

[a]*TMA* trimethylamine
[b]*DMA* dimethylamine
[c]*$MeNH_2$* monomethylamine
[d]G_s genome sequencing
[e]*nd* not determined

3 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). This cosmopolitan distribution of methanogens could be associated with their growth largely relied on only simple substrates such as H_2/CO_2, acetate, formate and other C1 compounds, which are widely available across ecosystems where complex substrates have to be degraded into simple substrates to drive the carbon cycle. A recent metagenomics survey has also predicted the presence of complex fermentation and β-oxidation pathways in the putative Bathyarchaeota methanogens, suggesting the ability of using complex substrates may be advantageous for methanogens that thrive in environments where degradation of complex substrates could be very slow (Evans et al. 2015). In addition, some methanogens as described in the taxonomy and phylogeny section can also survive extreme environmental conditions such as hyperthermophilic, psychrophilic, piezophilic, halophilic, alkaliphilic and acidophilic, which further expands their habitats.

In some natural habitats, methanogens are also present in microoxic 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 by O_2-uptake aerobes (Brusa et al. 1987; Belay et al. 1988; Leadbetter and Breznak 1996). *Methanocellales* methanogens are prevalent in rice rhizosphere, which is transiently oxic, and their genomes encode a unique set of antioxidant enzymes, which may explain an aerotolerant life style (Erkel et al. 2006; Sakai et al. 2011; Lü and Lu 2012a; Lyu and Lu 2015, 2017).

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 reductions of these compounds are 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 are their metabolic versatility as well as lower sensitivity to O_2. The ecology of each methanogen order is discussed below.

3.1 Methanobacteriales

Members of the *Methanobacteriales* are widely distributed in anaerobic habitats such as marine and freshwater sediments, soils, animal gastrointestinal tracts, anaerobic sewage digestors, and geothermal habitats. *Methanobacterium* has been cultivated from marine and freshwater sediments, groundwaters, soils, anaerobic digestors, and animal gastrointestinal tracts and has also been detected as endosymbionts in anaerobic ciliate (Embley et al. 1992). *Methanobrevibacter* has been isolated from rumens, feces, termite hindguts, human subgingival plaque, anaerobic digestors, and decaying wood tissues. *Methanosphaera* has only been isolated from animal gastrointestinal tracts but has been detected in anaerobic digestors (Weiss et al. 2008). *Methanothermobacter* has been cultivated from thermophilic anaerobic digestors and natural gas and oil fields (Nazina et al. 2006; Mochimaru et al. 2007). *Methanothermus* has only been isolated from solfarata hot springs.

3.2 Methanococcales

Members of the *Methanococcales* have all been isolated from marine environments. *Methanococcus* has been isolated from marine and salt marsh sediments.

Methanothermococcus has been isolated from coastal geothermally heated sea sediments, deep sea hydrothermal vents, and reservoir water from marine oil fields (Nilsen and Torsvik 1996) and has been detected in continental high-temperature oil reservoirs (Orphan et al. 2000) and tropical hypersaline coastal lagoons (Clementino et al. 2008). *Methanocaldococcus* has only been isolated from deep sea hydrothermal vents. *Methanotorris* has been isolated from shallow and deep sea hydrothermal vents. Environmental 16S rRNA sequences closely related to *Methanococcales* have also been detected in anaerobic granular sludge (Liu et al. 2002; Diaz et al. 2003). Quantitative real-time PCR assays have also recently shown possible presence of *Methanococcales* in forest and grassland soils, but how specific the primers were remain unknown (Hofmann et al. 2016). Since this finding is very much unexpected, sequence data is also needed to make conclusive taxonomy inference.

3.3 Methanomicrobiales

Members of the *Methanomicrobiales* are widely distributed in anaerobic habitats, including marine and freshwater sediments, anaerobic sewage digestors, rice paddies, oil fields, groundwaters, and animal gastrointestinal tracts. Anaerobic digestors and sewage sludge are common habitats of *Methanoculleus*, *Methanofollis*, *Methanocorpusculum*, *Methanospirillum*, and *Methanomicrobium*. From marine sediments, species belonging to *Methanoculleus*, *Methanogenium*, and *Methanolacinia* have been isolated. From freshwater sediments, species belonging to *Methanoculleus*, *Methanogenium*, and *Methanocorpusculum* have been isolated. From rice roots and rice-field soils, species belonging to *Methanoculleus* have been isolated, and environmental clone sequences closely related to *Methanoculleus* and *Methanogenium* have been identified (Kudo et al. 1997). *Methanomicrobium mobile* has been isolated from bovine rumen (Paynter and Hungate 1968). *Methanoplanus endosymbiosus* lives as endosymbiont of the marine ciliate *Metopus contortus* (Bruggen et al. 1986).

3.4 Methanosarcinales

Members of the *Methanosarcinales* are widely distributed in marine and freshwater sediments, anaerobic digestors, and animal gastrointestinal tracts. *Methanosarcina* has been isolated from marine and freshwater sediments, anaerobic digestors, and rumen and has been detected in rice paddies (Chin et al. 2004; Krüger et al. 2005; Lu et al. 2005). *Methanococcoides* and *Methanolobus* have been isolated from aquatic environments with salinity near that of seawater. The habitats of *Methanohalobium*, *Methanohalophilus*, and *Methanosalsum* are restricted to hypersaline environments. *Methanomethylovorans* has been isolated from freshwater sediments and bioreactors. *Methanosaeta* has been isolated from freshwater sediments and anaerobic digestors and has been detected in rice paddies (Chin et al.

2004; Krüger et al. 2005) and marine sediments (Purdy et al. 2002). *Methanimicrococcus* has been isolated from cockroach hindgut and has been detected in anaerobic digestors (Weiss et al. 2008).

3.5 Methanocellales

All members of the *Methanocellales* have been isolated from rice soils, but they are also widely distributed in terrestrial ecosystems such as wetland soils and freshwater sediments based on environmental DNA sequence surveys (Conrad et al. 2006; Sakai et al. 2008, 2010; Lü and Lu 2012b). *Methanocellales* have been studied extensively in rice soils both *in situ* and in microcosms, revealing the following unique ecophysiological features. (i) They are closely associated with rice roots where they can actively convert plant-derived carbon into biomass and methane (Lu and Conrad 2005); (ii) they are able to tolerate the microaerophilic conditions around the rice roots, probably due to a robust antioxidant system encoded in their genomes (Erkel et al. 2006; Conrad et al. 2008; Sakai et al. 2011; Lü and Lu 2012a; Lyu and Lu 2017); (iii) they tend to become more active under low H_2 but high temperature conditions (Lu et al. 2005; Wu et al. 2006; Peng et al. 2008; Sakai et al. 2009); and (iv) they frequently form syntrophic relationships with fatty acid degrading bacteria (Lueders et al. 2004; Liu et al. 2011; Rui et al. 2011; Gan et al. 2012). Additional ecophysiological features have also been revealed by studying *Methanocellales* in acidic peat soils, tank bromeliads and arid soils, suggesting that at least some members of *Methanocellales* could survive moderately acidic conditions, interact with plants other than rice such as *Sphagnum* in peat soil and tank bromeliads in neotropical forests, and tolerate desiccation (Sizova et al. 2003; Cadillo-Quiroz et al. 2010; Martinson et al. 2010; Angel et al. 2011, 2012).

3.6 Methanomassiliicoccales

Only one member of *Methanomassiliicoccales* has been isolated into pure culture from human feces (Dridi et al. 2012). Metagenomic analysis with human feces enrichment samples also revealed two new candidate species *Candidatus* 'Methanomassiliicoccus intestinalis' and *Candidatus* 'Methanomethylophilus alvus' (Borrel et al. 2012, 2013). This apparent common association with human suggests that *Methanomassiliicoccales* may play a role in human health. Due to their ability to metabolize trimethylamine into methane, it has been proposed that *Methanomassiliicoccales* may prevent or limit human diseases that are induced by trimethylamine (Brugere et al. 2014). However, distribution of *Methanomassiliicoccales* is not restricted to the human gut. An enrichment culture from anaerobic digester has led to the proposal of another candidate species *Candidatus* 'Methanogranum caenicola' (Iino et al. 2013). Environmental DNA sequence survey has suggested that *Methanomassiliicoccales* could be grouped into two clades, a

gastro-intestinal tract clade that is largely associated with animal samples, and an environmental clade which includes mainly aquatic and terrestrial samples.

3.7 Other Methanogen Candidates

Methanogenesis pathways have been predicted from a euryarchaeon, *Candidatus* 'Methanofastidiosa', members of the newly proposed archaeal phyla Verstraetearchaeota and Bathyarchaeota (Evans et al. 2015; Nobu et al. 2016; Vanwonterghem et al. 2016). *Candidatus* 'Methanofastidiosa' belongs to the uncultivated WSA2 or Arc I cluster, which has long been identified as a core euryarchaeal group in anaerobic digestion that was previously thought to use H_2/CO_2 or formate for methanogenesis (Hendrickson et al. 2004; Nakamura et al. 2013). However, genomic data has now proposed that WSA2 methanogens may conduct methylated thiol reduction with H_2 (Nobu et al. 2016). This suggests that they may be able to bridge the carbon and sulfur cycles, which may enable competition with CO_2 reducing methanogens and sulfate reducers. Previously loosely classified as the Terrestrial Miscellaneous Crenarchaeota Group or TMCG, members of Verstraetearchaeota methanogens also had their first metagenomes reconstructed from anaerobic digesters, but environmental DNA sequence survey could extend their distribution to wetlands, freshwater sediments, and hydrocarbon-rich environments (Vanwonterghem et al. 2016). Previously known as the MCG or Miscellaneous Crenarchaeotal Group, the recently proposed Bathyarchaeota have been found in deep ocean and freshwater sediments, and they are particularly present in high abundance within sulfate-methane transition zones (Vetriani et al. 1999; Inagaki et al. 2003; Gagen et al. 2013; Evans et al. 2015). Likewise, their first metagenomes were recovered from coal-bed methane wells in an ocean basin (Evans et al. 2015). Although those novel methanogen candidates suggest the diversity of methanogens would be much higher than previously anticipated, interpretation of their environmental distribution and ecophysiology should be cautious. This is because no pure cultures have been available so far, and it remains elusive if every member of the WSA2, Verstraetearchaeota and Bathyarchaeota could also be capable of methanogenesis as predicted from a limited number of metagenomes.

4 Research Needs

A few established methanogen orders are still underrepresented by cultivated members. *Methanocellales* is only represented by one genus, and both *Methanomassiliicoccales* and *Methanopyrales* are represented by just one species. Discovery and isolation of new strains will certainly add to our knowledge of the diversity of those orders. Isolations of new strains are also necessary to support the classification of *Methanimicrococcus blatticola* and *Methermicoccus shengliensis* as separate families within the order *Methanosarcinales* and expand our knowledge of the diversity of *Methanosarcinales*. On the other hand, since the *Methanosarcinales* can use a

relatively broad range of substrates for methanogenesis, isolation of new strains suitable for industrial purposes can be valuable.

Recent culture-independent studies have revealed the presence of novel phylogenetic groups of methanogens. Their isolation and characterization will also 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). In addition, many novel methanogen candidates are still only represented by metagenomes, such as the *Candidatus* 'Methanofastidiosa' and members of the archaeal phyla Verstraetearchaeota and Bathyarchaeota (Evans et al. 2015; Nobu et al. 2016; Vanwonterghem et al. 2016).

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. For instance, the genome-wide pairwise average nucleotide identity or ANI has been increasingly used to delineate species (Goris et al. 2007). However, convenient tools and methods will still need to be developed to meet the needs for analyzing large genome dataset. The Joint Genome Institute or JGI has been a pioneer in this filed, which has developed an Integrated Microbial Genome online pipeline to tackle the big data challenge (Markowitz et al. 2007a, b, 2009). Another grand challenge is to associate the environmental meta-data with the sequence data, which can provide enormous ecophysiological context for not only interpreting the sequence data from a single project but uncovering new trends across different projects.

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