16 The Family *Methanopyraceae*

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Abstract
The family *Methanopyraceae* currently consists of a single genus with a single species: *Methanopyrus kandleri*. It is a rod-shaped chemolithoautotrophic methanogenic archaeon that grows optimally at 98 °C and grows up to 110 °C and possibly higher under high hydrostatic pressure, making it the most extreme thermophile among the methanogens. No growth is observed below 84 °C. Strains of *M. kandleri* were isolated from marine hydrothermal vent systems. The organism contains 2,3-di-*O*-phytanyl-*sn*-glycerol and 2,3-di-*O*-geranylgeranyl-*sn*-glycerol lipids. In 16S rRNA phylogenetic trees, it forms a very deep branch near the root of the Archaea, remote from the other lineages of methanogens. However, in trees based on concatenated alignments of ribosomal proteins or proteins involved in transcription, *M. kandleri* consistently groups with other methanogens, suggesting that the methanogens are a monophyletic group of Archaea.

Taxonomy

Methanopyraceae Huber and Stetter 2002 (Validation List no. 85, 2002; Effective Publication: Huber and Stetter 2001a, 353)

Me.tha.no.py.ra.ce'ae. N.L. masc. n. *Methanopyrus* type genus of the family. suff. *–aceae*, ending to denote a family; N.L. fem. pl. n. *Methanopyraceae*, the *Methanopyrus* family. Type genus: *Methanopyrus*.

The family *Methanopyraceae* was created in 2001 to harbor a single species of hyperthermophilic hydrogenotrophic methanogenic Archaea, *Methanopyrus kandleri*. It was first isolated in the early 1990s from submarine hydrothermal vent systems in the Guaymas Basin in the Gulf of California, Mexico, and near Iceland (Kurr et al. 1991). In 16S rRNA-based phylogenetic trees, this species forms a very deep branch near the root of the Archaea, remote from the other lineages of methanogens.

Thus far, the monospecific genus *Methanopyrus* is the only genus described within the family. Because of the deep branch in the 16S rRNA-based phylogenetic tree, a separate class, the *Methanopyri*, a separate order, the *Methanopyrales*, and a separate family, the *Methanopyraceae*, were created for the classification of *M. kandleri* (Garrity and Holt 2001; Huber and Stetter 2001a, b).

Phylogenetic Structure of the Family and Its Genera

There are not many prokaryotes for which the phylogenetic position has been disputed so much as in the case of *Methanopyrus kandleri*. *Figure 16.1* presents a 16S rRNA phylogenetic tree that shows its location on a very deep branch near the root of the Archaea, remote from the other lineages of methanogens. A maximum likelihood tree shows a topology similar to that of the neighbor-joining tree illustrated. Early studies suggested that, although more euryarchaeal than crenarchaeal signature features are present in the 16S rRNA, it shows more crenarchaeal signature features than any other known euryarchaeal rRNA (Burggraf et al. 1991).

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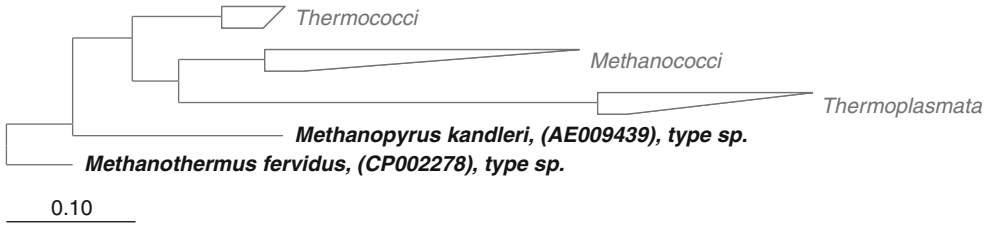

Fig. 16.1
Phylogenetic reconstruction of the family *Methanopyraceae* based on 16S rRNA and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). The sequence dataset and alignment were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as out-groups. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

Based on phylogenetic analyses of its 16S rRNA gene sequence, the *M. kandleri* lineage was proposed to branch off close to the universal root of life. Analyses of certain other genes strengthened the evidence for a *Methanopyrus*-proximal root of life. One such study was based on the analysis of transfer RNA and aminoacyl-tRNA synthetase genes. The intraspecies genetic distances between tRNAs cognate for different amino acids, between the initiator and elongator tRNAs for methionine, and between potentially paralogous pairs of aminoacyl-tRNA synthetases were found to be at a minimum within the *M. kandleri* genome. The findings were presented as evidence for a close genotypic relationship between *Methanopyrus* and the last universal common ancestor of life (Xue et al. 2005). A comparative study of valine- and isoleucine-tRNA synthetases was made for a number of *Methanopyrus* isolates from different geographic origins: the Mid-Atlantic Ridge, the Guaymas Basin, and Kolbeinsey Ridge, Iceland, as well as gene fragments cloned from environmental samples. Based on the genetic distance between the sequences, gene fragments recovered from the Central Indian Ridge were considered the most primitive among the sequences studied (Yu et al. 2009). Analysis of the elongation factor 1-α suggested that *Methanopyrus* may have diverged from the crenarchaeote branch before the eukaryote and the crenarchaeote lineages separated (Rivera and Lake 1996). A study of methyl coenzyme M reductase operon (*mcrBDCGA*) sequences also supported the placement of *M. kandleri* on a separate methanogen lineage (Nölling et al. 1996).

However, other more comprehensive analyses failed to confirm the special location of *Methanopyrus* in the universal tree of life. In trees based on concatenated alignments of ribosomal proteins and trees based on gene content, *M. kandleri* consistently groups with other methanogens (Slesarev et al. 2002). To solve the *M. kandleri* phylogeny paradox, concatenated datasets of 14 proteins involved in transcription and 53 ribosomal proteins were compared for *Methanopyrus* and members of the *Methanococcales* and the *Methanobacteriales*. *Methanopyrus* groups with the *Methanococcales* and the *Methanobacteriales* in the translation tree, whereas it emerged at the base of the euryarchaeotes in the transcription tree. It was concluded that the unusual placement of *M. kandleri* in the 16S rRNA tree may be the result of a reconstruction artifact due to the high evolutionary rates of the components of its transcription apparatus (Brochier et al. 2004). Placement of *Methanopyrus* together with the *Methanobacteriales* and the *Methanosarcinales* was therefore proposed (Luo et al. 2009).

Genome Analysis

The genome of the type strain of *Methanopyrus kandleri* (GenBank accession number AE009439) consists of a single circular chromosome of 1,694,969 base pairs, encoding for 1,692 proteins. The G+C content is 62.1 mol% (values of 59 and 60 mol% were earlier reported based on thermal denaturation and HPLC, respectively). Thirty-nine genes for structural RNAs were identified. The genome contains the same genes implicated in the pathway of the reduction of carbon dioxide to methane as found in the chemolithoautotrophic thermophilic methanogens *Methanocaldococcus jannaschii* and *Methanothermobacter thermautotrophicus*, suggesting that archaeal methanogens are monophyletic. Relatively few events of putative lateral gene transfer were identified. Among the unique enzymes present are type 1B DNA topoisomerase V and a two-subunit reverse gyrase. Two-component histidine kinase regulatory systems, which are present, e.g., in *Methanothermobacter thermautotrophicus*, are lacking. Overall, the regulatory and signaling systems are scaled down in comparison with other Archaea (Slesarev et al. 2002).

The proteins encoded by the genome show a high content of negatively charged amino acids, a possible adaptation to high intracellular ionic content; the enzymes typically require high ionic concentrations for activity and stability (see below). Accordingly, the median isoelectric point of the proteins encoded by the genome is ~5, close to the values found for the extremely halophilic Archaea of the family *Halobacteriaceae*.

Phages

No phages attacking *Methanopyrus* strains have yet been described.

Table 16.1

Comparison of the properties of *Methanopyrus kandleri*, the sole representative of the family *Methanopyraceae*, with the properties of the three other families of thermophilic methanogenic Archaea

	Methanopyrus	*Methanothermaceae*	*Methanocaldococcaceae*	*Methermicoccaceae*
Morphology	Long rigid rods	Straight to slightly curved rods	Cocci	Cocci
Cell wall	Pseudomurein with outer protein layer	Pseudomurein with outer protein layer	Protein S-layer	Probably protein S-layer
Core lipids	2,3-di-*O*-phytanyl-*sn*-glycerol (archaeol) and 2,3-di-*O*-geranylgeranyl-*sn*-glycerol	Archaeol, caldarchaeol, and others	Archaeol, hydroxyarchaeol, caldarchaeol, cyclic archaeol	NR
Substrates for methanogenesis	H_2/CO_2	H_2/CO_2	H_2/CO_2, (formate)	Methanol, methylamine, trimethylamine
Temperature range (°C)	84–110	55–97	45–91	50–70
Salinity range (% NaCl)	0.2–4	NR	0.4–6	1.2–6.4
G+C content of the DNA (mol%)	59–62	32–33	31–33	56

Data were derived from the sources cited in this chapter, from Oren (2014a, b, c) and from Whitman et al. (2006)
NR Not reported

Phenotypic Analyses

Methanopyrus Kurr et al. 1992 (Validation List no. 41, 1992; Effective Publication: Kurr et al. **1991**, 245)

Me.tha.no.py'rus. N.L. neut. n. *methanum* [from French n. *méth*(*yle*) and chemical suffix *–ane*] methane; N.L. pref. *methano-*, pertaining to methane; Gr. neut. n. *pur*, fire; N.L. masc. n. *Methanopyrus*, the "methane fire" (the hyperthermophilic methanogen). The type species, and thus far single species, is *Methanopyrus kandleri*, with type strain AV19 (= DSM 6324 = JCM 9639 = NBRC 100938).

Methanopyrus kandleri cells are rod-shaped, occurring singly and in chains. Cells are about 0.5 µm in diameter, and their length varies between 2 and 14 µm. Flocs and stringlike aggregates are formed when cultures are shaken. *Methanopyrus* is an obligately aerobic chemolithoautotroph that obtains its energy from the reduction of CO_2 with H_2 as the electron donor with the formation of methane. Formate, acetate, methanol, and methylamines are not used for methane formation. Growth is observed between 84 °C and 110 °C with an optimum at 98 °C. Under high hydrostatic pressure some strains may grow up to 122 °C (Takai et al. 2008). Cells grow within a wide range of salt concentrations (0.2–4 % NaCl, optimum 2 % NaCl) at pH values between 5.5 and 7 (optimum pH 6.5) (*Table 16.1*).

Cells divide by septum formation and stain Gram-positive (Huber et al. 1989). Cells are very rigid, and in the electron microscope they show a two-layered cell wall. The inner layer is composed of pseudomurein which contains ornithine in addition to lysine as diamino acid and lacks *N*-acetylglucosamine. It consists of L-glutamic acid, L-alanine acid, L-lysine acid, L-ornithine acid, *N*-acetylgalactosamine acid, and talosaminuronic acid. The pseudomurein layer is surrounded by an outer layer which is sensitive to treatment with sodium dodecyl sulfate and probably consists of protein (Kurr et al. 1991). According to Kurr et al. (1991), the thickness of each layer is about 20 nm, but Huber and Stetter (2001c) gave a total width of ~10–15 nm for the combined layers.

M. kandleri was reported to be motile by means of polar tufts of flagella (Huber et al. 1989; Kurr et al. 1991). However, no genes for archaellin/flagellin were annotated in the genome (Slesarev et al. 2002). A search of the genome sequence yielded two putative pili operons, but no signs for the presence of genes for archaella were found (Sonja-Verena Albers, Max Planck Institute for Terrestrial Microbiology, Marburg, personal communication).

The core lipids of *Methanopyrus* are 2,3-di-*O*-phytanyl-*sn*-glycerol and the unsaturated 2,3-di-*O*-geranylgeranyl-*sn*-glycerol (Hafenbradl et al. 1993). The presence of the latter unsaturated terpenoid lipid is considered to be a primitive feature. During the biosynthesis of polar lipids, the fully unsaturated pre-diethers (2,3-di-*O*-geranylgeranyl-*sn*-glycerol as core lipid) are linked stepwise with polar groups and finally reduced to saturated membrane lipids (Hafenbradl et al. 1996). Thus, hydrocarbon chains with one to four double bonds can be detected (Nishihara et al. 2002). Sprott et al. (1997) also reported the presence of minor amounts of caldarchaeol

tetraether lipids and hydroxyarchaeols. Nonpolar lipids account for about 50 % by weight of the total lipids, with a high proportion of 2,3-di-*O*-geranylgeranyl-*sn*-glycerol, 2,3-di-*O*-phytanyl-*sn*-glycerol, and geranylgeraniol. Two-dimensional thin layer chromatography of the polar lipids shows mostly glycolipids and minor amounts of aminophospholipids, phosphoglycolipids, and other phospholipids. Purification yielded three diglycosyl lipids, one triglycosyl lipid, and six lipids with five glycosyl groups. The glycosyl moieties contain glucose, galactose, and mannose (Hafenbradl et al. 1996). A more detailed study of the polar lipids showed an even greater variety of archaeol glycolipids with one to six hexose units composed primarily of mannose, with in addition glucose, galactose, and *N*-acetylglucosamine (Sprott et al. 1997). A choline-containing phospholipid is also present (Nishihara et al. 2002).

As polyamines *M. kandleri* contains spermidine, spermine, and agmatine. Long and branched polyamines are lacking (Hamana et al. 1999). Its tRNAs contain an exceptionally large number (31) of modified nucleosides. Many have a 2′-*O*-methyl group. The novel modified nucleoside N^6-acetyladenosine was characterized from *M. kandleri* tRNA (Sauerwald et al. 2005).

Elemental sulfur is reduced to H_2S, which causes the cells to lyse (Huber and Stetter 2001c; Kurr et al. 1991).

Isolation, Enrichment, and Maintenance Procedures

All extant *Methanopyrus* isolates were obtained from enrichment cultures based on mineral media in the presence of 1.5 % NaCl (e.g., 10 ml portions in 120 ml serum bottles) incubated at 100 °C under an atmosphere of H_2-CO_2 (4:1, 300 kPa) (Huber et al. 1989; Huber and Stetter 2001c; Kurr et al. 1991). Elemental sulfur should not be added: reduction of sulfur to H_2S leads to rapid lysis of *Methanopyrus* cells. Colonies can be isolated on medium solidified with Gelrite® gellan gum following 1 week of incubation at 98 °C under H_2/CO_2 in stainless steel anaerobic jars. Colonies appear after about 7 days.

Methanopyrus can survive under anaerobic conditions for long periods at low temperatures. Isolation was still possible from hydrothermal vent samples that had been stored anaerobically for 7 months at 4 °C (Kurr et al. 1991). For long-term storage cells can be suspended in liquid nitrogen in the presence of 5 % dimethyl sulfoxide (Huber and Stetter 2001c).

Physiological and Biochemical Features

Methanopyrus kandleri is a hyperthermophile that shows optimal growth at 98 °C. The doubling time under optimal conditions is 50 min. At 110 °C, near the maximum temperature enabling growth, a doubling time of 8 h is still possible (Huber et al. 1989). *M. kandleri* strain 116, isolated from the Kairei hydrothermal field on the Central Indian Ridge, can proliferate at 116 °C when incubated at a pressure of 0.4 MPa and even at 122 °C at 20 MPa pressure (Takai et al. 2008). This is still the highest temperature at which cell growth and multiplication has been documented to date.

The only mechanism for energy generation is the reduction of carbon dioxide to methane with hydrogen as the electron donor. None of the other substrates used by many other methanogenic archaea (acetate, formate, methanol, methylated amines) supports growth of *Methanopyrus* (Huber and Stetter 2001c). The enzymatic machinery involved in methanogenesis by *M. kandleri* is no different from that of the other autotrophic methanogens. All enzymes necessary for the operation of the pathway have been found by their activities in cell extracts (Rospert et al. 1991; Shima and Thauer 2001), and their genes were identified in the genome sequence (Slesarev et al. 2002). In the fluorescence microscope cells show the characteristic blue fluorescence due to the deazaflavin cofactor F_{420} (Huber et al. 1989; Kurr et al. 1991). Based on the genome sequence, the metabolic network of *M. kandleri* could be reconstructed (Chen et al. 2013).

A special feature of many enzymes of *Methanopyrus*, beyond their thermophilicity, is their marked requirement for high ionic concentrations for optimal activity. Concentrations >1 M of lyotropic salts such as K_2HPO_4/KH_2PO_4 or $(NH_4)_2SO_4$ are generally needed for optimal activity. NaCl, KCl, and NH_4Cl are not effective. Moreover, in the presence of 1.5 M salts, thermostability of the enzymes is dramatically increased. Enzymes showing this behavior include the *N*-formylmethanofuran:tetrahydromethanopterin formyltransferase and the methenyltetrahydromethanopterin cyclohydrolase (Breitung et al. 1992; Shima and Thauer 2001; Shima et al. 1998).

Inside the cells the high concentration of the osmotic solute cyclic 2,3-diphosphoglycerate (Martins et al. 1997) (estimated at ~1.1 M; Kurr et al. 1992) is responsible for the activation and stabilization of the thermophilic enzymes. At the concentrations present in the cell, the enzymes are highly active and thermostable (Shima et al. 1998).

Ecology

Cultures of *M. kandleri* have been obtained from different submarine hydrothermal vent systems worldwide: the Guaymas Basin in the Gulf of California, the Mid-Atlantic Ridge, the Kairei Field in the Central Indian Ridge, and shallow marine hydrothermal system of the Kolbeinsey Ridge, Iceland. However, no positive enrichments were obtained from samples from the near-shore hydrothermal field at Vulcano, Italy, in spite of the fact that the in situ water temperatures (98–103 °C) were in the range optimal for the growth of *Methanopyrus* (Huber and Stetter 2001c; Kurr et al. 1991; Takai et al. 2008).

Sequences of 16S rRNA genes with a high similarity to *M. kandleri* were recovered from 16S rRNA gene clone libraries from samples of sulfide structures from the hydrothermal vents on the East Pacific Rise (13°N) (Nercessian et al. 2003) and from the deep-sea hydrothermal systems of the Kairei Field

in the Central Indian Ridge (Takai et al. 2004). However, the Iheya North site in the Okinawa Trough off Japan did not yield such sequences (Takai et al. 2004).

To increase the sensitivity of detection of the presence of *Methanopyrus* in environmental samples, a protocol for the PCR amplification of its isoleucyl-tRNA synthetase gene was optimized following testing of different primer combinations, polymerase enzymes, and target DNA length (Yu 2010).

Pathogenicity, Clinical Relevance

As expected for a hyperthermophile that does not grow at temperatures below 84 °C, *Methanopyrus* is not known to be associated with humans, animals, or plants.

Application

No applications for *Methanopyrus* are currently known.

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