

1 Supplemental material

2

3 Subunit α

MCR111_M_thermolithotrophicus $\alpha 1$ TT $\eta 1$ $\alpha 2$ $\alpha 3$ TT $\beta 1$ TT $\beta 2$
1 10 20 30 40 50 60 70 80
MCR111_M_thermolithotrophicus ME AEK KLF PKALKEKFP EDPKRYRKFVTTGGWQGSARKREVEANEKTVAEKRGTFYVNDP...GVP L GQR K L M P V K L S G T D Y I V R
MCR111_M_formicicus MG AEK KLF PKALKEKFP EDPKRYRKFVTTGGWQGSARKREVEANEKTVAEKRGTFYVNDP...GVP L GQR K L M P V K L S G T D Y I V R
MCR111_M_maripaludis MD AEK KLF PKALKEKFP EDPKRYRKFVTTGGWQGSARKREVEANEKTVAEKRGTFYVNDP...GVP L GQR K L M P V K L S G T D Y I V R
MCR111_M_thermolithotrophicus ME AEK KLF PKALKEKFP EDPKRYRKFVTTGGWQGSARKREVEANEKTVAEKRGTFYVNDP...GVP L GQR K L M P V K L S G T D Y I V R
MCR111_M_formicicus MD NEK KLF PKALKEKFP EDPKRYRKFVTTGGWQGSARKREVEANEKTVAEKRGTFYVNDP...GVP L GQR K L M P V K L S G T D Y I V R
MCR111_M_wolfiellii MD NEK KLF PKALKEKFP EDPKRYRKFVTTGGWQGSARKREVEANEKTVAEKRGTFYVNDP...GVP L GQR K L M P V K L S G T D Y I V R
MCR111_M_marburgensis MA ...D KLF PKALKEKFP EDPKRYRKFVTTGGWQGSARKREVEANEKTVAEKRGTFYVNDP...GVP L GQR K L M P V K L S G T D Y I V R
MCR111_M_marburgensis SS SAE KLF PKALKEKFP EDPKRYRKFVTTGGWQGSARKREVEANEKTVAEKRGTFYVNDP...GVP L GQR K L M P V K L S G T D Y I V R
MCR111_M_kandleri MA ...D KLF PKALKEKFP EDPKRYRKFVTTGGWQGSARKREVEANEKTVAEKRGTFYVNDP...GVP L GQR K L M P V K L S G T D Y I V R
MCR111_M_barkeri MA ...D KLF PKALKEKFP EDPKRYRKFVTTGGWQGSARKREVEANEKTVAEKRGTFYVNDP...GVP L GQR K L M P V K L S G T D Y I V R

MCR111_M_thermolithotrophicus $\eta 2$ $\eta 3$ $\alpha 4$ $\beta 3$ $\alpha 5$ $\alpha 6$ $\eta 4$ $\beta 4$ $\alpha 7$
90 100 110 120 130 140 150 160 170 180
MCR111_M_thermolithotrophicus GDDLHFNNAAGQVDDIRRTVIGMDTGHAVVEKRLGVEVTPETINNYMETNHALPGGAVVOEHMVEHPFLAVDGVAKIFITGDELDADEDRKFLI
MCR111_M_formicicus GDDLHFNNAAGQVDDIRRTVIGMDTGHAVVEKRLGVEVTPETINNYMETNHALPGGAVVOEHMVEHPFLAVDGVAKIFITGDELDADEDRKFLI
MCR111_M_maripaludis GDDLHFNNAAGQVDDIRRTVIGMDTGHAVVEKRLGVEVTPETINNYMETNHALPGGAVVOEHMVEHPFLAVDGVAKIFITGDELDADEDRKFLI
MCR111_M_thermolithotrophicus GDDLHFNNAAGQVDDIRRTVIGMDTGHAVVEKRLGVEVTPETINNYMETNHALPGGAVVOEHMVEHPFLAVDGVAKIFITGDELDADEDRKFLI
MCR111_M_formicicus GDDLHFNNAAGQVDDIRRTVIGMDTGHAVVEKRLGVEVTPETINNYMETNHALPGGAVVOEHMVEHPFLAVDGVAKIFITGDELDADEDRKFLI
MCR111_M_wolfiellii GDDLHFNNAAGQVDDIRRTVIGMDTGHAVVEKRLGVEVTPETINNYMETNHALPGGAVVOEHMVEHPFLAVDGVAKIFITGDELDADEDRKFLI
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MCR111_M_kandleri GDDLHFNNAAGQVDDIRRTVIGMDTGHAVVEKRLGVEVTPETINNYMETNHALPGGAVVOEHMVEHPFLAVDGVAKIFITGDELDADEDRKFLI
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MCR111_M_formicicus DIKRFEBQAEOLKAAIGKRTYQSRVPLVGRVCGGTVSRWSAMQIMSEITAVKLCAGEAATADFSVAASKHADVIVQGNALPARRARGNPEPGGTR
MCR111_M_maripaludis DIKRFEBQAEOLKAAIGKRTYQSRVPLVGRVCGGTVSRWSAMQIMSEITAVKLCAGEAATADFSVAASKHADVIVQGNALPARRARGNPEPGGTR
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MCR111_M_wolfiellii DIKRFEBQAEMLKRYICNRTYQSRVPLVGRVCGGTVSRWSAMQIMSEITAVKLCAGEAATADFSVAASKHADVIVQGNALPARRARGNPEPGGTR
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MCR111_M_wolfiellii FVGLDQIVQTRV...SDPPVEQLSLEVVAVGAMIIYDGLWLGYSMSGGVGFQYAAAYVDDILDDFFAVYGVVYVKKYGINNS...TKP FMDVVDDIAT
MCR111_M_marburgensis FVGLDQIVQTRV...SDPPVEQLSLEVVAVGAMIIYDGLWLGYSMSGGVGFQYAAAYVDDILDDFFAVYGVVYVKKYGINNS...TKP FMDVVDDIAT
MCR111_M_kandleri FVGLDQIVQTRV...SDPPVEQLSLEVVAVGAMIIYDGLWLGYSMSGGVGFQYAAAYVDDILDDFFAVYGVVYVKKYGINNS...TKP FMDVVDDIAT
MCR111_M_barkeri FVGLDQIVQTRV...SDPPVEQLSLEVVAVGAMIIYDGLWLGYSMSGGVGFQYAAAYVDDILDDFFAVYGVVYVKKYGINNS...TKP FMDVVDDIAT

MCR111_M_thermolithotrophicus $\alpha 18$ $\alpha 19$ $\alpha 20$ $\alpha 21$ $\alpha 22$ $\alpha 23$ $\eta 7$
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MCR111_M_formicicus EVTFTSHQVDEYPLIDDFFGGSRRAVVAASACCTAFATGNSNAAGVNGWYLSQIIHKVEHSRLGFGYDLDQCCGASNSLSIRNDEGLIHEARGPNY
MCR111_M_maripaludis EVTFTSHQVDEYPLIDDFFGGSRRAVVAASACCTAFATGNSNAAGVNGWYLSQIIHKVEHSRLGFGYDLDQCCGASNSLSIRNDEGLIHEARGPNY
MCR111_M_thermolithotrophicus EVTFTSHQVDEYPLIDDFFGGSRRAVVAASACCTAFATGNSNAAGVNGWYLSQIIHKVEHSRLGFGYDLDQCCGASNSLSIRNDEGLIHEARGPNY
MCR111_M_formicicus EVTFTSHQVDEYPLIDDFFGGSRRAVVAASACCTAFATGNSNAAGVNGWYLSQIIHKVEHSRLGFGYDLDQCCGASNSLSIRNDEGLIHEARGPNY
MCR111_M_wolfiellii EVTFTSHQVDEYPLIDDFFGGSRRAVVAASACCTAFATGNSNAAGVNGWYLSQIIHKVEHSRLGFGYDLDQCCGASNSLSIRNDEGLIHEARGPNY
MCR111_M_marburgensis EVTFTSHQVDEYPLIDDFFGGSRRAVVAASACCTAFATGNSNAAGVNGWYLSQIIHKVEHSRLGFGYDLDQCCGASNSLSIRNDEGLIHEARGPNY
MCR111_M_kandleri EVTFTSHQVDEYPLIDDFFGGSRRAVVAASACCTAFATGNSNAAGVNGWYLSQIIHKVEHSRLGFGYDLDQCCGASNSLSIRNDEGLIHEARGPNY
MCR111_M_barkeri EVTFTSHQVDEYPLIDDFFGGSRRAVVAASACCTAFATGNSNAAGVNGWYLSQIIHKVEHSRLGFGYDLDQCCGASNSLSIRNDEGLIHEARGPNY

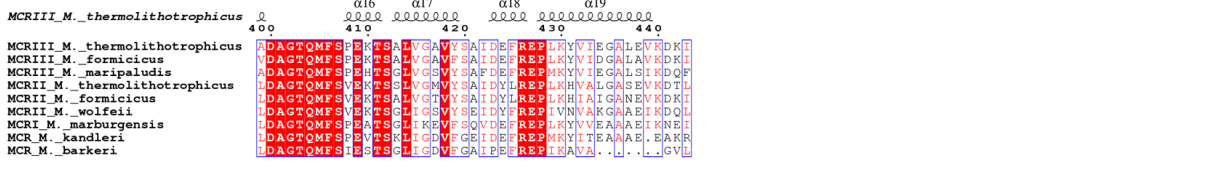
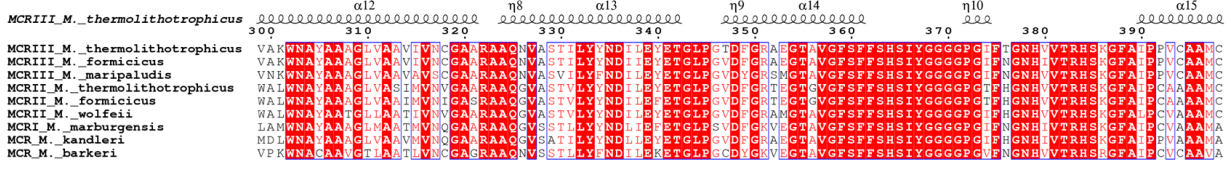
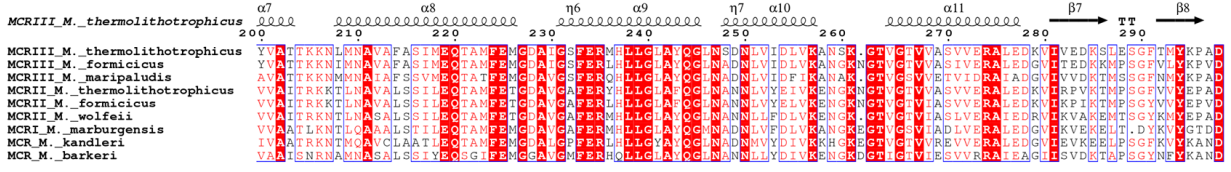
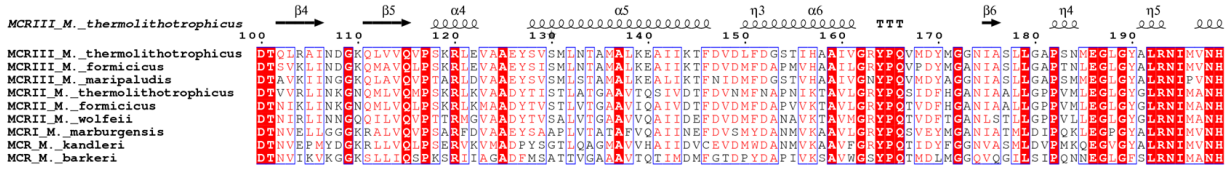
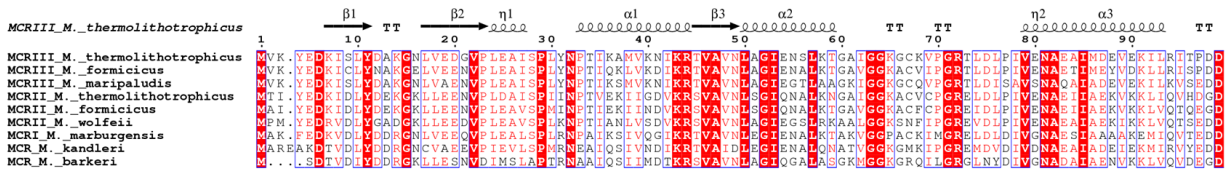
MCR111_M_thermolithotrophicus $\eta 8$ $\alpha 24$ $\alpha 25$ TT $\alpha 26$ $\eta 9$
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MCR111_M_thermolithotrophicus PNYAMNVGHGQVACIQAHAARADAFVNPVRIAFADPDLVDFPDRFRRECRGAREFEPAAGRDVILP...R
MCR111_M_formicicus PNYAMNVGHGQVACIQAHAARADAFVNPVRIAFADPDLVDFPDRFRRECRGAREFEPAAGRDVILP...R
MCR111_M_maripaludis PNYAMNVGHGQVACIQAHAARADAFVNPVRIAFADPDLVDFPDRFRRECRGAREFEPAAGRDVILP...R
MCR111_M_thermolithotrophicus PNYAMNVGHGQVACIQAHAARADAFVNPVRIAFADPDLVDFPDRFRRECRGAREFEPAAGRDVILP...R
MCR111_M_formicicus PNYAMNVGHGQVACIQAHAARADAFVNPVRIAFADPDLVDFPDRFRRECRGAREFEPAAGRDVILP...R
MCR111_M_wolfiellii PNYAMNVGHGQVACIQAHAARADAFVNPVRIAFADPDLVDFPDRFRRECRGAREFEPAAGRDVILP...R
MCR111_M_marburgensis PNYAMNVGHGQVACIQAHAARADAFVNPVRIAFADPDLVDFPDRFRRECRGAREFEPAAGRDVILP...R
MCR111_M_kandleri PNYAMNVGHGQVACIQAHAARADAFVNPVRIAFADPDLVDFPDRFRRECRGAREFEPAAGRDVILP...R
MCR111_M_barkeri PNYAMNVGHGQVACIQAHAARADAFVNPVRIAFADPDLVDFPDRFRRECRGAREFEPAAGRDVILP...R

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6 Subunit β

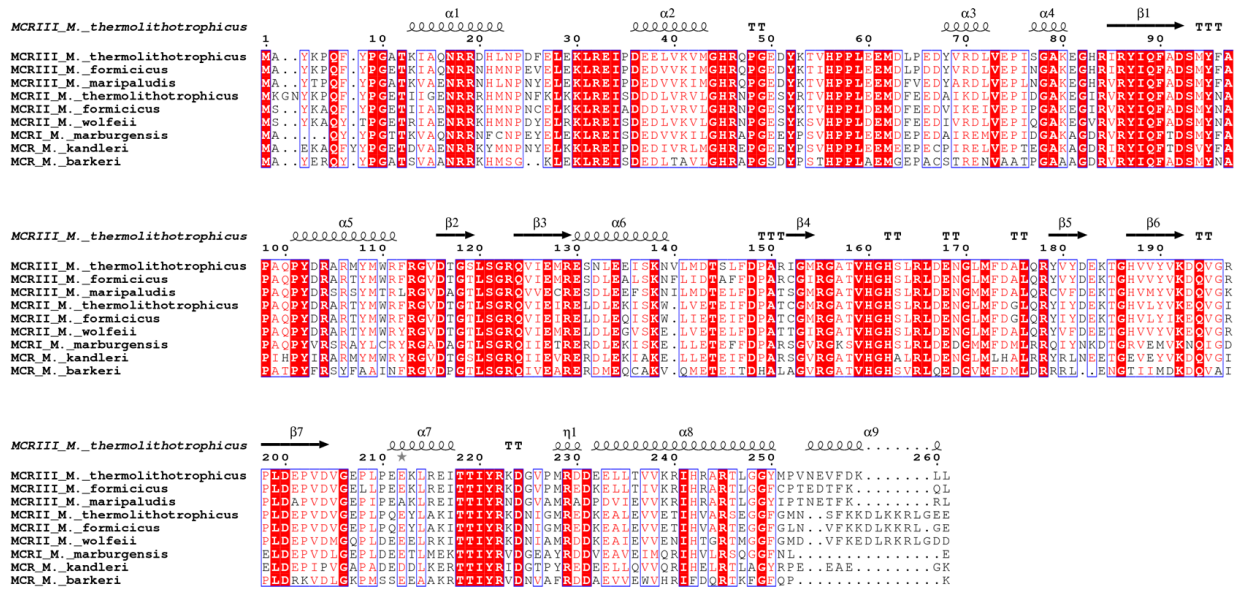
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10 Subunit γ



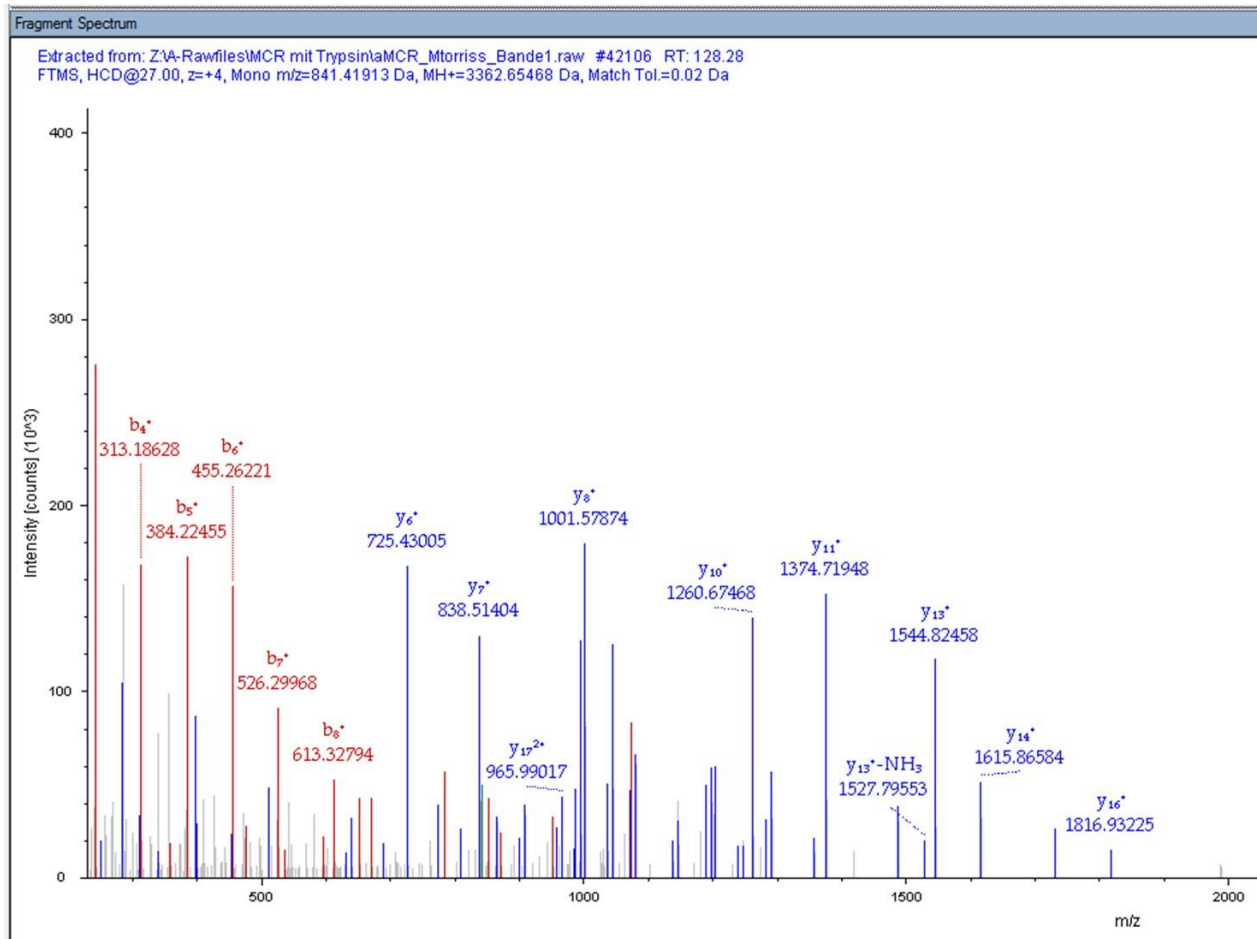
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13 **Fig. S1.** Primary structure comparison of subunits α , β and γ of MCR.

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17 **Fig. S2.** A part of MALDI-TOF-MS (MS/MS) mass fragmentation spectrum of a trypsin-digested
18 peptide, ⁴⁰³AAVAAAASGISVCMATGNSNAGLNGWYLSQILHK⁴³⁷ from the α -subunit of
19 MCR from *M. formicicus*. The amino acid sequence of the relevant peaks are given in Table 3.

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21 **Table S1.** Substrates and habitats of methanogenic archaea. If not indicated otherwise
 22 information was retrieved from Bergeys Manual of Systematics of Archaea and Bacteria (18, 65-
 23 69)

Group / Genera	Substrate range	Habitat / Isolated from	Environment / Temperature (pH) preference
Methanobacteriales			
<i>Methanobacterium</i> spp.	hydrogenotrophic, varying co-substrates with CO ₂ , formatotrophic	sewage sludge, anaerobic digestors, oil-bearing rock cores, rice fields	aquatic / soil, mesophilic
<i>Methanothermobacter</i> spp.	hydrogenotrophic, formatotrophic	sewage sludge, river sediments	aquatic, mesophilic
<i>Methanothermus</i> spp.	hydrogenotrophic	sulfurous mud springs	aquatic, thermophilic
<i>Methanosphaera</i> spp.	hydrogenotrophic, alcoholotrophic (methanol)	human large intestine, feces	intestinal tracts, mesophilic
<i>Methanobrevibacter</i> spp.	hydrogenotrophic, formatotrophic	rumen, anaerobic digestors, termite gut, gingiva, feces	intestinal tracts, mesophilic
Methanopyrales			
<i>Methanopyrus kandleri</i>	hydrogenotrophic	hydrothermal vents	aquatic, hyperthermophilic
Methanococcales			
<i>Methanocaldococcus</i> spp.	hydrogenotrophic	hydrothermal vents	aquatic, thermophilic
<i>Methanotorris</i> spp.	hydrogenotrophic	hydrothermal vents	aquatic, thermophilic
<i>Methanococcus</i> spp.	hydrogenotrophic, formatotrophic, methylamines, alcoholotrophic (methanol), methyl sulfides	marine environments (primarily sediments)	aquatic, mesophilic
<i>Methanothermococcus</i> spp.	hydrogenotrophic, formatotrophic	geothermally heated marine sediments	aquatic, thermophilic
Methanocellales			

<i>Methanocella</i> spp.	hydrogenotrophic	soils, especially rice fields	soil, mesophilic
Methanomicrobiales			
<i>Methanocalculus</i> spp.	hydrogenotrophic, formatotrophic	alkaline lakes	aquatic, mesophilic (alkaliphilic)
<i>Methanocorpusculum</i> spp.	hydrogenotrophic, formatotrophic	anaerobic digestors, waste water, coal beds, shale	aquatic / soil, mesophilic
<i>Methanoculleus</i> spp.	hydrogenotrophic, formatotrophic	marine sediments, biogas plants	aquatic, mesophilic
<i>Methanofollis</i> spp.	hydrogenotrophic, formatotrophic, alcoholotrophic (propanol, butanol, pentanol)	waste water reactors	aquatic, mesophilic
<i>Methanogenium</i> spp.	hydrogenotrophic, formatotrophic	freshwater sediments	aquatic, mesophilic
<i>Methanolacina</i> spp.	---	marine sediments	aquatic, mesophilic
<i>Methanomicrobium</i> spp.	hydrogenotrophic	marine sediments	aquatic, mesophilic
<i>Methanoplanus</i> spp.	hydrogenotrophic, formatotrophic, alcoholotrophic (propanol)	marine sediments, oil fields	aquatic, mesophilic
<i>Methanolinea</i> spp.	hydrogenotrophic, formatotrophic	sewage sludge, marine sediments, fen sediments	aquatic, mesophilic (acidophilic)
<i>Methanoregula</i> spp.	hydrogenotrophic	acidic bog	aquatic, mesophilic (acidophilic)
<i>Methanosphaerula</i> spp.	hydrogenotrophic, formatotrophic	acidic peatlands	aquatic, mesophilic (acidophilic)
<i>Methanospirillum</i> spp.	hydrogenotrophic, formatotrophic, alcoholotrophic (propanol)	sewage sludge, digestors	aquatic, mesophilic
Methanosarcinales			
<i>Methanosaeta</i> spp.	acetoclastic	anaerobic digestors, anoxic sediments / soils, hot springs, thermal lakes	aquatic / soil, mesophilic / thermophilic

<i>Methanimicrococcus</i> spp.	methylotrophic	cockroach gut	intestinal tracts, mesophilic
<i>Methanococcoides</i> spp.	methylotrophic	submarine canyon, Ace lake	aquatic, psychrophilic / mesophilic
<i>Methanohalobium</i> spp.	methylotrophic	highly saline environments	aquatic / sediment, mesophilic / thermophilic
<i>Methanohalophilus</i> spp.	methylotrophic	saline environments	aquatic / sediment, mesophilic
<i>Methanobolus</i> spp.	methylotrophic	mesophilic, anoxic environments (e.g. rocks)	terrestrial / aquatic, psychrophilic / mesophilic
<i>Methanomethylovorans</i> spp.	methylotrophic	sludge reactors, lake sediments	aquatic, mesophilic / thermophilic
<i>Methanosalsum</i> spp.	methylotrophic	hypersaline soda lakes	aquatic, mesophilic / moderately thermophilic
<i>Methanosarcina</i> spp.	hydrogenotrophic, acetoclastic, methylotrophic	almost ubiquitous in anoxic environments	ubiquitous, mesophilic / moderately thermophilic
<i>Methermicrococcus</i> spp.	methylotrophic	oil field	aquatic, thermophilic
Methanomassilicoccales			
<i>Methanomassilicoccus</i> spp.	methylotrophic	intestinal tracts	intestinal tracts / mesophilic
Bathyarchaeota			
Bathyarchaeota	methylotrophic	ubiquitous, fresh water, open ocean	-