SUPPLEMENTARY INFORMATION

Comparative Genome Analysis of “*Candidatus* Methanoplasma termitum” Indicates a New Mode of Energy Metabolism in the Seventh Order of Methanogens

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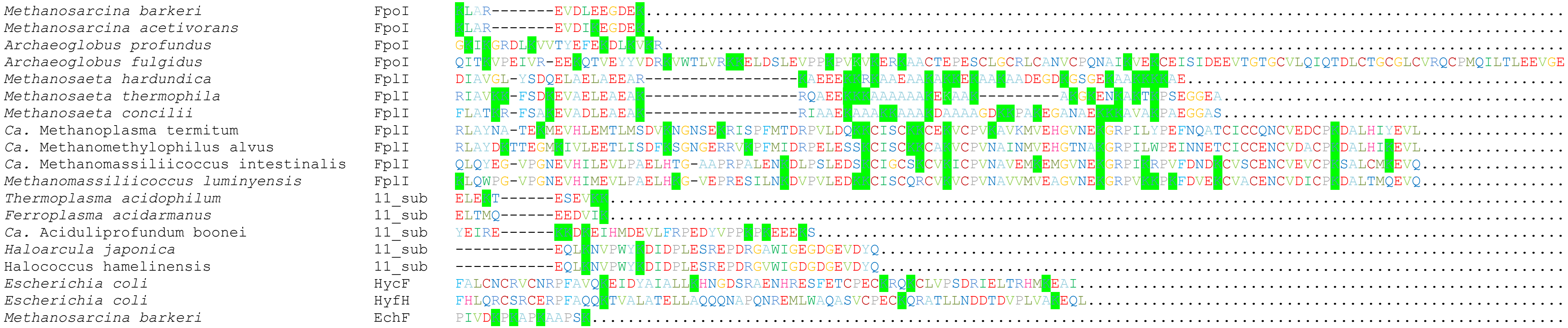

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Methanosarcina barkeri                    FpoI    KLAR-------EVDLEEGDEK......................................................................................................
Methanosarcina acetivorans                FpoI    KLAR-------EVDIKEGDEK......................................................................................................
Archaeoglobus profundus                   FpoI    GKIKGRDLKVVTYEFEKDLKVKR....................................................................................................
Archaeoglobus fulgidus                    FpoI    QITKVPEIVR-EEKQTVEYYVDRKVWTLVRKKELDSLEVPPKPVKVKERKAACTEPESCLGCRLCANVCPQNAIKVEKCEISIDEEVTGTGCVLQIQTDLCTGCGLCVRQCPMQILTLEEVGE
Methanosaeta hardundica                   FplI    DIAVGL-YSDQELAELAEEAR-----------------KAEEEKKRKAAEAAKAKKEKAAKAADEGDKGSGEKAAKKKKAE..........................................
Methanosaeta thermophila                  FplI    RIAVKK-FSDKEVAELEAEAK-----------------RQAEEKKKAAAAAAKEKAAK---------AKGKENKAKTKPSEGGEA......................................
Methanosaeta concilii                     FplI    FLATKR-FSAKEVADLEAEAK-----------------RIAAEKAAAKKAAAKDAAAAGDKKPAKEGANAEKKKAVAKPAEGGAS......................................
Ca. Methanoplasma termitum                FplI    RLAYNA-TEKMEVHLEMTLMSDVKNGNSEKRISPFMTDRPVLDQKKCISCKKCEKVCPVKAVKMVEHGVNEKGRPILYPEFNQATCICCQNCVEDCPKDALHIYEVL................
Ca. Methanomethylophilus alvus            FplI    RLAYDKTTEGMKIVLEETLISDFKSGNGERRVKPFMIDRPELESSKCISCKKCAKVCPVNAINMVEHGTNAKGRPILWPEINNETCICCENCVDACPKDALHIKEVL................
Ca. Methanomassiliicoccus intestinalis    FplI    QLQYEG-VPGNEVHILEVLPAELHTG-AAPRPALENKDLPSLEDSKCIGCSKCVKICPVNAVEMKEMGVNEKGRPIKRPVFDNDKCVSCENCVEVCPKSALCMKEVQ................
Methanomassiliicoccus luminyensis         FplI    KLQWPG-VPGNEVHIMEVLPAELHKG-VEPRESILNKDVPVLEDKKCISCQRCVKVCPVNAVVMVEAGVNEKGRPVKKPKFDVEKCVACENCVDICPKDALTMQEVQ................
Thermoplasma acidophilum                  11_sub  ELEKT------ESEVKK..........................................................................................................
Ferroplasma acidarmanus                   11_sub  ELTMQ------EEDVIK..........................................................................................................
Ca. Aciduliprofundum boonei               11_sub  YEIRE------KKDKEIHMDEVLFRPEDYVPPKPKEEEKS...................................................................................
Haloarcula japonica                       11_sub  -----------EQLKNVPWYKDIDPLESREPDRGAWIGEGDGEVDYQ............................................................................
Halococcus hamelinensis                   11_sub  -----------EQLKNVPWYKDIDPLESREPDRGVWIGDGDGEVDYQ............................................................................
Escherichia coli                          HycF    FALCNCRVCNRPFAVQKEIDYAIALLKHNGDSRAENHRESFETCPECKRQKCLVPSDRIELTRHMKEAI......................................................
Escherichia coli                          HyfH    FHLQRCSRCERPFAQQKTVALATELLAQQQNAPQNREMLWAQASVCPECKQRATLLNDDTDVPLVAKEQL.....................................................
Methanosarcina barkeri                    EchF    PIVDKPKAPKAAPSK............................................................................................................
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FIG S1 Alignment of the C-terminus of FpoI of *Methanosarcina* and *Archaeoglobales* species; FpoI-like (FplI) of *Methanosaeta* and *Methanomassiliicoccales* species; the I subunit of the 11-subunit complex (11_sub) of *Thermoplasmatales* species, *Ca.* Aciduliprofundum boonei, and *Halobacteriales* species; the ferredoxin-reducing subunits of the hydrogenases Hyc and Hyf of *Escherichia coli;* and the Ech hydrogenase of *Methanosarcina barkeri*. Amino acids of the same families have the same color; lysine is highlighted in green

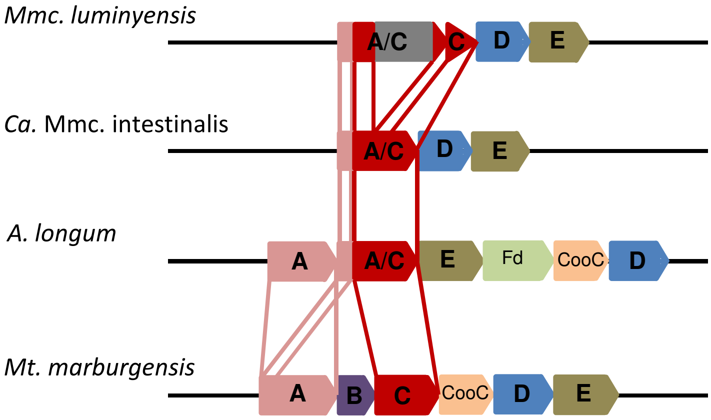

FIG S2 CO dehydrogenase/acetyl-CoA synthase operon structure of *Methanomassilii-coccus luminyensis*, *Ca. Methanomassiliicoccus intestinalis*, *Acetonema longum*, and *Methanothermobacter marburgensis*. Abbreviations: A, alpha subunit; C, beta subunit; A/C, fused alpha/beta subunit; D, delta subunit; E, gamma subunit; Fd, ferredoxin; CooC, CO dehydrogenase maturation factor; B, epsilon subunit; Fd, Fe-S-cluster-containing protein

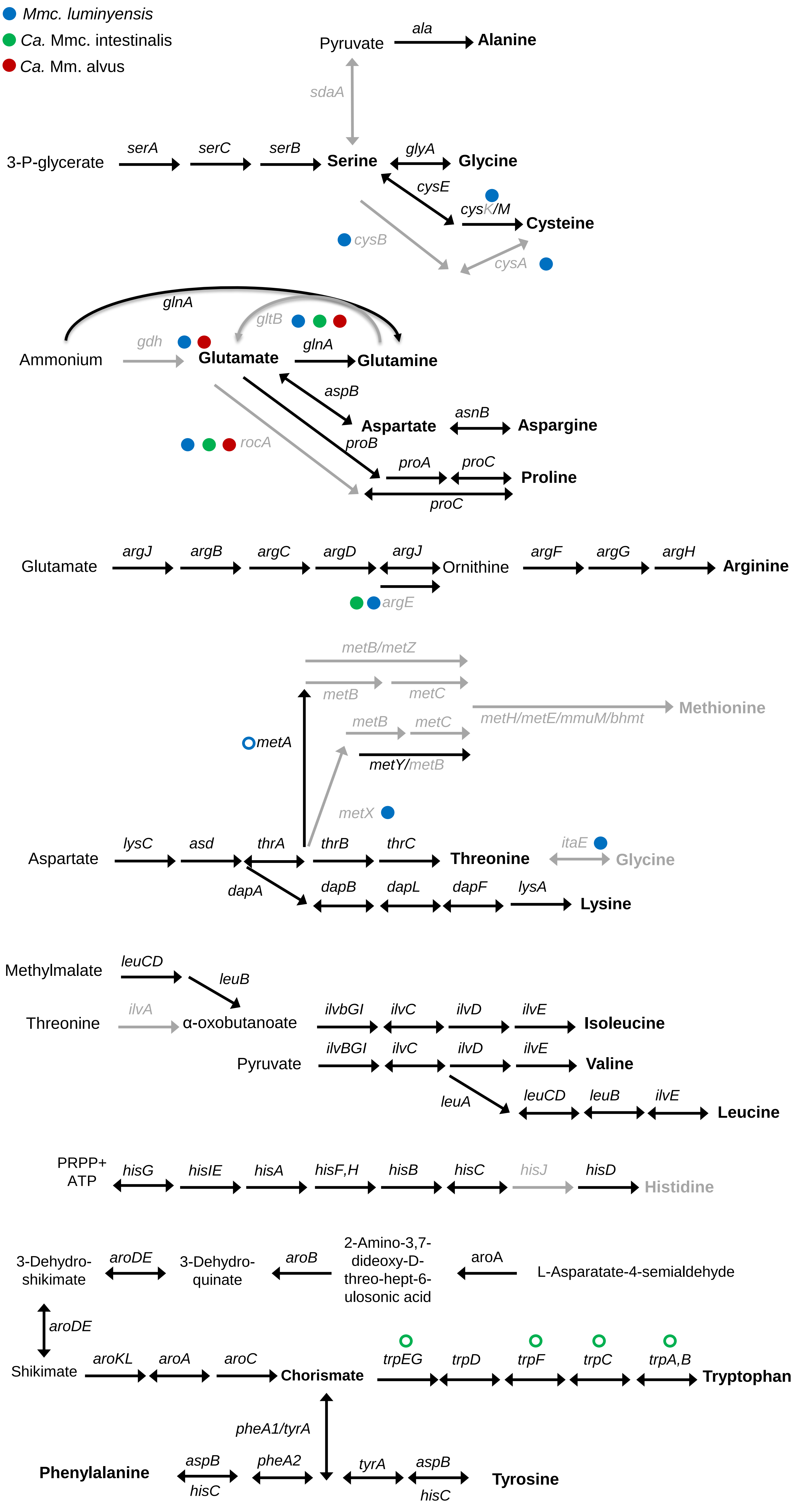

FIG S3 Schematic overview of the amino acid anabolism of *Ca*. Methanoplasma termitum and other species of *Methanomassiliicoccales*. Genes present in the genome of *Ca*. Mp. termitum are shown in black; an open circle indicates that the gene is absent in the other genomes. Amino acids that cannot be synthesized by *Ca*. Mp. termitum and the corresponding missing genes are shown in gray; colored, filled circles indicate that the genes are present in the other genomes.

TABLE S1 Annotated list of genes in the genome of *Ca*. Methanoplasma termitum. The gene product names and the predicted functions are manually curated. The genes are listed by gene ID but can be sorted as desired. In addition to the enzyme, the COG, Pfam, Tigrfam, KO, and IMG assignment of each gene product are given. Indicated are the number of nucleotides and deduced amino acids, start and stop coordinates, strand, locus type, and presence of signal peptides and transmembrane helices. **Please download Table_S1.xlsx for interactive spreadsheet.**

TABLE S2 Genes encoding the different subunits of Ech hydrogenases in *Methanosarcina barkeri* and their homologs in *Methanomassiliicoccus luminyensis* and *Ca.* Methanomassiliicoccus intestinalis (*Methanomassiliicoccaceae*). Ech hydrogenases are absent in members of the intestinal cluster.

	Ech	Ech-1	Ech-1	Ech-2	Ech-2
	Ms. barkeri	*Mmc. luminyensis*	*Ca.* Mmc. intestinalis	*Mmc. luminyensis*	*Ca.* Mmc. intestinalis
Large subunit	*echE*	WP_019178475	MMINT_01070	WP_019176382	MMINT_17010
Small subunit	*echC*	WP_019178473	MMINT_01050	WP_019176384	MMINT_17030
4Fe/4S - Fd	*echF*	WP_019178476	MMINT_01080	–[a]	MMINT_17000
Small protein	*echD*	WP_019178474	MMINT_01060	WP_019176383	MMINT_17020
Transmembrane proteins	*echA*	WP_019178471	MMINT_01030	WP_019176386	MMINT_17050
	echB	WP_019178472	MMINT_01040	WP_019176385	MMINT_17040

[a] Not present

TABLE S3 Genes encoding the different subunits of Hyc and Hyf hydrogenases in *Escherichia coli* and their homologs in *Ca*. Methanoplasma termitum and *Methanomassiliicoccus luminyensis*. Hyf-like hydrogenases are absent in *Ca.* Methanomethylophilus alvus and *Ca.* Methanomassiliicoccus intestinalis.

	Hyc	Hyf	Hyf-like	Hyf-like
	E. coli	*E. coli*	*Ca*. Mp. termitum	*Mmc. luminyensis*
Large subunit	*hycE*	*hyfG*	Mpt1_c08770	WP_019178467
Small subunit	*hycG*	*hyfI*	Mpt1_c08760	WP_019178469
4Fe/4S - Fd	*hycF*	*hyfH*	Mpt1_c08760	WP_019178468
Small protein	*hycE*	*hyfG*	Mpt1_00880	WP_019178467
Transmembrane proteins	*hycC*	*hyfD*	Mpt1_c08780/8810	WP_019178466/63
	hycD	*hyfC*	Mpt1_c08800	WP_019178464
	– [a]	*hyfB,F*	Mpt1_c8810/8780	WP_019178463/66
	–	*hyfE*	Mpt1_c08790	WP_019178465
Other subunits	*hycBH*	*hyfAJR*	–	–

[a] Not present