Supplementary Material

Function and regulation of isoforms of carbon monoxide dehydrogenase/acetyl-CoA synthase in *Methanosarcina acetivorans*

Nicole Matschiavelli, Ellen Oelgeschläger, Berardino Cocchiararo, Johannes Finke, and Michael Rother

Supplementary Table S1: Primers used in this study.

Primer	Sequence[a]	Restriction site(s) introduced
5-up1016	CCGAAGCTCGAGGGCGCGCCGCAAATTAACTACAGGGAATTTTTCTATAG	XhoI, AscI
3-up1016	GAACCCAAGCTTGAAATTACACACTTACAAACAAAATTAAGTAC	HindIII
5-do1011	CGGGATCCCTCAATTTTCGACTACAGCAAAAATC	BamHI
3-do1011	GACTAGTGGCGCGCCGCTGTTTTAAGTTCTCCATCAAACTTTC	SpeI, AscI
5-up3860	CCGAAGCTCGAGGGCGCGCCCGAAGTTCTTATGTTTGGAAACTTTTTTC	XhoI, AscI
3-up3860	GAACCCAAGCTTCGCCTTAAAGTTACAACCGTTTAAATG	HindIII
5-do3865	CGGGATCCCGCAGGTAATTCTTTAAGGATTCAC	BamHI
3-do3865	GACTAGTGGCGCGCCCAGCAGAATGAACACGGAAACAG	SpeI, AscI
5-up4399	CCGAAGCTCGAGGGCGCGCCGCCTTAGACCTGAGTTCAAGTC	XhoI, AscI
3-up4399	GAACCCAAGCTTACGCAGTTGAAGGTAAAAATC	HindIII
5-do4399	CGGGATCCGGTTCAATGCTCGGTTCAATGC	BamHI
3-do4399	GACTAGTGGCGCGCCCTGGATAAACCAGGATATGACTTC	SpeI, AscI
ocdhA-SB/forw	GTAGGGGCAGCAAAGGAG	
ocdhA-SB/rev	CGTGGTCAAGGCTGCCG	

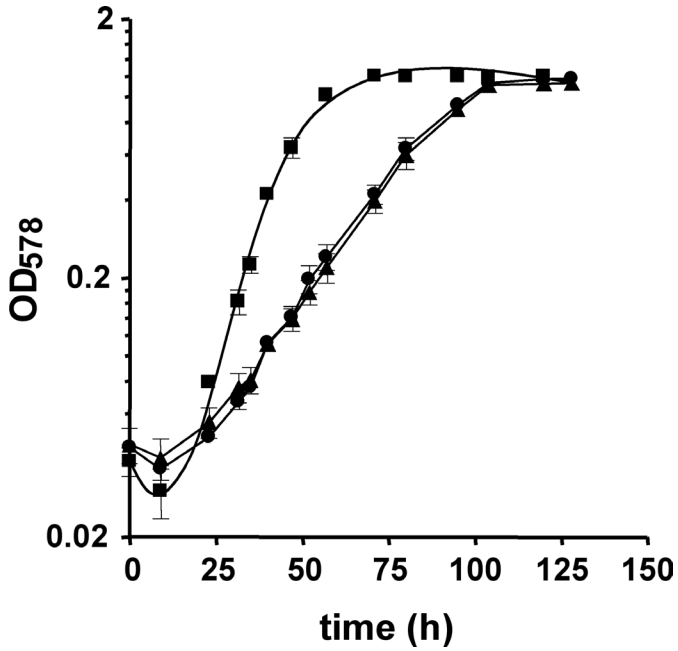
a: 5' → 3'; restriction site(s) introduced are underlined.

Supplementary Table S2: Substrate-dependent growth parameters of *cdh*-mutants.

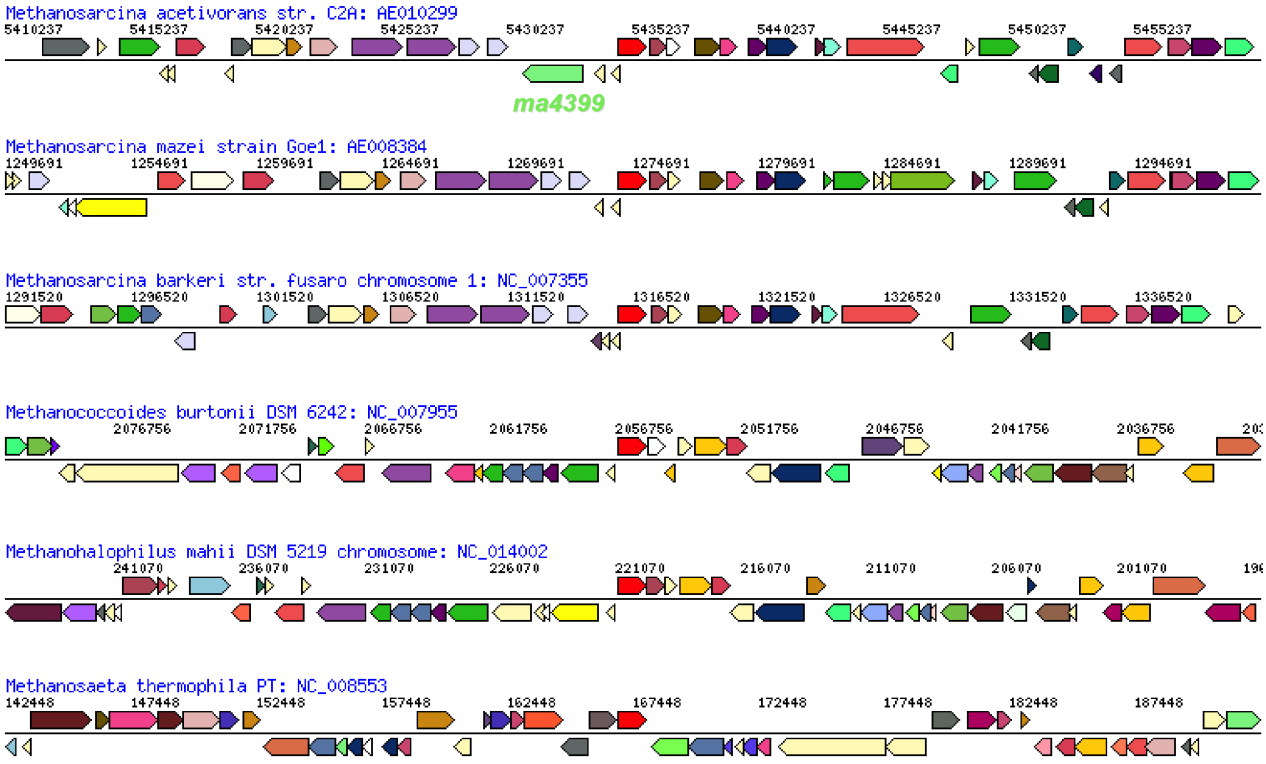
Strain	Growth substrate[a]					
	Methanol		CO		Acetate	
	t_d	final OD_{578}	t_d	final OD_{578}	t_d	final OD_{578}
WWM1	9.2 ± 0.8	1.2 ± 0.1	26.6 ± 2.6	0.6 ± 0.04	46.8 ± 1.7	0.7 ± 0.02
MCD1	8.8 ± 0.6	1.2 ± 0.3	29.3 ± 2.1	0.6 ± 0.03	48.4 ± 2.1	0.7 ± 0.01
MCD2	9.8 ± 0.8	1.2 ± 0.1	35.5 ± 3.4	0.6 ± 0.1	46.6 ± 4.3	0.7 ± 0.03
MCD3	8.9 ± 1.2	1.1 ± 0.1	23.5 ± 1.5	0.9 ± 0.01	44.4 ± 4.5	0.7 ± 0.01
MCD31	10.4 ± 0.4	1.3 ± 0.2	23.6 ± 2.2	0.7 ± 0.02	47.8 ± 3.6	0.5 ± 0.02
MCD32	10.2 ± 0.6	1.3 ± 0.2	25.5 ± 1.3	0.7 ± 0.02	48.0 ± 3.0	0.7 ± 0.02
MCD21	23.2 ± 0.4	1.2 ± 0.1	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]
MCD213	23.1 ± 0.3	1.1 ± 0.02	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]

a: *M. acetivorans* was cultivated on methanol (125 mM), CO (150 kPa) or acetate (120 mM); doubling time (t_d) in h and final optical density were determined photometrically at 578 nm (OD_{578}). Shown are average values from at least three independent cultures, ± indicates standard deviation; the experiments were qualitatively reproduced at least once.

b: n.d.: not detectable as strains cannot grow on the respective substrate.



Supplementary Figure S1: Substrate-dependent growth of *M. acetivorans cdh* null mutants. Growth of the *cdh* wild type WWM1 (squares), of the *Δcdh1 Δcdh2* double mutant MCD21 (circles) and of the *Δcdh1 Δcdh2 ΔcdhA3* triple mutant MCD213 (triangles) in the presence of 125 mM methanol and 40 mM acetate was monitored by measuring the optical density at 578 nm (OD_{578}). Shown are average values and standard deviations of three independent cultures for each strain; the experiment was qualitatively reproduced at least twice.



Supplementary Figure S2: Comparison of local gene synteny in methanogenic archaea. The genomic environment of *cdhA3* (*ma4399,* light green) from *M. acetivorans* (top) was compared to the respective genomic regions in *M. mazei* (second from top) and *M. barkeri* (third from top) using the *Display Conserved Neighborhood* function of the Integrated Microbial Genomes database at the DOE Joint Genome Institute (http://img.jgi.doe.gov); as the latter two do not encode stand-alone *cdhA* subunits *ma4401* was used as the query, which also allowed to include *Methanococcoides burtonii* (third from bottom), *Methanohalophilus mahii* (second from bottom) and *Methanosaeta thermophila* (bottom) in the comparison; orthologous genes are colored identically; numbers depict position in the respective genome sequence.