Supplemental materials for

The genetic basis for metabolism of methylated sulfur compounds in *Methanosarcina*

He Fu and William W. Metcalf

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TABLE S1. Plasmids used in this study

Plasmid	Description and/or construction	Reference
pMP44	Vector used to construct up- and down-region cassettes to delete genes in the *M. acetivorans* C2A chromosome using the markerless exchange method	(1)
pFH002	KpnI/SpeI-digested fusion PCR product of up-stream PCR product (with primers 5-upMA4164 and 3-upMA4164) and down-stream PCR product (with primers 5-dnMA4166 and 3-dnMA4166) cloned into KpnI/SpeI-digested pMP44	This study
pFH003	KpnI/SpeI-digested fusion PCR product of up-stream PCR product (with primers 5-upMA4164 and 3-upMA4164) and down-stream PCR product (with primers 5-dnMA4164 and 3-dnMA4164) cloned into KpnI/SpeI-digested pMP44	This study
pFH004	KpnI/SpeI-digested fusion PCR product of up-stream PCR product (with primers 5-upMA4165 and 3-upMA4165) and down-stream PCR product (with primers 5-dnMA4165 and 3-dnMA4165) cloned into KpnI/SpeI-digested pMP44	This study
pFH005	KpnI/SpeI-digested fusion PCR product of up-stream PCR product (with primers 5-upMA4166 and 3-upMA4166) and down-stream PCR product (with primers 5-dnMA4166 and 3-dnMA4166) cloned into KpnI/SpeI-digested pMP44	This study
pFH006	KpnI/SpeI-digested fusion PCR product of up-stream PCR product (with primers 5-upMA4167 and 3-upMA4167) and down-stream PCR product (with primers 5-dnMA4167 and 3-dnMA4167) cloned into KpnI/SpeI-digested pMP44	This study
pJK027A	Vector used for construction of complementation of deletion mutants in *M. acetivorans* C2A	(2)
pFH009	NdeI/SbfI-digested MA4164 PCR product (with primers MA4164-F01 and MA4164-R01) cloned into NdeI/NsiI-digested pJK027A	This study
pFH010	NdeI/SbfI-digested MA4165 PCR product (with primers MA4165-F01 and MA4165-R01) cloned into NdeI/NsiI-digested pJK027A	This study
pFH011	NdeI/SbfI-digested MA4166 PCR product (with primers MA4166-F01 and MA4166-R01) cloned into NdeI/NsiI-digested pJK027A	This study
pFH012	NdeI/SbfI-digested MA4164-66 PCR product (with primers MA4164-F03 and MA4166-R03) cloned into NdeI/NsiI-digested pJK027A	This study
pFH013A	NdeI/SbfI-digested fusion PCR product of PCR product 1 (with primers MA4167-F01a and MA4167-R01) and product 2 (with primers MA4167-F02 and MA4167-R02) cloned into NdeI/NsiI-digested pJK027A	This study

TABLE S2. Primers used in this study

Primer	Sequence	Added site(s)
5-up4164	GGCGGCCGGTACCGAATCTTTGCTGGATTTCCTTTTTTATCAAACTGA	KpnI
3-up4164	TCAACTCCATCTCACGCTTTCAGACGGGAATTCCTGGACTTCTTCCGGTAGGTCATCCAT	None
5-dn4164	ATGGATGACCTACCGGAAGAAGTCCAGGAATTCCCGTCTGAAAGCGTGAGATGGAGTTGA	None
3-dn4164	GGCGCGCCACTAGTTCTTCCATATTAACAGCCTGTTCTACACTGA	SpeI
5-up4165	GGCGCGCCGGTACCTTAACATGTTTATTTTTCAGAGCTCTCTGT	KpnI
3-up4165	TCAAAGCTTATCTGCAGACCAGTCAGCTGCCCTTTCTTTTGAGGTCATCTCAGATACCAT	None
5-dn4165	ATGGTATCTGAGATGACCTCAAAAGAAAGGGCAGCTGACTGGTCTGCAGATAAGCTTTGA	None
3-dn4165	GGCGCGCCACTAGTAGGACTTTCTTTCTGGACTTGAGCACTTTAT	SpeI
5-up4166	GGCGCGCCGGTACCCTTGACAATATTGTAATGCCTTTTGGAATGT	KpnI
3-up4166	TTATCTCTGTGACCCGACAGGTACTTCCCCAATAACAAAAACCACCCAGCGGTATTTCAT	None
5-dn4166	ATGAAATACCGCTGGGTGGTTTTTGTTATTGGGGAAGTACCTGTCGGGTCACAGAGATAA	None
3-dn4166	GGCGCGCCACTAGTAGCAGAAGAGAATAGGGAAACATTAGTTGA	SpeI
5-up4167	GGCGCGCCGGTACCATAAGAAGTAAAGATAAGGAAAAGATAGAA	KpnI
3-up4167	TCAGAATTTACTGGCTTTTGTGATATTATATTTTATCAGGCAAGATGGGTCCAATCTCAA	None
5-dn4167	TTGAGATTGGACCCATCTTGCCTGATAAAATATAATATCACAAAAGCCAGTAAATTCTGA	None
3-dn4167	GGCGCGCCACTAGTAATACACTTAAAATAGTGTGGTACTACTTC	SpeI
MA4164-F01	GGCCATATGGATGACCTACCGGAAGAAG	NdeI
MA4164-R01	GGCCTGCAGGCAAAAACACCTGTAAAGTTCAACTCC	SbfI
MA4165-F01	GGCCATATGGTATCTGAGATGACCTCAAAAGAAAG	NdeI
MA4165-R01	GGCCTGCAGGCAAAGCTTATCTGATCAAAGCTTATCTGC	SbfI
MA4166-F01	GGCCATATGAAATACCGCTGGGTGGTTTTTG	NdeI
MA4166-R01	GGCCTGCAGGCCTGATTTTTGCTCCAAACCTTTCC	SbfI
MA4167-F01a	GGCCATATGAGATTGGACCCATCTTGCCTG	NdeI
MA4167-R01	TTCCCGATCCTTTTTCTCATGTGATACGGGATTTCACTAAGGTCG	None
MA4167-F02	CTTAGTGAAATCCCGTATCACATGAGAAAAAGGATCGGGAACCTG	None
MA4164-F03	GGCGCGCCCATATGGATGACCTACCGGAAGAAGTCCAG	AscI, NdeI
MA4166-R03	GGCGCGCCTGCAGGCCGGAAGACTAAGGTTTTTCAGATACTCTGC	SbfI
16sFor	ATTCTGGTTGATCCTGCCAGAGGTTAC	None
T7_16sRev	GCCGGGAATTTAATACGACTCACTATAGGGGGTCAGGTTCGAACACGGCACG	None
23sFor	CAAACGTCTGGCGGTAAAAT	None
T7_23sRev	GCCGGGAATTTAATACGACTCACTATAGGGCCTATCGGGGTCCTCTTCTC	None

Table S3. Generation time for *Methanosarcina* strains on various substrates

	Generation time (h) on various substrates[a]						
Strain	**MeOH**	**TMA**	**DMA**	**MMA**	**Acetate**	**DMS**	**MeSH**
WT C2A	9.6 ± 0.6	16 ± 3.5	18.2 ± 3.3	25.6 ± 3.4	46.3 ± 9.6	20.7 ± 3.6	44.8 ± 5.7
WWM82	10.1 ± 1.0	15.5 ± 2.5	26.4 ± 3.1	29.1 ± 6.6	49.5 ± 13.8	24.1 ± 4.7	52.8 ± 1.2
Δ*mtpCAP*	9.0 ± 0.4	18.4 ± 0.3	23.7 ± 3.7	29.1 ± 7.5	47.2 ± 13.1	25.7 ± 5.0	45.3 ± 7.0
Δ*mtpC*	10.2 ± 0.6	13.1 ± 1.4	20.9 ± 1.2	39.9 ± 3.5	48.6 ± 9.2	23.4 ± 2.4	30.7 ± 0.3
Δ*mtpA*	9.9 ± 0.2	13.4 ± 0.8	22.7 ± 2.6	28.3 ± 4.1	48.7 ± 8.2	21.5 ± 2.1	47.8
Δ*mtpP*	9.3 ± 0.5	14.3 ± 1.2	21.4 ± 2.6	28.3 ± 3.0	56.4 ± 1.4	23.8 ± 1.3	46.1 ± 1.5
Δ*msrH*	9.3 ± 0.6	14.8 ± 2.7	22.4 ± 1.9	32.0 ± 2.6	61.0 ± 7.2	25.6 ± 5.2	35.4

[a] Growth was measured as indicated in Materials and Methods. Values represent the average and standard deviations of at least three replicates, with the exception of Δ*mtpA* and Δ*msrH* on MeSH, which were measured once. Abbreviations: methanol (MeOH), trimethylamine (TMA), dimethylamine (DMA), monomethylamine (MMA), dimethylsulfide (DMS), methanethiol (MeSH). Strains used are described in Table 1.

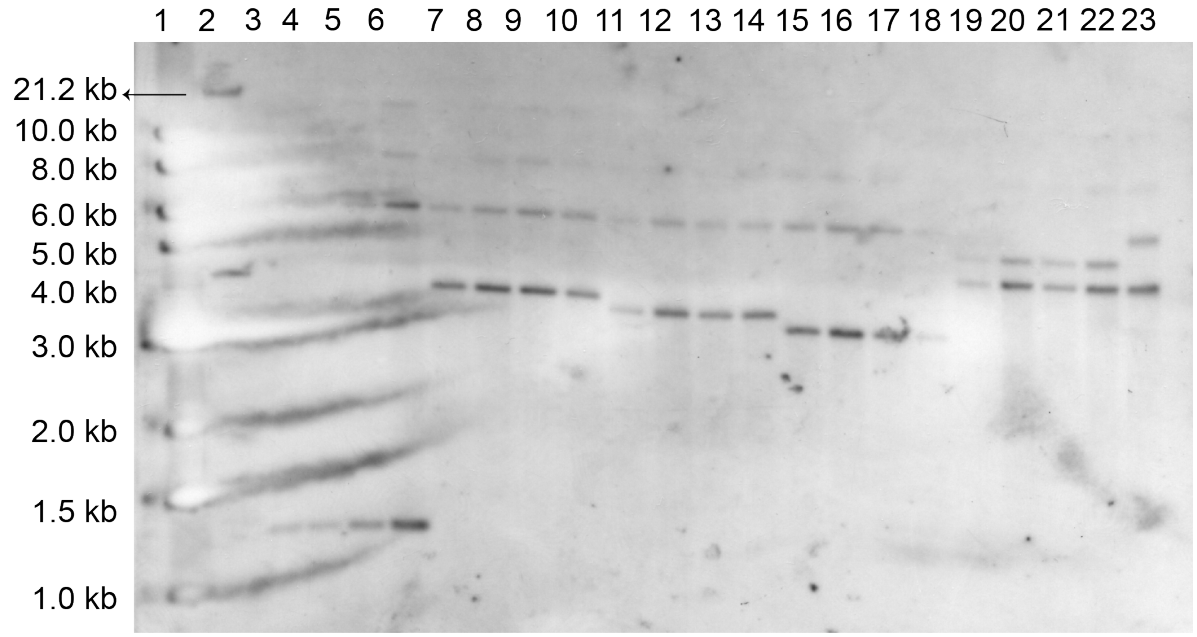

Fig. S1. Verification of the genotypes of *mtpCAP-msrH* mutants via Southern hybridization. Genomic DNA from several independent isolates was prepared, restricted with BstBI, separated by agarose gel electrophoresis, blotted onto membranes and probed with DIG-labeled pFH002 as described in the main text. Lane 1, 1-kb DNA ladder (NEB); Lane 2, DIG-labeled EcoRI/HindIII lambda DNA (Roche); Lane 3-6: Δ*mtpCAP* (predicted bands size: 6.4 kb, 1.3 kb); Lane 7-10: Δ*mtpC* (predicted bands size: 6.4kb, 4.0 kb); Lane 11-14: Δ*mtpA* (predicted bands size: 6.4 kb, 3.8 kb); Lane 15-18: Δ*mtpP* (predicted bands size: 6.4 kb, 3.5 kb)*;* Lane 19-22: Δ*msrH* (predicted bands size: 5.6 kb, 4.8 kb); Lane 23: Wild-type C2A (predicted bands size: 6.4 kb, 4.8 kb).

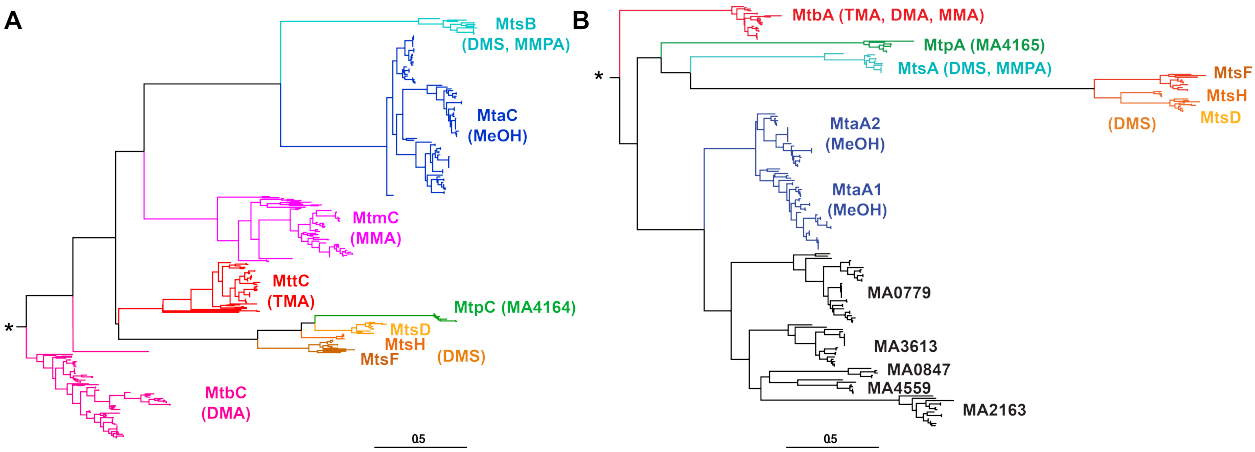

Fig. S2. Phylogeny of *Methanosarcina* MT1 corrinoid subunits and MT2 proteins. Homologous proteins from thirty sequenced *Methanosarcina* genomes were retrieved and analyzed using the ITEP bioinformormatics tools (3). Clusters of orthologous genes were generated in ITEP using MCL clustering with stringent parameters. Protein alignments were then generated with CLUSTALW prior to construction of phylogenetic trees using FastTree (4) using WAG model of evolution. Substrate-specific clades were identified based on the inclusion of biochemically or genetically characterized proteins. *Panel A:* Phylogeny of MT1 corrinoid subunits. Methionine synthase (MetH, GenBank accession number NP418443) from *E. coli* was used as an out-group to root the tree (shown in asterisk). *Panel B:* Phylogeny of MT2 proteins. UroD from *Desulfobacterium autotrophicum* (GenBank accession number ACN17787) was used as an out-group to root the tree (shown in asterisk). Scale bar refers to 0.5 substitutions per site. Abbreviations: TMA, Trimethylamine; DMA, Dimethylamine; MMA, Monomethylamine; MeOH, Methanol; DMS, Dimethylsulfide; MMPA, Methylmercaptopropionate. Genomes analyzed include: *M. acetivorans C2A, M. barkeri Fusaro, M. mazei Go1, M. sp.* MTP4, *M. thermophila* TM1, *M. thermophila* CHTI55, *M. thermophila* MSTA1, *M. vacuolata* Z761, *M. sp.* Kolskee, *M. barkeri* Weismoor, *M. barkeri* MS, *M. barkeri* 227, *M. barkeri* 3, *M. siciliae* C2J, *M. siciliae* HI350, *M. siciliae* T4M, *M. sp.* WH1, *M. sp.* WWM586, *M. lacustris* Z7289, *M. horonobensis* HB1, *M. mazei* SarPi, *M. mazei* LYC, *M. mazei* S6, *M. mazei* TMA, *M. mazei* WWM610, *M. mazei* C16, *M. baltica* GS1, *M. sp.* Naples 100, *M. lacustris* ZS and *M. calensis* Cali. Full data can be found in NCBI BioProjects 230935-230962, and GenBank accession numbers NC_003901.1, NC_003552.1 and NC_007355.1).

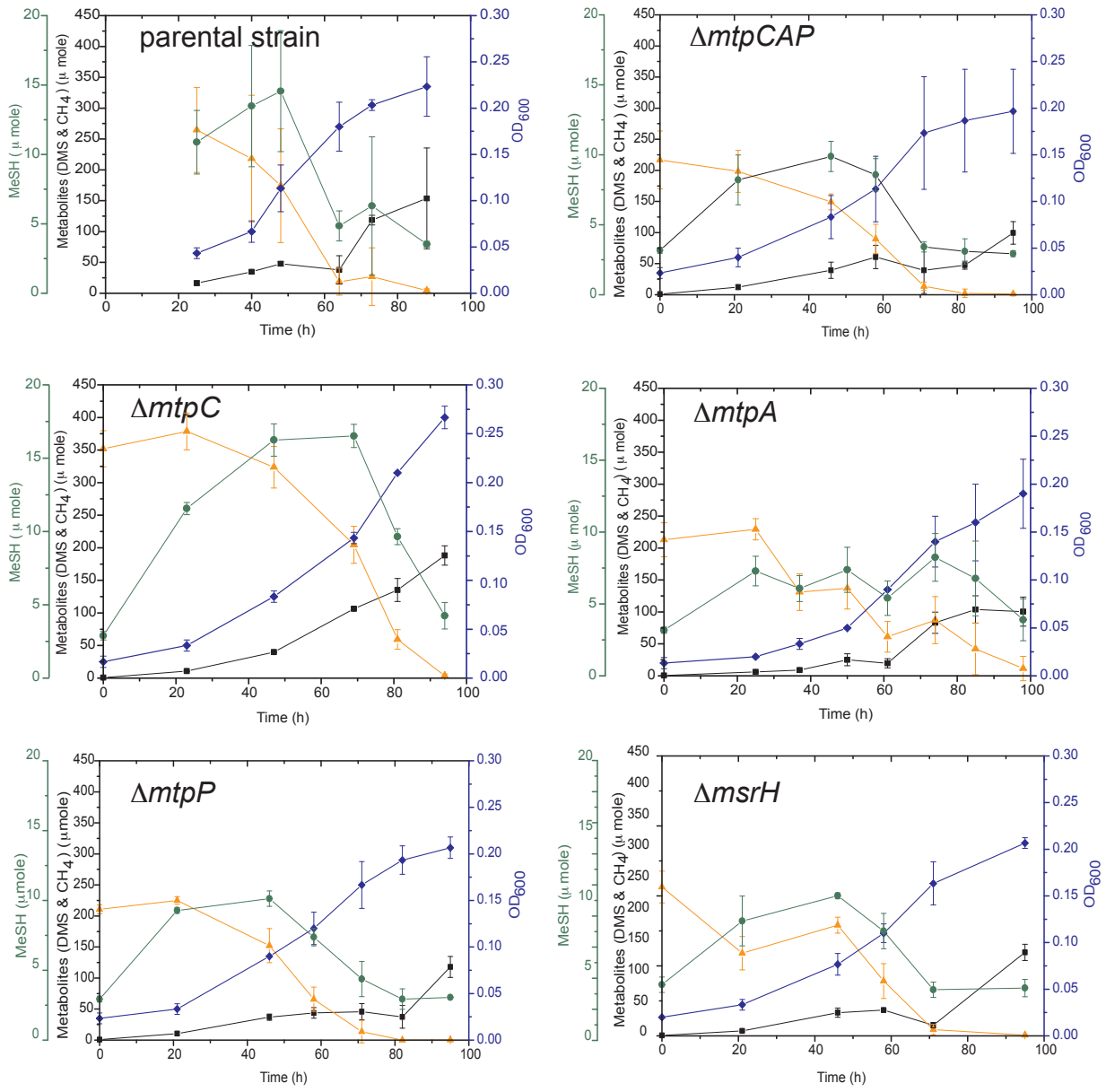

Fig. S3. Growth and metabolite production of *M. acetivorans* strains in DMS medium. The indicated mutants were grown in HS-MA medium with 20 mM DMS. Metabolites were measured via gas chromatography as described. Strains used were: WWM82 (parental strain), WWM829 (Δ*mtpCAP*), WWM830 (Δ*mtpC*), WWM831 (Δ*mtpA*), WWM832 (Δ*mtpP*), WWM833 (Δ*msrH*). Error bars represent standard deviation of triplicate cultures. Symbols: ■, Methane; ●, MeSH; ▲, DMS; ♦, OD_{600}. Error bars represent standard deviation of triplicate cultures.

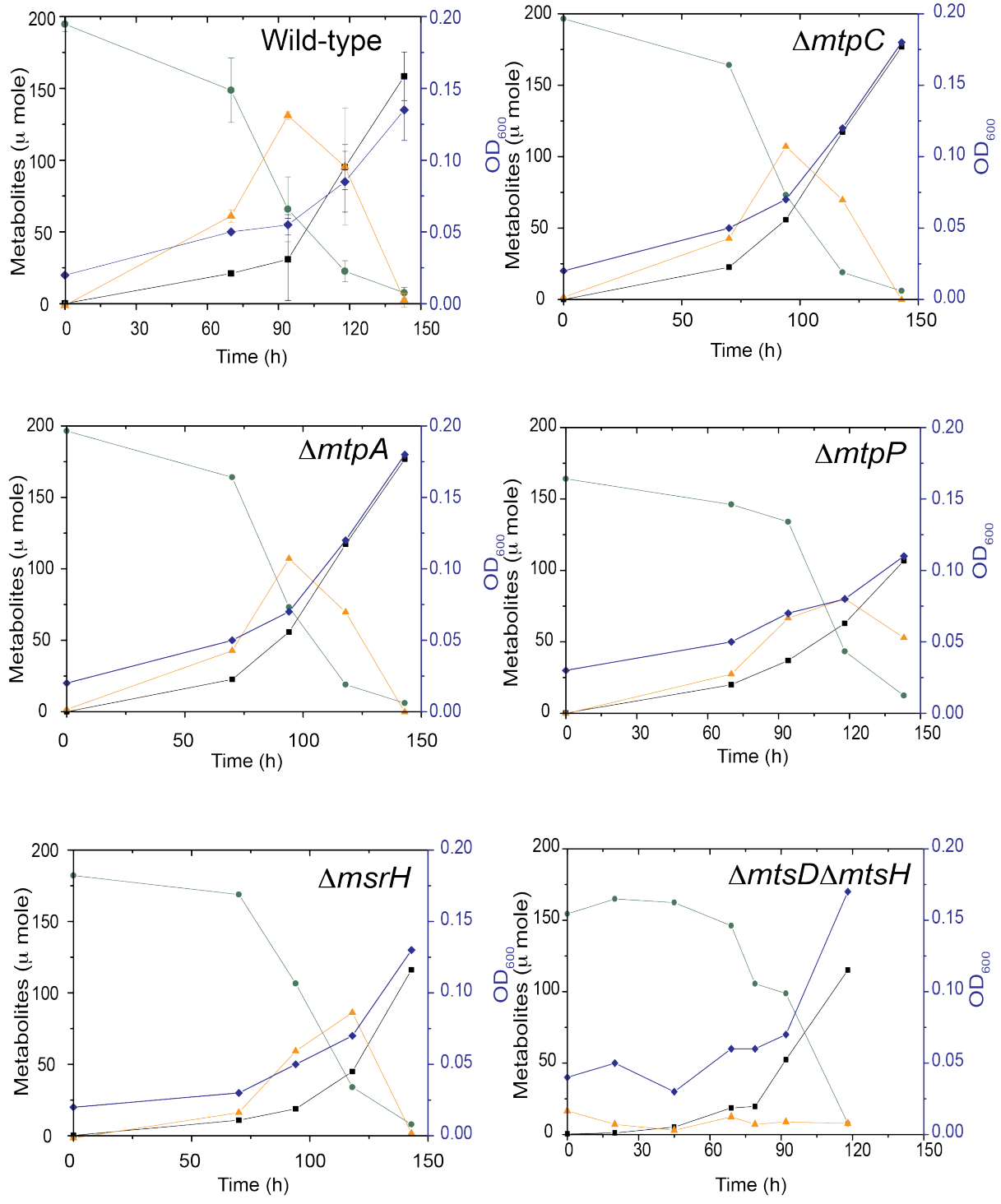

Fig. S4. Growth and metabolite production of *M. acetivorans* strains in MeSH medium. The indicated mutants were grown in HS-MA medium with 20 mM MeSH. Metabolites were measured via gas chromatography as described. Strains used were: WWM82 (parental strain), WWM830 (Δ*mtpC*), WWM831 (Δ*mtpA*), WWM832 (Δ*mtpP*), WWM833 (Δ*msrH*) and WWM814 (Δ*mtsD*Δ*mtsH*). Symbols: ■, Methane; ●, MeSH; ▲, DMS; ♦, OD_{600}. Error bars represent standard deviation of triplicate cultures.

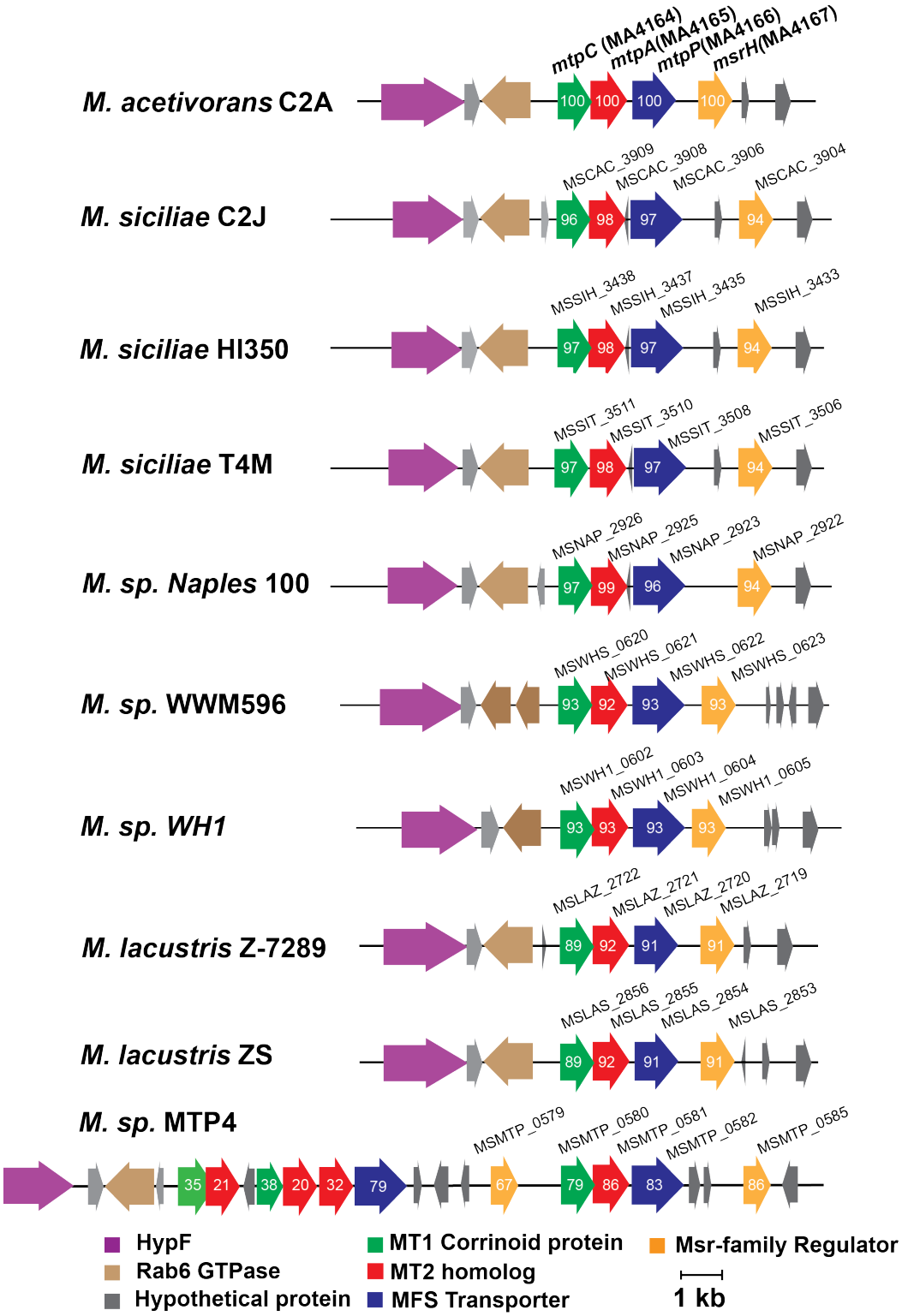

Fig. S5. The genomic neighborhoods of the *mtpCAP-msrH* ten sequenced *Methanosarcina* genomes. The *M. acetivorans* locus is shown at the top with the homologous loci on subsequent lines. The locus tags and percent identity to the corresponding *M. acetivorans* genes are shown for each. The general protein families were color coded as indicated based on annotations found in the published *M. acetivorans* genome sequence (5).

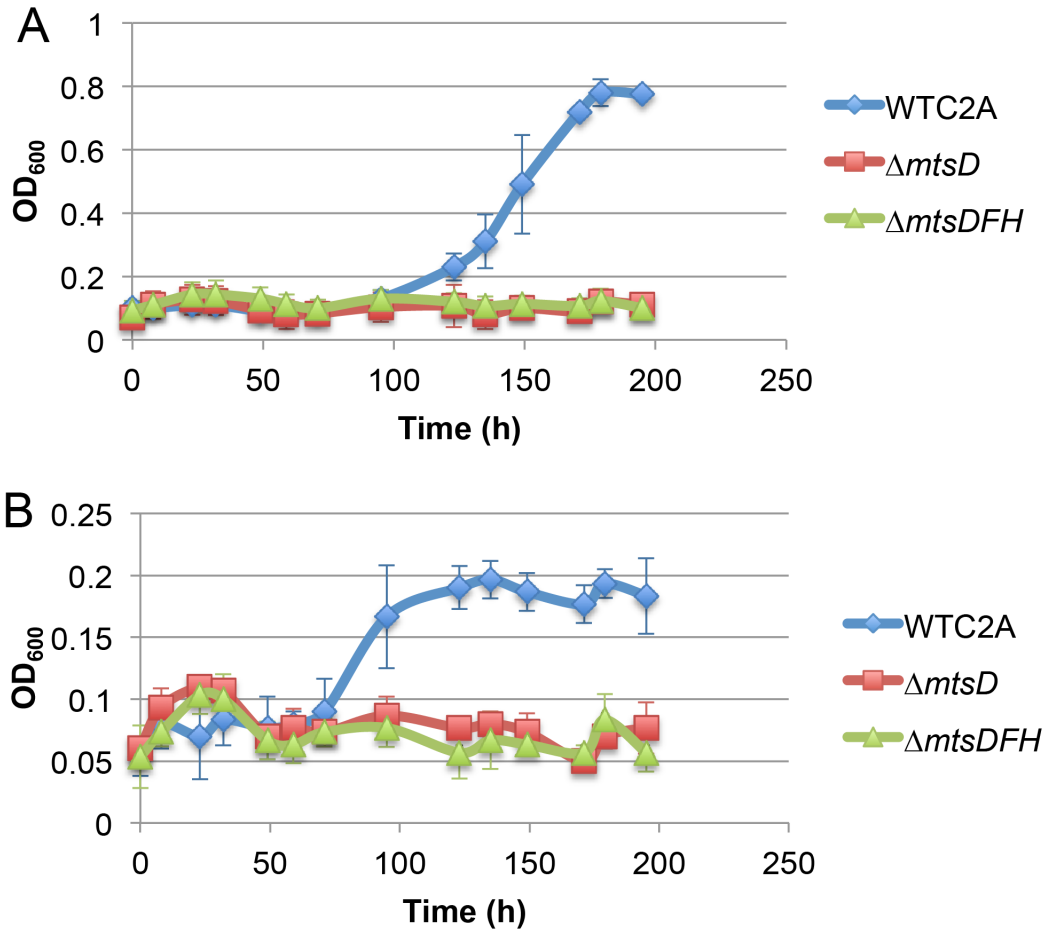

Fig. S6. Growth of *M. acetivorans* strains in DMS medium. The indicated mutants were grown in HS medium with 20 mM DMS (A) and 5 mM DMS (B). Values are the average of triplicates cultures, and error bars are the standard deviations.

Table S4 Legend

The whole dataset of RNA-seq analysis of *M. acetivorans* grown on MMPA, DMS, MeSH and MeOH. For pair-wise comparison, our criteria for differentially expressed genes: fold expression change > 4, P-value < .01.

References

1. **Pritchett MA, Zhang JK, Metcalf WW.** 2004. Development of a markerless genetic exchange method for *Methanosarcina acetivorans* C2A and its use in construction of new genetic tools for methanogenic archaea. Appl. Environ. Microbiol. **70:**1425-1433.
2. **Guss AM, Rother M, Zhang JK, Kulkarni G, Metcalf WW.** 2008. New methods for tightly regulated gene expression and highly efficient chromosomal integration of cloned genes for *Methanosarcina* species. Archaea **2:**193-203.
3. **Benedict MN, Henriksen JR, Metcalf WW, Whitaker RJ, Price ND.** 2014. ITEP: an integrated toolkit for exploration of microbial pan-genomes. BMC Genomics **15:**8.
4. **Price MN, Dehal PS, Arkin AP.** 2010. FastTree 2--approximately maximum-likelihood trees for large alignments. Plos One **5:**e9490.
5. **Galagan JE, Nusbaum C, Roy A, Endrizzi MG, Macdonald P, FitzHugh W, Calvo S, Engels R, Smirnov S, Atnoor D, Brown A, Allen N, Naylor J, Stange-Thomann N, DeArellano K, Johnson R, Linton L, McEwan P, McKernan K, Talamas J, Tirrell A, Ye WJ, Zimmer A, Barber RD, Cann I, Graham DE, Grahame DA, Guss AM, Hedderich R, Ingram-Smith C, Kuettner HC, Krzycki JA, Leigh JA, Li WX, Liu JF, Mukhopadhyay B, Reeve JN, Smith K, Springer TA, Umayam LA, White O, White RH, de Macario EC, Ferry JG, Jarrell KF, Jing H, Macario AJL, Paulsen I, Pritchett M, Sowers KR, et al.** 2002. The genome of *M. acetivorans* reveals extensive metabolic and physiological diversity. Genome Res. **12:**532-542.