

Supplemental Material:

Highlighting the unique roles of radical SAM enzymes in methanogenic archaea

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Table S1. Proteins with CX₃CX₂C domain in <i>M. maripaludis</i> S2 genome (tax ID 267377)				
UniProtKB Accession number	Locus tag	Annotation	Comments	Selected references
Q6M170	MMP0047	Hypothetical protein; TIGR04013 family B12-binding domain/radical SAM domain-containing protein		
Q6M161	MMP0056	CofH1, F ₀ synthase subunit 2		Philmus et al., 2015 (1)
Q6M160	MMP0057	CofH2; F ₀ synthase subunit 2		Philmus et al., 2015 (1)
Q6M141	MMP0077	QueE, 7-carboxy-7-deazaguanine synthase		McCarty et al., 2013 (2)
Q6M124	MMP0094	tRNA pseudouridine (54/55) synthase, Pus10	Not a radical SAM	
Q6M0T1	MMP0188	Dph2, dipthamide biosynthesis enzyme		Su et al., 2013 (3)
Q6M096	MMP0375	Conserved hypothetical protein		
Q6M059	MMP0412	MtaB, tRNA-t(6)A37 methylthiotransferase		Arragain et al., 2010 (4)
Q6LZX9	MMP0494	helicase family member with a unique C-terminal domain including a metal-binding cysteine cluster		
Q6LZS9	MMP0544	MoaA/nifB/pqqE family, AmmeMemoRadiSam system radical SAM enzyme		
Q6LZQ3	MMP0571	GTP 3',8-cyclase MoaA		
Q6LZP4	MMP0580	Radical activating enzyme		
Q6LZH0	MMP0658	NifB, nitrogen fixation protein		Hu and Ribbe, 2016 (5); Kang et al., 2021 (6)

Q6LZC5	MMP0704	Iron-sulfur cluster carrier protein, Mrp/NBP35 family ATP-binding protein	Likely not radical SAM	
Q6LYX4	MMP0861	KAM, lysine 2,3-aminomutase		Pflüger et al., 2003 (7)
Q6LYV9	MMP0876	CofG, F ₀ synthase subunit 1		Philmus et al., 2015 (1)
Q6LYA5	MMP1086	Radical SAM domain containing protein		
Q6LYA4	MMP1087	Uncharacterized protein		
Q6LY68	MMP1123	B12-binding domain-containing radical SAM protein		
Q6LY47	MMP1146	PurF, Amidophosphoribosyltransferase	Likely not radical SAM	
Q6LY40	MMP1153	nickel-dependent hydrogenase large subunit	Likely not radical SAM	
Q6LY13	MMP1180	Radical SAM domain containing protein		
Q6LY01	MMP1192	Radical SAM domain containing protein		
Q6LY00	MMP1193	Hypothetical protein		
Q6LXV8	MMP1238	Biotin synthase		
Q6LXV1	MMP1245	FwdF, Tungsten containing formylmethanofuran dehydrogenase, subunit F	Not a radical SAM	
Q6LXQ2	MMP1297	FdhB, Formate dehydrogenase beta subunit	Not a radical SAM	
Q6LXK0	MMP1350	Radical SAM core domain-containing protein	This is likely QCMT, MCR glutamine C-methyl-transferase	Gagsteiger et al., 2022 (8)
Q6LXB2	MMP1440	Taw1, 4-demethylwyosine synthase		Young and Bandarian, 2015 (9)
Q6LX72	MMP1480	3-isopropylmalate dehydratase large subunit	Not a radical SAM	

Q6LX00	MMP1554	Conserved hypothetical archaeal protein	This is Mmp10, MCR arginine methylase	Radle et al., 2019 (10)
Q6LWR8	MMP1638	MoaA/nifB/pqqE family, unknown function		
Known radical SAM enzymes in <i>M. maripaludis</i> with non-canonical cysteine motif				
Q6LWX7	MMP1577	Elp3, tRNA uridine(34) 5-carboxymethylaminomethyl modification radical SAM/GNAT enzyme	CX ₄ CX ₂ C motif	Selvadurai et al., 2014 (11)
Q6LYH7	MMP1014	RaSEA, archaeosine biosynthesis protein	CX ₇ CX ₂ C motif	Yokogawa et al, 2019 (12)
P61428	MMP0187	ThiC, Phosphomethylpyrimidine synthase	CX ₂ CX ₃ C motif	
Q6M0Z2	MMP0126	5,10-methenyltetrahydromethanopterin hydrogenase cofactor biosynthesis protein HmdB	This is now called HcgA, mis-annotated in some places as biotin synthase, CX ₅ CX ₂ C motif	McGlynn et al., 2010 (13); Schick et al., 2012 (14)

Table S2. Proteins with CX₃CX₂C domain in <i>M. acetivorans</i> C2A genome (tax ID 188937)				
UniProtKB Accession number	Locus tag	Annotation	Comments	Selected references
Q8TUM6	MA_0040	Tyw1/Taw1, 4-demethylwyosine synthase		Young and Bandarian, 2015 (9)
Q8TUV6	MA_0071	Radical activating enzyme		
Q8TUG2	MA_0105	MoaA, GTP 3',8-cyclase		
Q8TUB9	MA_0154	PylB, (3R)-3-methyl-D-ornithine synthase		Krzycki, 2013 (15)
Q8TUB3	MA_0160	Hypothetical protein		
Q8TU32	MA_0242	Hypothetical protein		
Q8TTX0	MA_0305	FmdF, formylmethaofuran dehydrogenase, subunit F	Ferredoxin, not radical SAM	
Q8TTT9	MA_0336	cell-surface protein		
Q8TTJ5	MA_0436	Hypothetical protein		
Q8TT64	MA_0573	NirJ/AhbD, Heme d1 biosynthesis protein		Kuhner et al., 2014 (16); Kuhner et al., 2016 (17)
Q8TT18	MA_0622	Hypothetical protein		
Q8TSX9	MA_0664	Rnf electron transport complex subunit B	Not radical SAM	
Q8TSK2	MA_0794	Tungsten-containing aldehyde ferredoxin oxidoreductase cofactor modifying protein		
Q8TSK1	MA_0795	Hypothetical protein		
Q8TRQ9	MA_1114	MoaA/nifB/pqqE family protein, lipid biosynthesis(?)		Zeng et al., 2022 (18)
Q8TRM2	MA_1153	MtaB, 2-methylthioadenine synthase		Arragain et al., 2010 (4)
Q8TRH4	MA_1204	Hypothetical protein		
Q8TR79	MA_1302	Tungsten-containing aldehyde ferredoxin oxidoreductase cofactor modifying protein, MoaA/NifB/PqqE/SkfB family		

Q8TQW6	MA_1421	Predicted protein		
Q8TQQ4	MA_1486	MoaA/nifB/pqqE family protein, Tetraether synthase (Tes)		Zeng et al., 2022 (18)
Q8TQQ1	MA_1489	cofH2, F ₀ synthase subunit 2		Philmus et al., 2015 (1)
Q8TQQ0	MA_1490	cofH1, F ₀ synthase subunit 2		Philmus et al., 2015 (1)
Q8TQP9	MA_1491	cofG, F ₀ synthase subunit 1		Philmus et al., 2015 (1)
Q8TQM0	MA_1520	Hypothetical protein		
Q8TPT1	MA_1824	thymidylate synthase, DUF166 superfamily (functions unknown)	Thymidylate synthase annotation likely incorrect	
Q8TNN8	MA_2245	ferredoxin	Likely not radical SAM	
Q8TNN6	MA_2248	Predicted protein		
Q8TNM4	MA_2260	Predicted protein		
Q8TN83	MA_2410	Formate acetyltransferase activating enzyme, pfl		
Q8TMU4	MA_2557	Predicted protein		
Q8TML0	MA_2647	AtsB, Arylsulfatase regulator	Two genes annotated as AtsB in MA genome	
Q8TMI5	MA_2672	ATP-dependent helicase, YprA superfamily, C-term metal binding domain		
Q8TME9	MA_2709	Predicted protein		
Q8TMB1	MA_2753	Predicted protein		
Q8TMA9	MA_2755	Predicted protein		
Q8TM98	MA_2768	Predicted protein		
Q8TLS9	MA_2949	Predicted protein		
Q8TLS6	MA_2952	Hypothetical protein		
Q8TL56	MA_3185	MoaA/nifB/pqqE family protein, unknown function		
Q8TK83	MA_3533	Predicted protein		
Q8TLK1	MA_3035	PqqE, PQQ synthesis protein E	This is not PqqE, it is AhbC (12,18-didecarboxysiroheme deacetylase)	Kuhner et al., 2014 (16); Kuhner et al., 2016 (17)
Q8TKS9	MA_3317	AtsB, Arylsulfatase regulator	Two genes annotated as AtsB in MA genome	

Q8TKF9	MA_3448	MoaA/nifB/pqqE family protein, unknown function		
Q8TKC6	MA_3482	MoaA/nifB/pqqE family protein, unknown function		
Q8TKD3	MA_3475	Predicted protein		
Q8TJR7	MA_3711	Hypothetical protein		
Q8TJ11	MA_3979	KAM, lysine 2,3-aminomutase		Pflüger et al., 2003 (7)
Q8TIZ8	MA_3991	Carboxymuconolactone decarboxylase	Likely not radical SAM	
Q8TIJ8	MA_4151	Metallo cofactor biosynthesis protein		
Q8TIH5	MA_4174	FmdF, Formylmethanofuran dehydrogenase, subunit F	Not radical SAM	
Q8TIF7	MA_4195	NifB, nitrogen fixation protein		Hu and Ribbe, 2016 (5); Kang et al., 2021 (6)
Q8TIF5	MA_4197	QueE, 7-carboxy-7-deazaguanine synthase		McCarty et al., 2013 (2)
Q8TI77	MA_4279	PqqE, PQQ synthesis protein E	Methanogens likely do not synthesize PQQ	
Q8TIB7	MA_4238	Hypothetical protein		
Q8THT9	MA_4422	MoaA/nifB/pqqE family protein, unknown function		
Q8THI2	MA_4534	Hypothetical protein		
Q8THG6	MA_4551	MCR arginine C-methyltransferase (Mmp10)		Radle et al., 2019 (10)
Q8THA2	MA_4618	UPF0313		
Known radical SAM enzymes in <i>M. acetivorans</i> with non-canonical cysteine motif				
Q8TSH0	MA_0826	Elp3, tRNA uridine(34) 5-carboxymethylaminomethyl modification radical SAM/GNAT enzyme	CX ₄ CX ₂ C motif	Selvadurai et al., 2014 (11)
Q8THK1	MA_4513	RaSEA, archaeosine biosynthesis protein	CX ₇ CX ₂ C motif	Yokogawa et al, 2019 (12)
Q8TPW4	MA_1790	ThiC1, Phosphomethylpyrimidine synthase 1	C-terminal CX ₂ CX ₃ C motif	

References:

1. Philmus B, Decamps L, Berteau O, Begley TP. 2015. Biosynthetic versatility and coordinated action of 5'-deoxyadenosyl radicals in deazaflavin biosynthesis. *J Am Chem Soc* 137:5406-13.
2. McCarty RM, Krebs C, Bandarian V. 2013. Spectroscopic, steady-state kinetic, and mechanistic characterization of the radical SAM enzyme QueE, which catalyzes a complex cyclization reaction in the biosynthesis of 7-deazapurines. *Biochemistry* 52:188-98.
3. Su X, Lin Z, Lin H. 2013. The biosynthesis and biological function of diphthamide. *Crit Rev Biochem Mol Biol* 48:515-21.
4. Arragain S, Handelman SK, Forouhar F, Wei FY, Tomizawa K, Hunt JF, Douki T, Fontecave M, Mulliez E, Atta M. 2010. Identification of eukaryotic and prokaryotic methylthiotransferase for biosynthesis of 2-methylthio-N⁶-threonylcarbamoyladenosine in tRNA. *J Biol Chem* 285:28425-33.
5. Hu Y, Ribbe MW. 2016. Maturation of nitrogenase cofactor-the role of a class E radical SAM methyltransferase NifB. *Curr Opin Chem Biol* 31:188-94.
6. Kang W, Rettberg LA, Stiebritz MT, Jasniewski AJ, Tanifuji K, Lee CC, Ribbe MW, Hu Y. 2021. X-Ray Crystallographic Analysis of NifB with a Full Complement of Clusters: Structural Insights into the Radical SAM-Dependent Carbide Insertion During Nitrogenase Cofactor Assembly. *Angew Chem Int Ed Engl* 60:2364-2370.
7. Pfluger K, Baumann S, Gottschalk G, Lin W, Santos H, Muller V. 2003. Lysine-2,3-aminomutase and beta-lysine acetyltransferase genes of methanogenic archaea are salt induced and are essential for the biosynthesis of N^ε-acetyl-β-lysine and growth at high salinity. *Appl Environ Microbiol* 69:6047-55.
8. Gagsteiger J, Jahn S, Heidinger L, Gericke L, Andexer JN, Friedrich T, Loenarz C, Layer G. 2022. A Cobalamin-Dependent Radical SAM Enzyme Catalyzes the Unique Cα-Methylation of Glutamine in Methyl-Coenzyme M Reductase. *Angew Chem Int Ed Engl* doi:10.1002/anie.202204198.
9. Young AP, Bandarian V. 2015. Mechanistic Studies of the Radical S-Adenosyl-L-methionine Enzyme 4-Demethylwyosine Synthase Reveal the Site of Hydrogen Atom Abstraction. *Biochemistry* 54:3569-72.
10. Radle MI, Miller DV, Laremore TN, Booker SJ. 2019. Methanogenesis marker protein 10 (Mmp10) from *Methanosarcina acetivorans* is a radical S-adenosylmethionine methylase that unexpectedly requires cobalamin. *J Biol Chem* doi:10.1074/jbc.RA119.007609.
11. Selvadurai K, Wang P, Seimetz J, Huang RH. 2014. Archaeal Elp3 catalyzes tRNA wobble uridine modification at C5 via a radical mechanism. *Nat Chem Biol* 10:810-2.
12. Yokogawa T, Nomura Y, Yasuda A, Ogino H, Hiura K, Nakada S, Oka N, Ando K, Kawamura T, Hirata A, Hori H, Ohno S. 2019. Identification of a radical SAM enzyme involved in the synthesis of archaeosine. *Nat Chem Biol* 15:1148-1155.
13. McGlynn SE, Boyd ES, Shepard EM, Lange RK, Gerlach R, Broderick JB, Peters JW. 2010. Identification and characterization of a novel member of the radical AdoMet enzyme superfamily and implications for the biosynthesis of the Hmd hydrogenase active site cofactor. *J Bacteriol* 192:595-8.

14. Schick M, Xie X, Ataka K, Kahnt J, Linne U, Shima S. 2012. Biosynthesis of the iron-guanylylpyridinol cofactor of [Fe]-hydrogenase in methanogenic archaea as elucidated by stable-isotope labeling. *J Am Chem Soc* 134:3271-80.
15. Krzycki JA. 2013. The path of lysine to pyrrolysine. *Curr Opin Chem Biol* 17:619-25.
16. Kuhner M, Haufschildt K, Neumann A, Storbeck S, Streif J, Layer G. 2014. The alternative route to heme in the methanogenic archaeon *Methanosarcina barkeri*. *Archaea* 2014:327637.
17. Kuhner M, Schweyen P, Hoffmann M, Ramos JV, Reijerse EJ, Lubitz W, Broring M, Layer G. 2016. The auxiliary [4Fe-4S] cluster of the Radical SAM heme synthase from *Methanosarcina barkeri* is involved in electron transfer. *Chem Sci* 7:4633-4643.