SUPPLEMENTARY MATERIALS

Efficient sulfide assimilation in *Methanosarcina acetivorans* is mediated by the MA1715 protein

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TABLE S1.Detection of sulfur-containing compounds in *M. acetivorans*

strain	acid labile sulfide (nmol/mg protein)	tRNA thiolation?
<i>wt</i> -like	-	yesa
Δ <i>ma</i> 1715	-	yes ^a
∆ <i>oass</i> ∆oahs	14.4 ± 1.9	yes ^a
∆oass∆oahs∆ma1715	14.0 ± 0.8	yesa

^a see Fig 5 for details

TABLE S2. List of primers used

- 1. CCCGGAAACACTAGTATTGTGTCGCCAGAATAAGC
- 2. GTGATTTTCGGCACCTCCGCCAGCTTGTCTGGCGGCCCTTCAC
- 3. CAAGCTGGCGGAGGTGCCGAAAATCACAAGAGCCGAGGTCAGGCATG
- 4. CATCGGGAGCTGGTACCGGGTTCTCCC
- 5. CCCCAAGTGAATCCATATGCCAGAACCC
- 6. GCCGCCCCAACACTCGAGTTTTGTAATATACTCATGCC
- 7. GGAGATATACGCATGCCAGAACCCAC
- 8. CCTTTCGGGCCCTGTTAGCAGC
- 9. GAGGCAAAGTACAGATTCTTCTGGACAGGTGG
- 10. CCTATGTACTCCCCCTGAATCACAAGTGCCC



FIG S1. Growth of *M. acetivorans* strains on HS_{DTT} medium supplemented with 0.05 mM sodium sulfide. The $\Delta ma1715$ +pBR60 experiment was conducted in the presence of 0.05 mg/ml tetracycline·HCI. All data points represent the mean of at least three experiments. Error bars reflect standard deviations. Starter cultures were grown to late exponential phase (0.3 < A600 < 0.7) on the HS_{Met} medium, which contains 0.4 mM sulfide, 3 mM cysteine and 3 mM methionine. These were diluted into experimental HS_{DTT} media so that A₆₀₀ would equal 0.01 upon inoculation.



FIG S2. Growth of *M. acetivorans* strains with different concentrations of sodium sulfide, present as the sole sulfur source. All data points represent the mean of at least three experiments. Error bars reflect standard deviations. Prior to each experiment, strains were grown for at least 12 generations with 0.8 mM sodium sulfide present as the sole sulfur source. Data for the $\Delta ma 1715$ experiments were re-plotted from Fig. 3.



FIG S3. Growth of the $\Delta oass \Delta oahs \Delta ma 1715$ strain with varying concentrations of homocysteine as the sole sulfur source.

Α

D. vulgaris (190-234)CPVSLCSSSATIGHS-----LSLGVGNIAAVRARDASLADAAATLFGNMLM. acetivoarns (156-199)SITGVCTSAGTVGPS-----ISFGMADAAAIFSDDVSLADAAATALGN-EM. maripaludis (146-190)NGYGICTSSGTVGHS-----VSLGNADSITVFSKSAIIADAAATSIGNFAA. cellulolyticus (155-199)TPMGICTSSGTVGHS-----VSLGNADSITVFSKSAIIADAAATSIGNFAT. pallidum (255-315)RDCSVVTSGAYERFFERDGVRYHHIIDPVTGF-PAHTDVDSVSIFAPRSTDADALATACFVL-S. enterica (228-288)NGHGISTSGSYRNYYELDGKRVSHVIDPQTGR-PIEHNLVSVTVIAPTALEADGWDTGLMVL-D. vulgaris (242-300)TECAIATSGTSEVYFDAR-HQHHHLITPVAGRSP--ASTGSVSVIAPTVMEADALATALSVI-T. maritima (253-312)KSGAVATSGDYERYFVVDGVRYHHILDPSTGY-PA-RGVWSVTIIAEDATTADALSTAGFVM-



FIG S4. Structural comparison of COG2122a and ApbE. **(A)** Sequence alignment of representatives of ApbE (black) and COG2122a (blue). Consensus identities of ApbE are shaded grey, and are rarely conserved in COG2122a. Residues D306 and T310 (shaded black) of *T pallidum* ApbE may coordinate the magnesium ions required for FAD cleavage. The highly conserved cysteine residue of COG2122a (yellow highlight) is not conserved in ApbE. **(B-C)** The X-ray crystal structure of *T. pallidum* ApbE (grey and blue cartoon) is shown bound to FAD (yellow sticks) and two Mg²⁺ ions (green spheres visible in panel C). Regions of ApbE that are not conserved in COG2122a (gaps > 4 amino acids; blue cartoon) form approximately half of the FAD-binding interface, and contact the adenylate and flavin moieties directly. D306 and T310 are depicted as sticks.