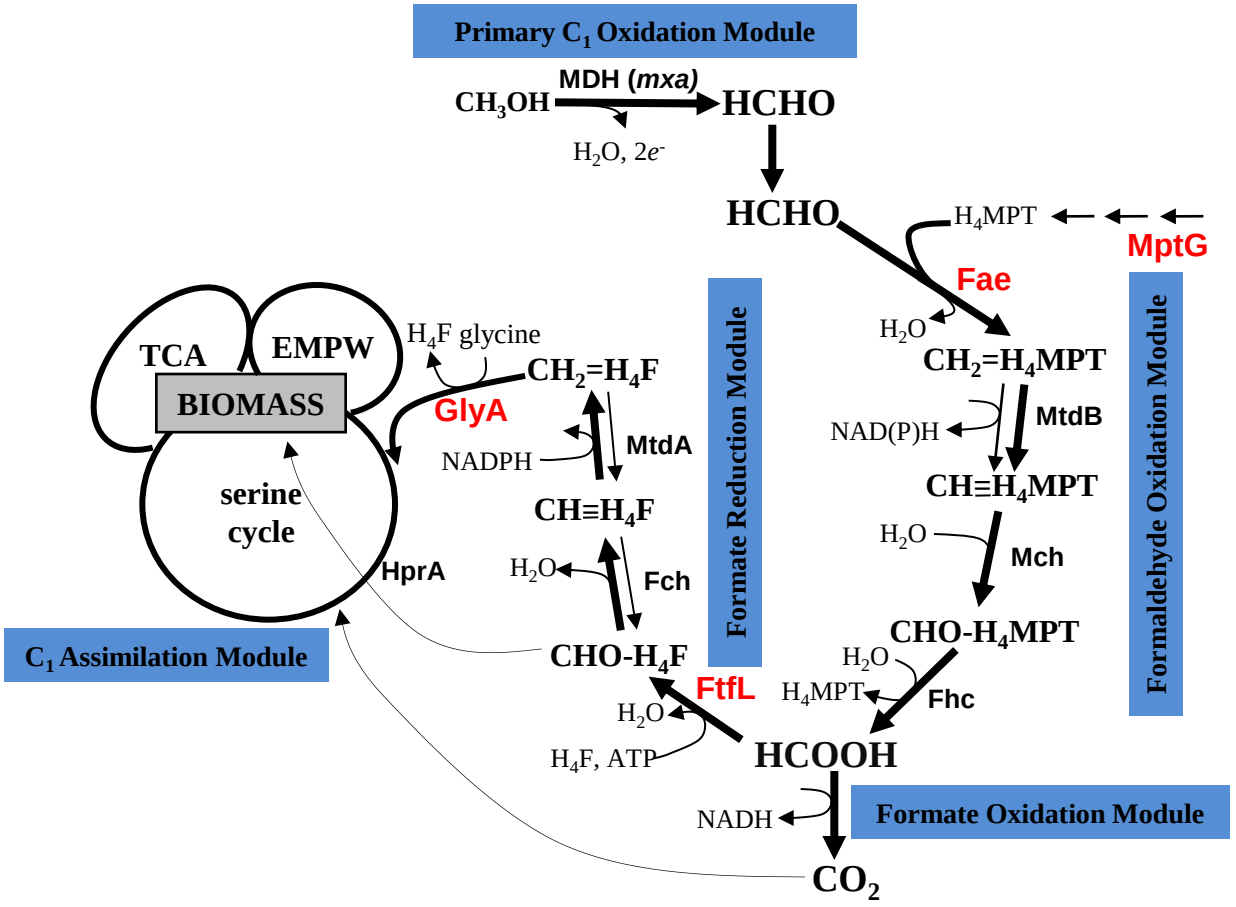
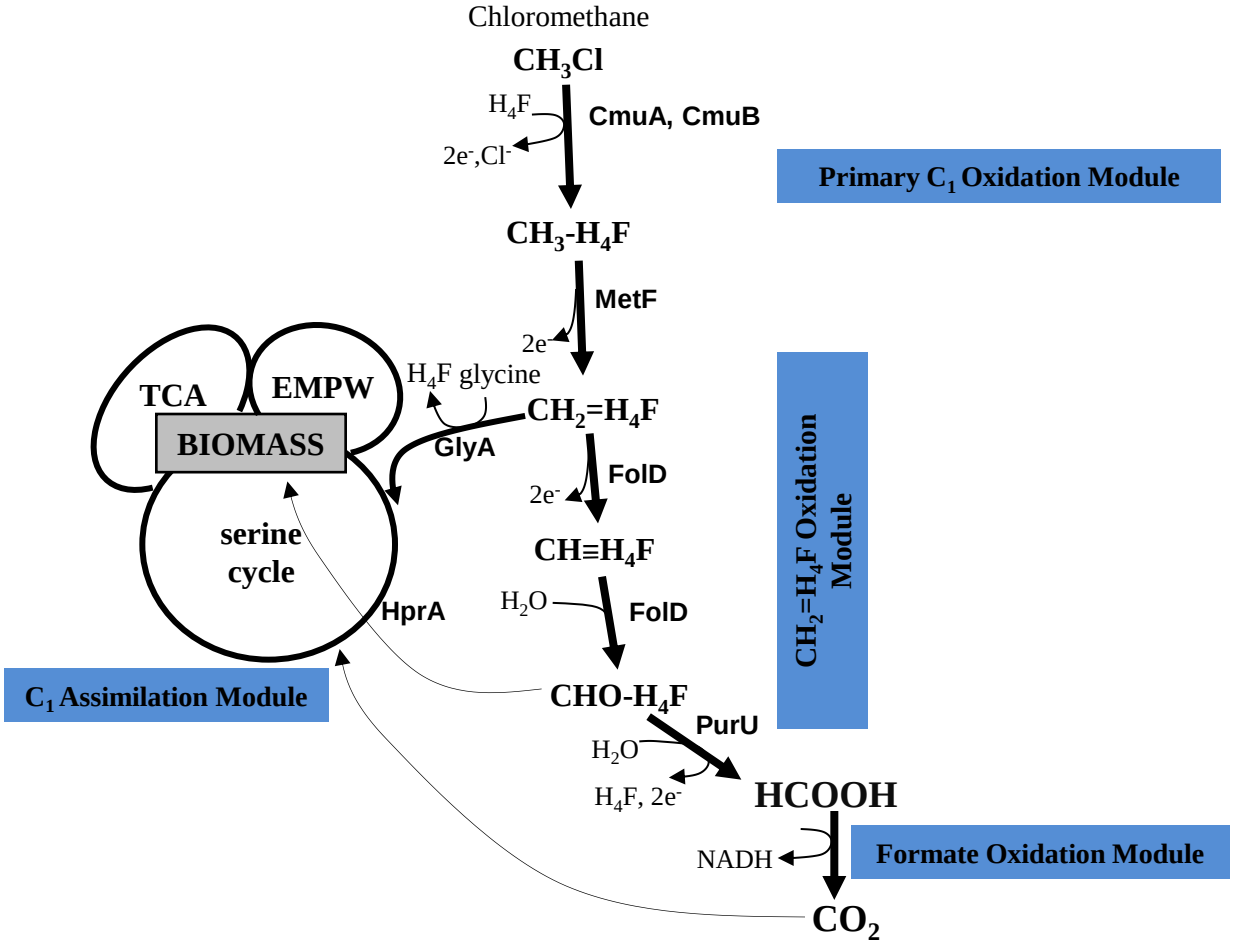
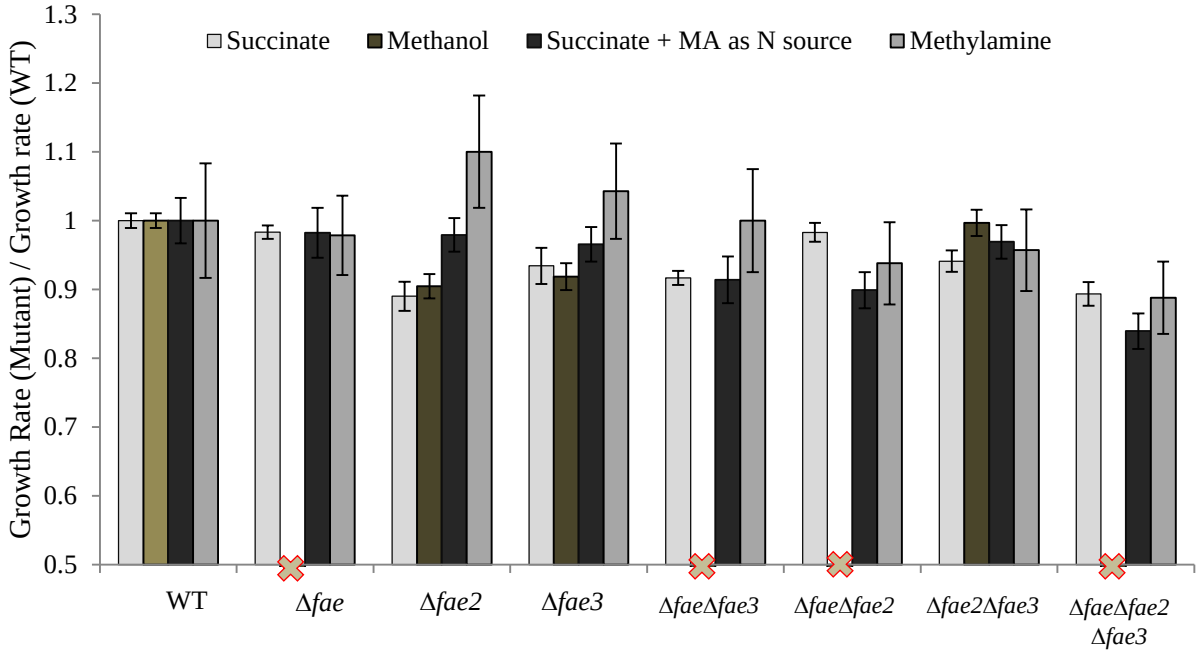
Supplementary Figure 1: A metabolic map illustrating metabolic modules that are involved in methanol growth in *M. extorquens* PA1. Methanol dehydrogenase serves as the primary C_1 oxidation module that oxidizes methanol to formaldehyde. Formaldehyde is further oxidized to formate by a H_4MPT mediated pathway which acts as the formaldehyde oxidation module. Formate is the branch point for metabolism - a part gets oxidized to CO_2 by formate dehydrogenases and the rest gets reduced and assimilated via a H_4F mediated pathway and serine cycle. Genes highlighted in red, representative of a particular module, were deleted to test the role of the specific module in methylamine growth via the *N*-methylglutamate pathway.

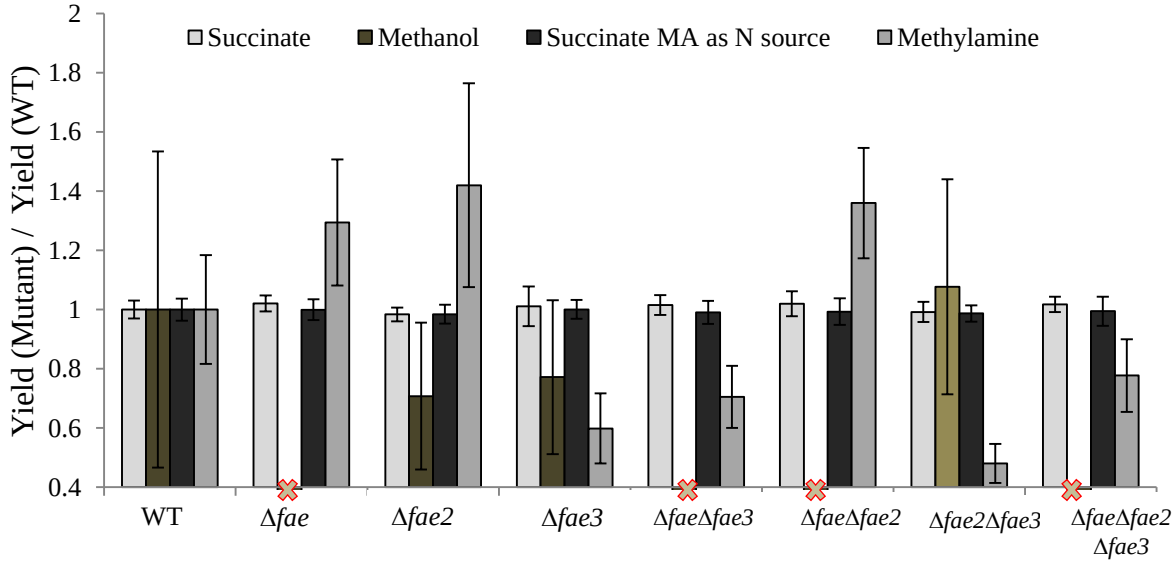


Supplementary Figure 2: A metabolic map illustrating the various metabolic modules that are involved in chloromethane growth in *M. extorquens* CM4. The primary C_1 oxidation module consists of CmuA and CmuB- that catalyze dehalogenation of chloromethane and subsequent methyl transfer to tetrahydrofolate (H_4F) to form methyl tetrahydrofolate (CH_3-H_4F) - and MetF that catalyzes the reduction of CH_3-H_4F to methylene tetrahydrofolate (CH_2=H_4F). CH_2=H_4F is the branch point of metabolism –a part gets oxidized by methylene tetrahydrofolate reductase (*folD*) and formyl tetrahydrofolate hydrolase (*purU*) to formate, which subsequent gets oxidized to CO_2 by a panel of formate dehydrogenases, and the rest gets assimilated via the serine cycle. ***metF, folD, and purU** are absent in *M. extorquens* PA1

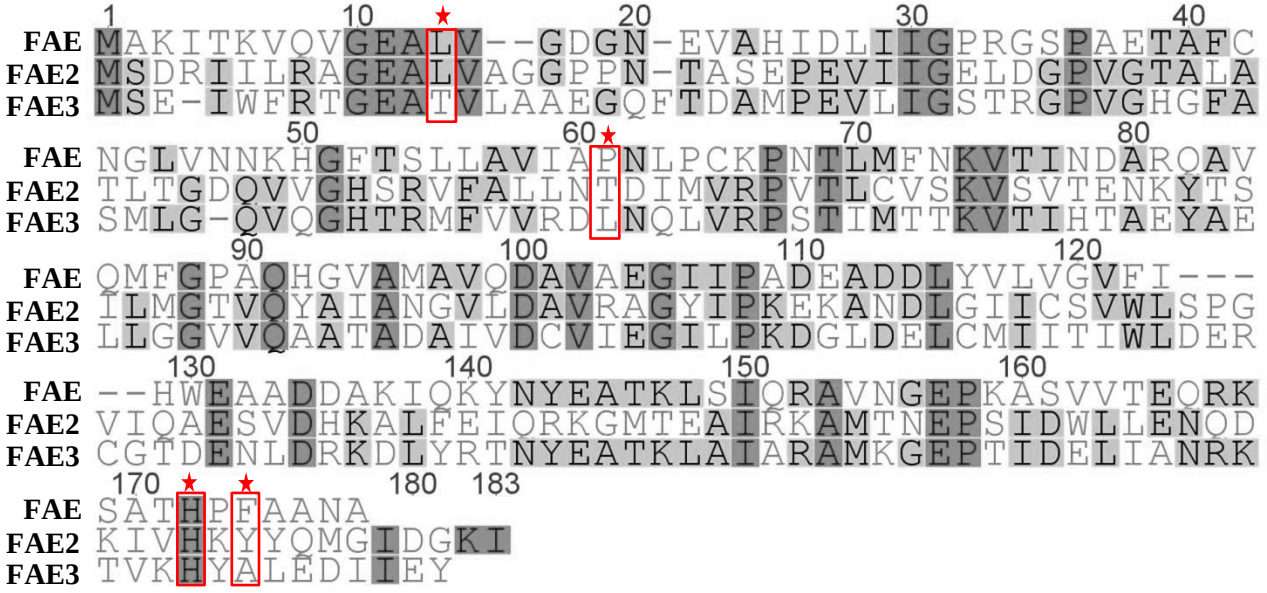


Supplementary Figure 3: A) Ratio of growth rates, of various knockout mutants versus the Δ*cel* 'wildtype' strain of PA1 and, in 3.5 mM succinate (white), 15mM methanol (light brown),nitrogen free media with 3.5 mM succinate and 7.66 mM methylamine (dark gray) and 15mM methylamine (blue) **B)** Ratio of yield (measured as the maximum OD_{600} value during growth) of various knockout mutants versus Δ*cel* 'wildtype' strain of PA1, in 3.5 mM succinate (white), 15mM methanol (light brown), nitrogen free media with 3.5 mM succinate and 7.66 mM methylamine (dark gray) and 15mM methylamine (blue). Error bars represent the 95% C.I. of the average ratio of three biological replicates grown in each condition. A X indicates that the mutants did not grow in methanol. **NOTE: The y-axis doesn't start at 0.0 but at 0.5 (for growth rate ratios) and 0.4 (for yield ratios). This was done to highlight subtle changes in growth/yield for these mutants**





Supplementary Figure 4: Amino acid alignment of FAE, FAE2 and FAE3 from *M. extroquens* PA1 (using MUSCLE: https://www.ebi.ac.uk/Tools/msa/muscle/ with standard settings and 150 iterations). The highlighted residues in FAE interact with methyl groups present in tetrahydromethanopterin (H_4MPT), absent in H_4F. These interactions are responsible for the specificity of FAE to H_4MPT. Two of these residues are identical and one residue (Y173) has similar properties in FAE2. Only one residue is identical in FAE3.



Supplementary Table 1: Growth rates of the Δ*cel* 'wildtype' strain of PA1 and the Δ*mptG* mutant (in the WT background) in 3.5 mM succinate with either 7.66 mM NH_4^+ as the nitrogen source or 7.66 mM methylamine as the nitrogen source. S.E.M. represents the standard error of the mean growth rate for three biological replicates in each condition.

Strain	Nitrogen Source	Mean Growth Rate (h^{-1})	S.E.M. (h^{-1})
WT	NH_4^+	0.213	0.002
Δ*mptG*	NH_4^+	0.184	0.002
WT	Methylamine	0.188	0.001
Δ*mptG*	Methylamine	0.148	0.002

Supplementary Table 2: Growth rates and maximum OD_{600} of the Δ*cel* 'wildtype' strain of PA1 and single-, double-, triple-knockout mutant (in the WT background) lacking Δ*fae*, Δ*fae2*, *and/or* Δ*fae3* in 15mM methylamine

Strain	Mean Growth Rate (h^{-1}) ± 95%C.I. (h^{-1})	Average Max. OD_{600} ± 95% C.I.
WT	0.0422±0.0018	0.712±0.071
Δ*fae*	0.0407±0.0004	1.044±0058
Δ*fae* 2	0.0476±0.0015	1.165±0.146
Δ*fae* 3	0.0427±0.0011	0.413±0.0.49
Δ*fae* -Δ*fae2*	0.0386±0.0008	1.003±0.033
Δ*fae* -Δ*fae3*	0.0406±0.0006	0.558±0.021
Δ*fae*2-Δ*fae3*	0.0409±0.0015	0.357±0.011
Δ*fae* -Δ*fae2*-Δ*fae*3	0.0375±0.0003	0.555±0.040

Supplementary Table 3: List of primers used to construct knockout mutants in the *N*-methylglutamate pathway as well as FAE-homologs in this study

Primer	Primer Sequence	Primer Description
Δfae2_us_f	ATGGATGCATATGCTGCAGCTCGAGCGGCCGCT CTGCATCAGGTCGTGCAGG	Gibson forward linker with Not1 and 24 bp of pCM433 backbone at 5' end. Product size = 311 bp
Δfae2_us_r	CTTTTCGACACTCAAACGCATCGAAGCGCGAGG ATGATGCGGTCGCTCATGG	30 bp of ds region linker at 5'end. Product size = 311 bp
Δfae2_ds_f	CGCGCTTCGATGCGTTTGAG	Product size = 412 bp
Δfae2_ds_r	GGTTAACACGCGTACGTAGGGCCCGCGGCCGCC GACACCTCGTCGTTGCTCAAG	Gibson reverse linker with Not1 and 24 bp of pCM433 backbone at 5' end. Product size = 412 bp
Δfae3_us_f	GCGGAACCAGATCTCGGACATG	Product size = 252 bp
Δfae3_us_r	GGTTAACACGCGTACGTAGGGCCCGCGGCCGCT TCCCTCGCCTGATCCACTG	Gibson reverse linker with Not1 and 24 bp of pCM 433 backbone at 5' end. Product size = 252 bp
Δfae3_ds_f	ATGGATGCATATGCTGCAGCTCGAGCGGCCGCC GATGGTCACCCTTCAGGTG	30 bp of us region linker at 5'end. Product size = 420 bp
Δfae3_ds_r	ATGACCGACATGTCCGAGATCTGGTTCCGCGCC TCACGCGAGAAGACTAC	Gibson forward linker with Not1 and 24 bp of pCM433 backbone at 5' end. Product size = 420 bp
Δmgs1,2,3_us_f	ATGGATGCATATGCTGCAGCTCGAGCGGCCGCG GCAATGGAGGCGAATCTCG	Gibson forward linker with Not1 and 24 bp of pCM433 backbone at 5'end. Product size =433 bp
Δmgs1,2,3_us_r	TCGCGGCCCTCGTGCCAATCGTCATAGGCGCGT TCGATCACTGTCGCGAG	30 bp of ds region linker at 5'end. Product size = 433 bp
Δmgs1,2,3_ds_f	CGCCTATGACGATTGGCACG	Product size = 441 bp
Δmgs1,2,3_ds_r	GGTTAACACGCGTACGTAGGGCCCGCGGCCGCC AGATCGGTGTAGGACACGAGG	Gibson reverse linker with Not1 and 24 bp of pCM 433 backbone at 5' end. Product size = 441 bp
Δgmas_us_f	ATGGATGCATATGCTGCAGCTCGAGCGGCCGCT CGCAGTGATGACGCTGGAG	Gibson forward linker with Not1 and 24 bp of pCM433 backbone at 5'end. Product size =492 bp
Δgmas_us_r	CGCTTGACGCCCTCCACCGTGTGCCCGTCGGTGT ACATGTTTCCAGGGGAGCTGGATCAG	30 bp of ds region linker at 5'end. Product size = 492 bp
Δgmas_ds_f	ACATGTACACCGACGGGCAC	Product size = 435 bp
Δgmas_ds_r	GGTTAACACGCGTACGTAGGGCCCGCGGCCGCT GGCCACGATTAGGGCGAAG	Gibson reverse linker with Not1 and 24 bp of pCM 433 backbone at 5' end. Product size = 435 bp
Δmgd1,2,3,4_us_f	GACGATCACCACGTCGTAGG	Product size = 493 bp
Δmgd1,2,3,4_us_r	GGTTAACACGCGTACGTAGGGCCCGCGGCCGCT AGAGTTCGCAGCCCGACAG	Gibson reverse linker with Not1 and 24 bp of pCM 433 backbone at 5'end. Product size = 493 bp
Δmgd1,2,3,4_ds_f	ATGGATGCATATGCTGCAGCTCGAGCGGCCGCC TCGACTCGTACGCGCATTG	30 bp of us region linker at 5'end. Product size = 607 bp
Δmgd1,2,3,4_ds_r	CCGCAGGAGGCCTACGACGTGGTGATCGTCTGA CGAGCATCGCCCATCTC	Gibson forward linker with Not1 and 24 bp of pCM433 backbone at 5' end. Product size = 607 bp