

SUPPLEMENTARY INFORMATION

Reversing Methanogenesis to Produce Ethanol

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Table S1. Strains used in this study. Amp^R indicates ampicillin resistance, Pur^R indicates puromycin resistance.

Strains	Description	Source
<i>M. acetivorans</i> C2A	Wildtype <i>M. acetivorans</i>	<u>DSMZ</u>
C2A/pES1(Pmat)	Amp ^R , Pur ^R , R6K <i>ori</i> , C2A <i>ori</i> , P _{mcr_ANME-1}	6
C2A/pES1-MATmcr3	Amp ^R , Pur ^R , R6K <i>ori</i> , C2A <i>ori</i> , P _{mcr_ANME-1::mcrBGA_{ANME-1}}	6
C2A/pES1-EtOH	Amp ^R , Pur ^R , R6K <i>ori</i> , C2A <i>ori</i> , P _{mcr_ANME-1::mcrBGA_{ANME-1}} , P _{mcr_ANME-1::car} , P _{mcr_ANME-1::adh}	This study
<i>E. coli</i> DH5α (λ pir)	F ⁻ <i>endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG</i> φ80 <i>dlacZ</i> ΔM15 Δ(<i>lacZYA-argF</i>)U169 <i>hsdR17</i> (r _K ⁻ m _K ⁺) λpir	6

Table S2. Contribution of each carbon source in ethanol formed. Day 6 ethanol production by the engineered *M. acetivorans* at a turbidity of 40 is analyzed based on **Fig. 1** and **Fig. S3**. (1) indicates condition “+ CH₄ + YE + HA + 10 mM Fe(III)” i.e., HS medium supplemented with headspace methane, yeast extract, humic acids, and, 10 mM iron(III). (2) indicates condition “No CH₄ + YE + 10 mM Fe(III)” i.e., HS medium supplemented with only yeast extract and 10 mM iron(III). (3) indicates condition “No CH₄ + HA + 10 mM Fe(III)” i.e., HS medium supplemented with only humic acids and 10 mM iron(III). (4) represents condition “No CH₄ + 10 mM Fe(III)” i.e., HS medium supplemented with only 10 mM iron(III). “CH₄” indicates ethanol contributed by methane, calculated based on the conditions 1 to 4. Headspace methane was maintained at 1 atm. The concentration of yeast extract was 2.5 g/L, and the concentration of humic acids was 0.1 g/L. The uncertainties represent standard deviations calculated from three independent cultures.

Condition	Ethanol produced (μmol)	Source of ethanol	(%)	Calculation
C2A/pES1-MATmcr3				
+ CH ₄ + YE + HA + 10 mM Fe(III) (1)	370 ± 20	CH ₄ + YE + HA	100	
No CH ₄ + YE + 10 mM Fe(III) (2)	54 ± 4	YE	15 ± 1	(2/1 *100)
No CH ₄ + HA + 10 mM Fe(III) (3)	50 ± 10	HA	13 ± 4	(3/1 *100)
No CH ₄ + 10 mM Fe(III) (4)	9 ± 7	HS medium	3 ± 2	(4/1 *100)
From CH ₄ [1-(2+3+4)]	260 ± 20	CH ₄	69 ± 5	[1-(2+3+4)]/1 * 100
C2A/pES1(Pmat)				
+CH ₄ + YE + HA + 10 mM Fe(III) (1)	205 ± 9	CH ₄ + YE + HA	100	
No CH ₄ + YE + 10 mM Fe(III) (2)	60 ± 20	YE	30 ± 10	(2/1 *100)
No CH ₄ + HA + 10 mM Fe(III) (3)	30 ± 10	HA	16 ± 6	(3/1 *100)
No CH ₄ + 10 mM Fe(III) (4)	19 ± 4	Unknown	9 ± 2	(4/1 *100)
From CH ₄ [1-(2+3+4)]	90 ± 30	CH ₄	50 ± 10	[1-(2+3+4)]/1 * 100

Table S3. Carbon and electron recovery analysis for ethanol. Day 6 methane consumption and ethanol production by engineered *M. acetivorans* at a turbidity of 40 from methane as the sole carbon source with and without 10 mM iron(III) based on **Fig. 1**.

Media condition	Strains	Methane consumed (μmol)	Ethanol produced (μmol)	Carbon recovery (%)	Electron recovery (%)
CH ₄	C2A/pES1-MAT <i>mcr3</i>	130 ± 30	120 ± 10	130 ± 30	100 ± 20
	C2A/pES1(Pmat)	71 ± 9	50 ± 20	90 ± 30	70 ± 30
CH ₄ + 10 mM Fe(III)	C2A/pES1-MAT <i>mcr3</i>	150 ± 40	180 ± 20	160 ± 50	150 ± 40
	C2A/pES1(Pmat)	90 ± 10	60 ± 20	90 ± 30	90 ± 30

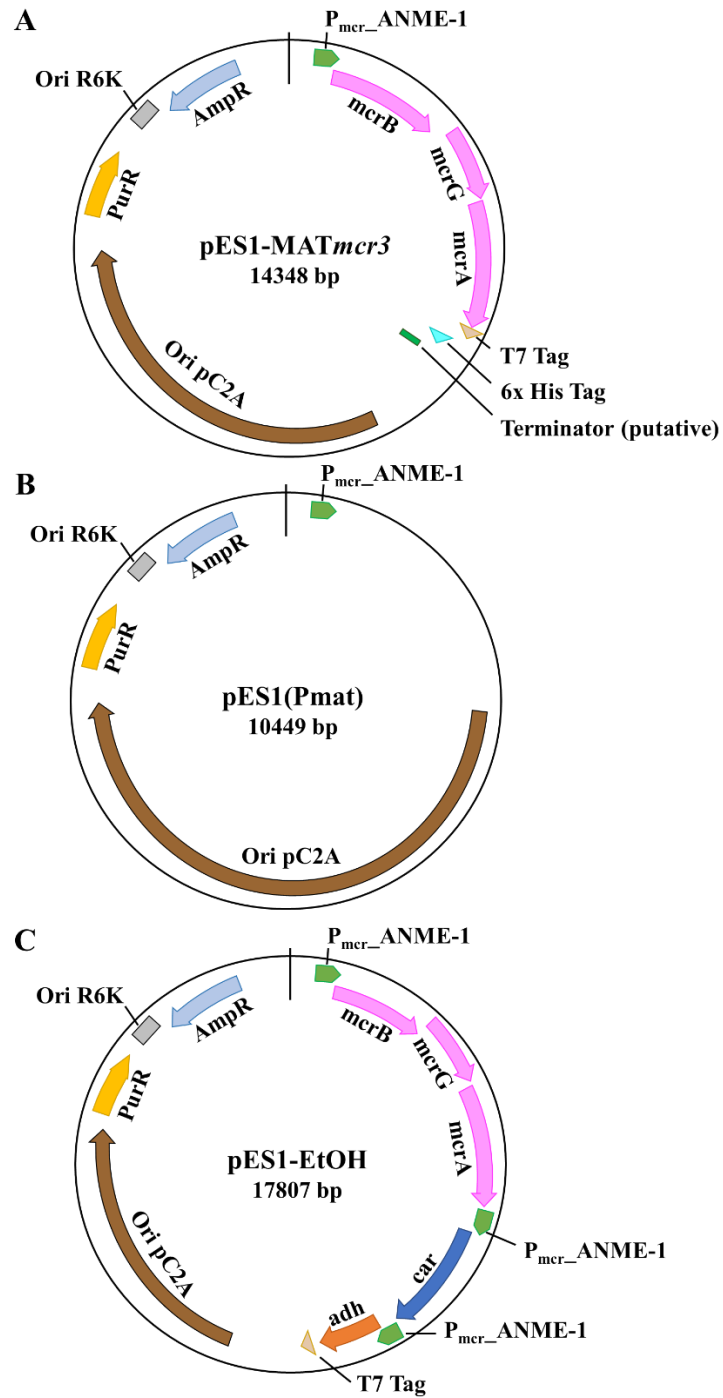


Figure S1. Plasmid maps for engineered *M. acetivorans* C2A strains. **A.** Plasmid map of pES1-MAT $mcr3$ with ANME $mcrBGA$ from the Black Sea driven by the native mcr promoter from ANME-1 (P_{mcr_ANME-1}). **B.** Plasmid map of empty pES1 plasmid. **C.** Plasmid map of pES1-EtOH with ANME $mcrBGA$, car from *M. thermoacetica*, and adh from *Synechocystis* sp. PCC 6803 each driven by the native mcr promoter from ANME-1 (P_{mcr_ANME-1}).

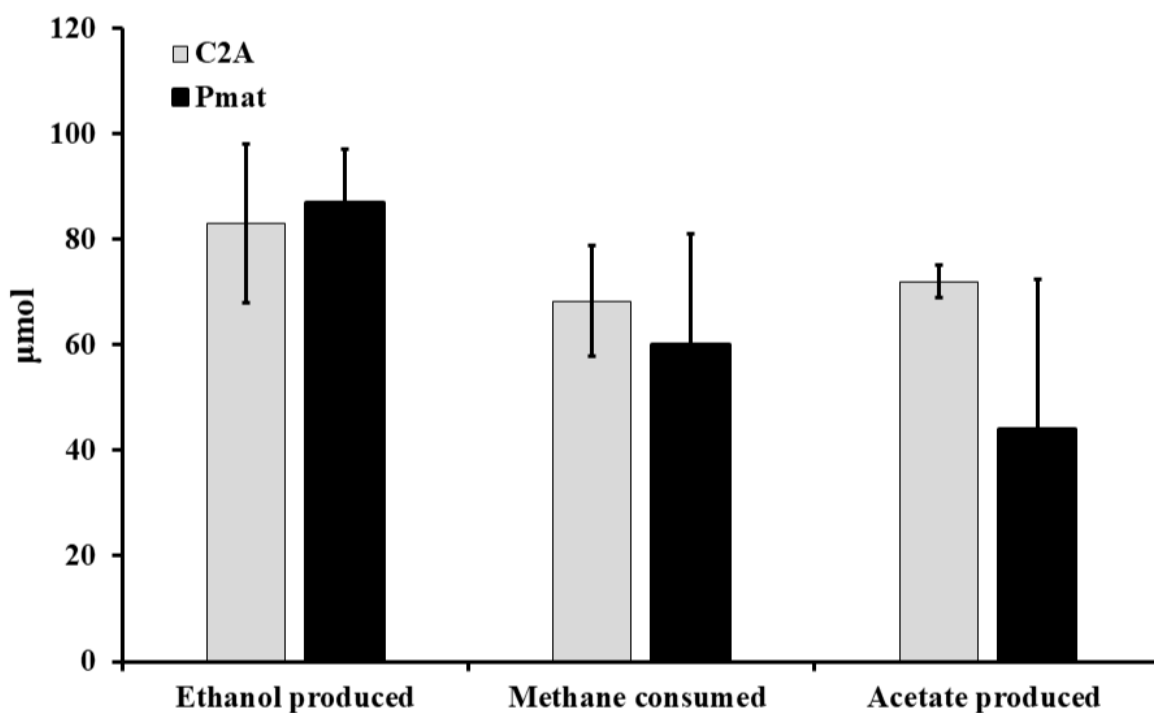


Figure S2. Ethanol production, methane consumption, and acetate production by *M. acetivorans* at a turbidity of 40 using methane as the sole carbon source in presence 10 mM iron(III) after six days of incubation in the absence of ANME-1 Mcr. C2A represents wild type *M. acetivorans* C2A without plasmid pES1(Pmat). Pmat represents C2A/pES1(Pmat). Bars and error bars are the mean and standard deviation of three independent cultures.

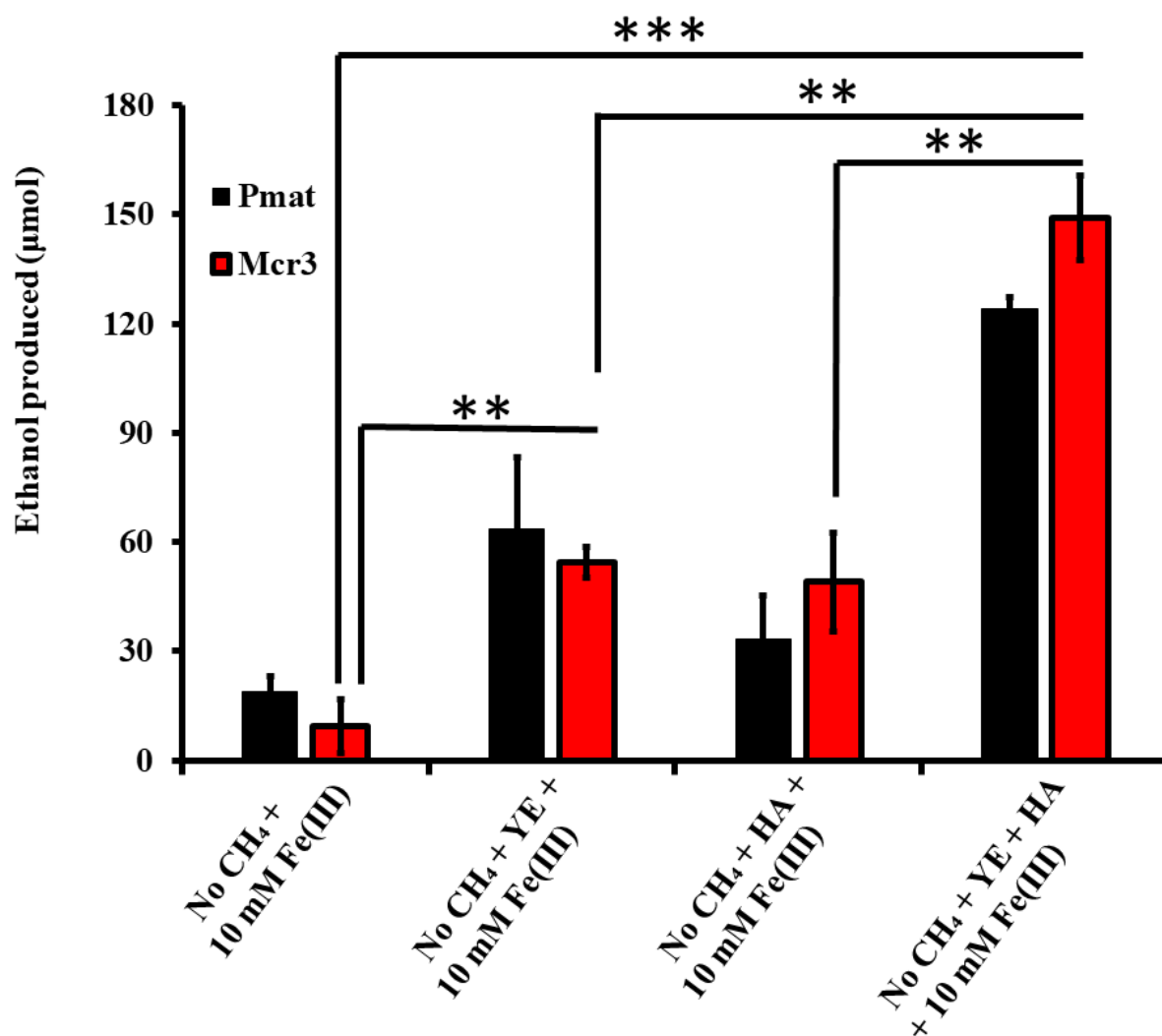


Figure S3. Ethanol production in the absence of methane by *M. acetivorans* C2A/pES1-MATmcr3 at a turbidity of 40 after six days of incubation. Bars and error bars are the mean and standard deviation of three independent cultures. ** p < 0.05, *** p < 0.005.