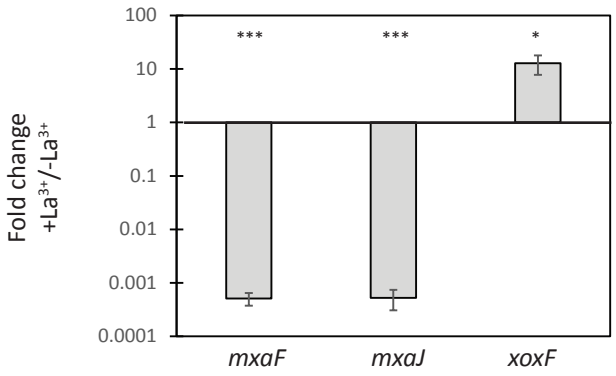
SUPPLEMENTAL MATERIALS AND METHODS

Complementation strain construction

All complementation strains are listed in Table S1. All complementation strains were constructed by conjugation of a pCM433-based suicide plasmid harboring a wild-type copy of the gene of interest, into the mutant strains (1). Conjugation was performed with *Escherichia coli* S17-1 λpir acting as the donor strain as previously described (1). Δ*mxaF*, Δ*mxaI*, and Δ*mxaB* strains were complemented using the genes under the control of their native promoter. Multiple attempts were made to complement the Δ*xoxF* mutant using its native promoter, without success. Therefore, the Δ*xoxF* mutant was complemented using the *tac* promoter, a strong promoter in *M. buryatense* 5GB1 (1). This complementation technique results in the integration of the complementation construct into the *M. buryatense* 5GB1C chromosome in regions known to be transcriptionally silent (2). The *mxa* complementation constructs were integrated between genes METBUDRAFT_2794 and METBUDRAFT_2795. The *xoxF* complementation construct was integrated between genes METBUDRAFT_1431 and METBUDRAFT_1432. Sucrose counter-selection was used to unmark all strains (1).

Strain FC52 was conjugated with plasmid pFC37 to create the *mxaF* complementation strain (FC59). Strain FC53 was conjugated with plasmid pFC40 to create the *xoxF* complementation strain (FC64). The *tac* promoter used in pFC40 was amplified from pAWP89 (1). Strain FC54 was conjugated with plasmid pFC38 to create the *mxaI* complementation strain (FC60). Strain FC63 was conjugated with pFC39 to create the mxaB complementation strain (FC65). All plasmids were constructed using Gibson assembly (3). All primers used for plasmid construction are listed in Table S2.



Supplemental Figure S1. Complementation of Δ*mxaB* restores wild-type lanthanum-dependent gene expression. qRT-PCR was performed on RNA harvested from the Δ*mxaB* complementation strain grown in the absence or presence of 30μM supplemental lanthanum. Results shown represent the fold change in gene expression in Δ*mxaB* complemented cells grown with lanthanum compared to gene expression in Δ*mxaB* complemented cells grown without lanthanum. Unpaired t-tests were used to determine significance (***=$p < 0.001$, **=$p < 0.01$, *=$p < 0.05$) between gene expression levels. Data represent means from three replicates ± standard deviations.

Table S1. *M. buryatense* 5GB1C strains and plasmids used in this study

Strain name		Genotype	Antibiotic resistance
M. buryatense 5GB1C		Wild-type	None
	FC31	METBUDRAFT_2794::P_{mxaF}-*xylE*	Unmarked
	FC52	Δ*mxaF*	Unmarked
	FC53	Δ*xoxF*	Unmarked
	FC54	Δ*mxaI*	Unmarked
	FC57	Δ*xoxFS*	Zeocin
	FC59	Δ*mxaF*; METBUDRAFT_2794::P_{mxaF}-*mxaF*	Unmarked
	FC60	Δ*mxaI*; METBUDRAFT_2794:: P_{mxaF}-*mxaI*	Unmarked
	FC63	Δ*mxaB*	Unmarked
	FC64	Δ*xoxF*; METBUDRAFT_::P_{tac}-*xoxF*	Unmarked
	FC65	Δ*mxaB*; METBUDRAFT_2794::P_{mxaB}-*mxaB*	Unmarked
Plasmids			
	pFC30	METBUDRAFT_2794::P_{mxaF}-*xylE*	kanamycin
	pFC37	METBUDRAFT_2794::P_{mxaF}-*mxaF*	kanamycin
	pFC38	METBUDRAFT_2794::P_{mxaF}-*mxaI*	kanamycin
	pFC39	METBUDRAFT_2794::P_{mxaB}-*mxaB*	kanamycin
	pFC40	METBUDRAFT_1431::P_{tac}-*xoxF*	kanamycin

26 Table S2. Primers used in this study*

For function	Primer name	Sequence
Gene knockouts		
FRT-zeo-FRT cassette	FC294_zeoR_F	*gaagttcctattctctagaaagtataggaacttc*CTCTGAAATGAGCTGTTGACAATTAATCAT
	FC295_zeoR_R	*gaagttcctatactttctagagaataggaacttc*CGTTCATGTCTCCTTTTTTATTCAGTCCTG
Δ*xoxF*	FC397_xoxFLF_F	AATAACGATTCGCCCTCTACCAATACC
	FC398_xoxFLF_R	tcagag*gaagttcctatactttctagagaataggaacttc*GTTGAGGCAATCAGCCAGCTTT
	FC399_xoxFRF_F	tgaacg*gaagttcctattctctagaaagtataggaacttc*TGGGCGGCACATTAACTGTATTC
	FC400_xoxFRF_R	GCTTGGTTGTCAAGGTTCGATC
Δ*mxaF*	FC413_mxaF_LF_F	AGTTGTTGCGCTAATTCGGGTTC
	FC414_mxaF_LF_R	tcagag*gaagttcctatactttctagagaataggaacttc*CAACAAGACGCCCGACACTAAT
	FC415_mxaF_RF_F	tgaacg*gaagttcctattctctagaaagtataggaacttc*TTAAGGAATTGGCGCATCACACC
	FC416_mxaF_RF_R	GATTCCTTGACATAGCGTGCTGCA
Δ*mxaI*	FC434_mxaILF_F	TGCAGCACGCTATGTCAAGGAATC
	FC435_mxaILF_R	tcagag*gaagttcctatactttctagagaataggaacttc*GAAGACGACAAAGCAACCGCGAAACT
	FC436_mxaIRF_F	tgaacg*gaagttcctattctctagaaagtataggaacttc*ACCGGCAAGTTTGTTTATGAGGTAG
	FC437_mxaIRF_R	GTTTCTTCTTTCTCGACCCGGTTG
Δ*mxaB*	FC404_367mxaF_R	GCTTCGGTTTGAATTGCCACA
	FC465_mxaBLF_R	tcagag*gaagttcctatactttctagagaataggaacttc*GTGTAGAGTTGGCAAGCGGTTT
	FC466_mxaBRF_F	ttgaac*ggaagttcctattctctagaaagtataggaacttc*AAACTCGGTGTCAAATCGGTCG
	FC467_mxaBRF_R	CAAGGAGAATGTCAAGGCGCTA
pFC30		
xylE gene	FC328_mdhxylE_F	tgcgaaaaatcaatctggaggaattATGAACAAAGGTGTAATGCGA
	FC329_xylE_R	tagccatgtttcctcaatggTCAGGTGAGCACGGTCATGAAT
P_{mxaF}	FC130_AP98mdh_R	AATTCCTCCAGATTGATTTTTCGCA
	AP235_62I_fwd_1	tcatgcgcttcatggttaaactgccgaattAATTAAACCGGGAATGATGTC
pFC37		
P_{mxaF} and	FC454_PmxaF_F	tcatgcgcttcatggttaaactgccgaattGTTGTTACTTCTCCATACAATTAAACCGGG
mxaF gene	FC458_mxaFORF_R	atcttagccatgtttcctcaatggTTACAACGAGAACACCATCACGC
pFC38		
P_{mxaF}	FC454_PmxaF_F	tcatgcgcttcatggttaaactgccgaattGTTGTTACTTCTCCATACAATTAAACCGGG
	FC459_PmxaF_R	gcgccgagcaataatgtttttttcatAATTCCTCCAGATTGATTTTTCGCATAA
mxaI gene	FC460_mxaIORF_F	ttatgcgaaaaatcaatctggaggaattATGAAAAAAACATTATTGCTCGGCGC
	FC461_mxaIORF_R	atcttagccatgtttcctcaatggTTAATTGATTTCTTCTACCTCATAAACAAACTTGC
pFC39		
P_{mxaB} and	FC468_mxaBc_F	ttcatggttaaactgccgaattAATTCCTCCAGATTGATTTTTCGCATAATG
mxaB gene	FC469_mxaBc_R	tagccatgtttcctcaatggTTAGTGCGCCAGATTGTCTATGATG
pFC40		
P_{tac}	MH15_dTom_F_new	gtagcaagccattccaacgagtatatttcCTCTGAAATGAGCTGTTGACAATTAATC
	FC343_pMH15R	AGCTGTTTCCTGTGTGAATACCTCC
xoxF gene	FC508_Ptacxox_F	ggaggtattcacacaggaaacagctATGAAGAAGCCTGTCAAAAGCTGG
	FC509_xoxORF3_R	gtactcttgtcgactttatgtctattgTTAATTAGGCAATGCGAATACAGTTAATGT
qRT-PCR		
xoxF	FC401_276xoxF_F	ATTCACACTCCATTCCCTAACACC
	FC402_550xoxF_R	CTAATGGAGCTTGAGTGTTGGTCATG

mxaF	FC403_169mxaF_F	AGTTGTACGACATCAACATCACG
	FC404_367mxaF_R	GCTTCGGTTTGAATTGCCACA
mxaJ	FC405_73mxaJ_F	AAGAGCCTCTGAAAGTGTGCAATG
	FC406_313mxaJ_R	GATAATACGGTTCAGTCGTCAGC
16S	FC409_34816S3_F	ATATTGGACAATGGGCGCAAG
	FC410_62316S3_R	CAAATGCCGTTCCCAGGTTAAG
mxaB	FC413_mxaF_LF_F	AGTTGTTGCGCTAATTCGGGTTC
	FC464_mxaB_R	CAACCGAATATCGTCGTGATGGAC

*Primer regions used for Gibson or PCR stitching junctions are in lower case, FRT sites are italicized

Table S3. Doubling times of mutant and complementation strains†

Strain	Without La^{3+}	With La^{3+}
WT 5GB1C	2.97 ± 0.2	2.83 ± 0.10
Δ*mxaF*	3.87 ± 0.45	2.88 ± 0.24
Δ*mxaF*; P_{mxaF}-*mxaF*	2.79 ± 0.05	3.13 ± 0.11
Δ*mxaI*	3.87 ± 0.16	2.86 ± 0.12
Δ*mxaI*; P_{mxaF}-*mxaI*	2.70 ± 0.03	2.90 ± 0.07
Δ*mxaB*	3.07 ± 0.19	2.71 ± 0.08
Δ*mxaB*; P_{mxaB}-*mxaB*	2.74 ± 0.03	2.87 ± 0.14
Δ*xoxF*	2.93 ± 0.07	10.3 ± 6.6
Δ*xoxF*; P_{tac}-*xoxF*	3.11 ± 0.24	2.86 ± 0.11

†Doubling times are in hours and represent the means of at least three technical replicates with standard deviations. Doubling times were calculated from three time points during the exponential phase of growth.

REFERENCES

1. **Puri AW, Owen S, Chu F, Chavkin T, Beck DA, Kalyuzhnaya MG, Lidstrom ME.** 2015. Genetic tools for the industrially promising methanotroph Methylomicrobium buryatense. Appl Environ Microbiol **81:**1775-1781.
2. **Yan X, Chu F, Puri AW, Fu Y, Lidstrom ME.** Submitted. Electroporation-Based Genetic Manipulation In Type I Methanotrophs. Appl Environ Microbiol.
3. **Gibson DG, Young L, Chuang RY, Venter JC, Hutchison CA, 3rd, Smith HO.** 2009. Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Methods **6:**343-345.