

TABLE S1: Primers used in this study	
Primer	Sequence (5' → 3')
pJDG2z	
JG39_pAWP87_backbone_fwd	TAGTTGTCGGGAAGATGCGTGATCTG
JG40_pAWP87_backbone_rev	TAATTCCTCCAGATTGATTTTTTCGC
JG49_zeo_F	cattatgcgaaaaatcaatctggaggaattATGGCCAAGTTGACCAGTGCC
JG50_zeo_R	ggatcagatcacgcatcttcccgacaactaTCAGTCCTGCTCCTCGGCCA
Knockout vector backbone primers	
AP88	TACGTACGCGTGTTAACCGG
JG88_backbone_rev	CTGTCAGACCAAGTTTACTCATATATACTTTAGATTG
pJDG14 <i>lanA</i> knockout plasmid	
JG119_screening_F	caacacatgccaactgctctt
JG120_up_130812del_F	CAATCTAAAGTATATATGAGTAACTTGGTCTGACAGcatttcgctccatccgcca
JG121_up_130812del_R	atgagtggtagcgcacgggtatttttaaagtgcgacagc
JG122_dn_130812del_F	ttaaaaaatcaccgtgctaccactcataagccac
JG123_dn_130812del_R	CTCACCGGTTAACACGCGTACGTAggcatcattggcatgcacatg
JG124_screening_R	tcaaaaagcgcgcttggtg
pJDG16 <i>fecR1</i> knockout plasmid	
JG169_up	CAATCTAAAGTATATATGAGTAACTTGGTCTGACAGgctacgaagtgcgttgggt
JG170_up_R	catccgatcaactatgtgatgctgtagagtagc
JG171_dn_F	agtacagcatcacatagttgatcggtggcgatgaataatg
JG172_dn_R	ACCGGTTAACACGCGTACGTAgcgaatggctgttaattgctg
pJDG17 <i>fecR2</i> knockout plasmid	
JG173_130847_up_F	CAATCTAAAGTATATATGAGTAACTTGGTCTGACAGagttaaactgataggaaattcc
JG174_130847_up_R	gggctaccatgccagagagtggtttaaatactatgacgtatcac
JG175_130847_dn_F	atttaaaccactctctggcgatggtagcccgatg
JG176_130847_dn_R	TCACCGGTTAACACGCGTACGTAttcggctgcccacaaggttg
pJDG18 <i>fecR3</i> knockout plasmid	
JG177_50004_up_F	CAATCTAAAGTATATATGAGTAACTTGGTCTGACAGcaaggtataacaactgggatcgg
JG178_50004_up_R	ctagccgactataacgctgctgttattattgggtcccac
JG179_50004_dn_F	aataataacgacagcgttatagtcggctagttgcatg
JG180_50004_dn_R	TCACCGGTTAACACGCGTACGTAgccagattgaaatgtagctgt
pJDG27 PJ23119-<i>lanA</i> complementation plasmid	
JG226_lanA_23119_F	CTAAAGAGGAGAAAGGATCTatgtctttaactctataataaaaaatcgaatctaac
JG227_lanA_R	GCTTACTATCTTAGCCATGTTTCCTCAATGGttagaaagtccacttcacaccg
JG228_J23119_lanA_R	cgattttattatagaagttaaagacatAGATCCTTTCTCCTCTTTAGATCtATTATAC
JG182_F	CCATTGAGGAAACATGGCTAAGATAG
pJDG34 <i>tonB</i> knockout plasmid	
JG269_TonB_LF_F	CAATCTAAAGTATATATGAGTAACTTGGTCTGACAGggtttcgagcagcaaatcgggt
JG270_TonB_LF_R	cgaatcgaatcatgaagggtccggctc
JG271_TonB_RF_F	cgggaccctcatgatttcgattcgcctgaggaaca
JG272_TonB_RF_R	CTCACCGGTTAACACGCGTACGTAggtataacttccaagccatttac
pJDG49 <i>PlanA-lanA</i> complementation plasmid	
JG35_backbone_fwd	TAGTTGTCGGGAAGATGCGTG
JG318_compl_bkbn_R	CAGCTCACTCAAAGGCGGTAAT
JG320_lanA_R	CAGATCACGCATCTTCCCGACAACtAttagaaggtccacttcacaccg
JG321_PlanA_F	CGTATTACCGCCTTTGAGTGAGCTGggttaatggaagcagtgagca
pJDG51 <i>fecI2</i> knockout plasmid	
JG344_130846_LF_F	CAATCTAAAGTATATATGAGTAACTTGGTCTGACAGggttcgcatagctcgagttgc
JG345_130846_LF_R	ggcaatttgaaccatgatgaaacgggtccaactcgt
JG346_130846_RF_F	ggaccgttcatcatggttcaaaattgaccttttctcgct
JG347_130846_RF_R	TCACCGGTTAACACGCGTACGTAggcatgttccaataccggttac

pJDG52 P_{xoxF}-xyIE knock-in	
FC431	TCATGCGCTTCATGGTTAAACTGCCGAATTaaccgcaaatgcttggtaac
FC463	CGGTGCGATTACACCTTTGTTTCATatatttctccttggtatcctgttacac
FC292_F	ATGAACAAAGGTGTAATGCGACCG
JG183_R	AATTCGGCAGTTTAACCATGAAG
pSMF8 PJ23119-xyIE plasmid	
SF1_pDL_F	AACGAACGATTCATGACCGTGCTCACCTGACtgcgagtaaggatctccagg
SF4_pDL014_R	CCCGGTGCGATTACACCTTTGTTTCATagatcctttctcctcttagatctattatacc
FC292_F	ATGAACAAAGGTGTAATGCGACCG
JG70	TCAGGTGAGCACGGTCATGAATC
cJDG1 P_{mxoF}-ble knock-in	
FC351_F	ACAGTCCGAACAACGATTTTCG
AP193	AATTCGGCAGTTTAACCATG
AP235	TCATGCGCTTCATGGTTAAACTGCCGAATTAATTAACCCGGGAATGATGTC
JG61_zeo_R	GCCATGCTTACTATCTTAGCCATGTTTCCTCAATGGtgcagtcctgctcctcgcca
AP192	CCATTGAGGAAACATGGCTA
FC352_R	AGTGATTCTGGTCGCGCACA
cJDG3 linear <i>fecI1</i> knockout	
CM33	AAAGTACGCCATTATCGGCAG
CM34	aactggtccacctacaacaaagctctcatcAGGTTCAACCGCATTTCATACG
CM35	gatgagagctttgttaggtggaccagtt
CM36	tctcgagtccgctcaagtcagcgtaatgct
CM37	agcattacgctgacttgacgggactcgagaTATTTCCGGTGAGTTGGGTCCG
CM38	GCTTGCGGACAATAAACTCG
cJDG13 linear <i>fecI3</i> knockout	
JG293_km_50001_LF_F	CGGAAATAGAACgatgagagctttgttaggtgg
JG294_km_50001_RF_R	GATTTACTTTTCATTTTCACCTctcgcgagtcctcctcaagtc
JG295_50001_LF_F	GCCTATCGACACGAAATAATGAA
JG296_50001_LF_km_R	acaacaaagctctcatcGTTCTATTTCCGGTTTTTGACG
JG297_50001_RF_km_F	gggactcgagaGGTGAAAATGAAAGTAAATCGTCTTATTC
JG298_50001_RF_R	AGTGTGTTTGTAAACTTTACGC
qRT-PCR primers	
FC401_276xoxF_F	ATTCACACTCCATTCCCTAACACC
FC402_550xoxF_R	CTAATGGAGCTTGAGTGTTGGTCATG
FC403_169mxoF_F	AGTTGTACGACATCAACATCACG
FC404_367mxoF_R	GCTTCGGTTTTGAATTGCCACA
LH_RPOD_F	TGCAGGGCTTAGAGAATCAAC
LH_RPOD_R	AAACTGCTTACCGACCTCTTC

TABLE S2 Point mutations in nitrosoguanidine-mutagenized lanthanide repression mutants. Eight JG3 mutants resistant to zeocin in the presence of lanthanum were subjected to whole genome resequencing, and seven more mutants were sequenced at the *lanA* locus. The basepair mutations, the amino acid residues changed, and any additional mutations discovered are shown.

Strain	Mutation	Amino acid change	Gene	Additional unique mutations
JG3 μ 2/JG3 μ 3	(C)5 \rightarrow 6	coding (1356/2199 nt)	MBURv2_130812	21
JG3 μ 5	G \rightarrow A	W643* (TGG \rightarrow TGA)	MBURv2_130812	0
JG3 μ 7/JG3 μ 8	G \rightarrow A	W643* (TGG \rightarrow TGA)	MBURv2_130812	38
JG3 μ 11	C \rightarrow T	Q657* (CAA \rightarrow TAA)	MBURv2_130812	56
JG3 μ 15	C \rightarrow T	Q720* (CAG \rightarrow TAG)	MBURv2_130812	2
JG3 μ 16	G \rightarrow A	W643* (TGG \rightarrow TGA)	MBURv2_130812	56
JG3 μ 1	C \rightarrow T	Q657* (CAA \rightarrow TAA)	MBURv2_130812	Not sequenced
JG3 μ 4	C \rightarrow T	Q336* (CAA \rightarrow TAA)	MBURv2_130812	Not sequenced
JG3 μ 6	G \rightarrow A	W396* (TGG \rightarrow TGA)	MBURv2_130812	Not sequenced
JG3 μ 9	G \rightarrow A	W643* (TGG \rightarrow TGA)	MBURv2_130812	Not sequenced
JG3 μ 10	C \rightarrow T	Q383* (CAG \rightarrow TAG)	MBURv2_130812	Not sequenced
JG3 μ 12	G \rightarrow A	W396* (TGG \rightarrow TGA)	MBURv2_130812	Not sequenced
JG3 μ 13	G \rightarrow A	W643* (TGG \rightarrow TGA)	MBURv2_130812	Not sequenced

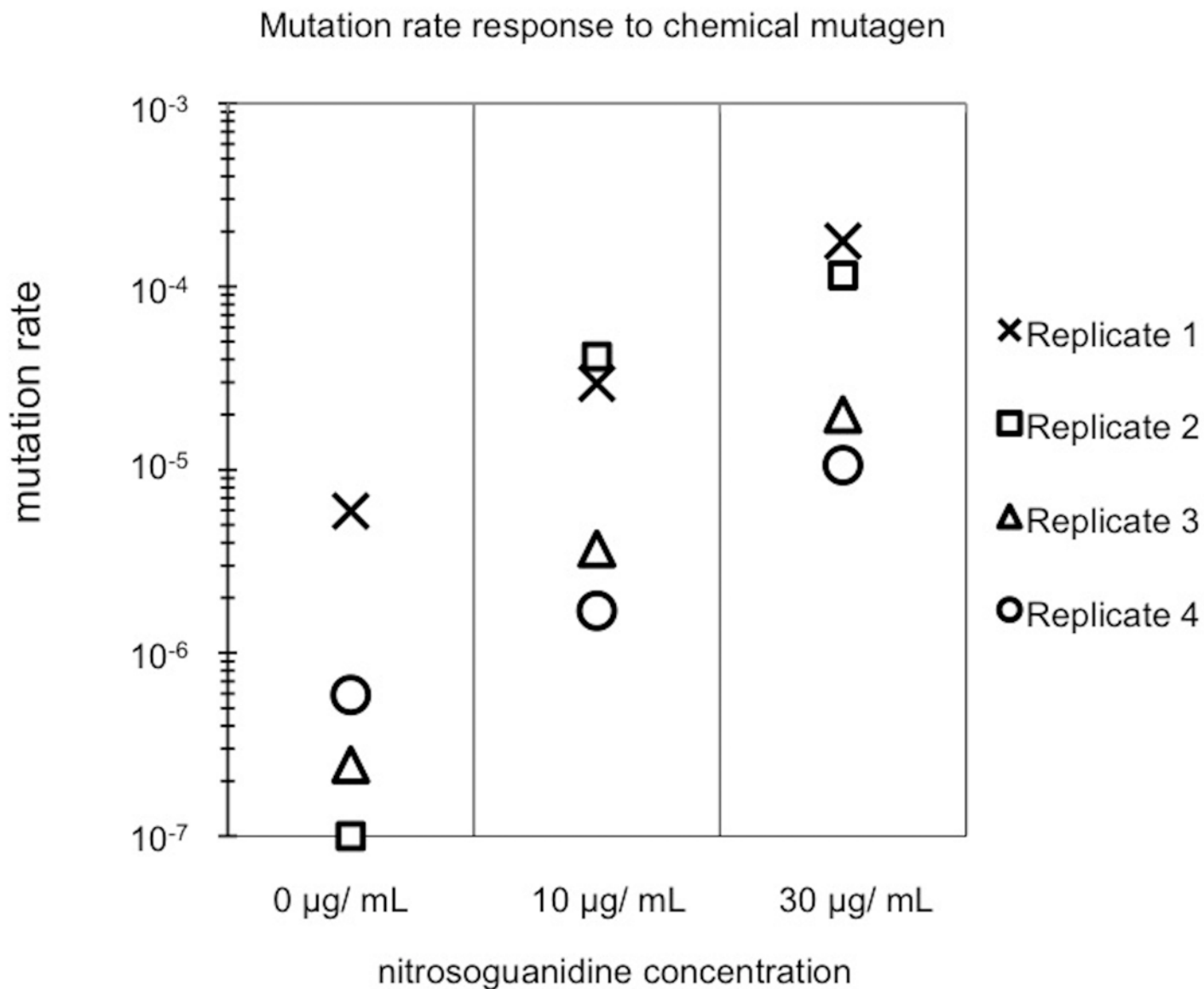


Fig S1 Nitrosoguanidine increases the mutation rate of *Methylomicrobium buryatense* 5GB1C. Data represent results from the mutagenized P_{mxoF} -*zeo* reporter strain of *M. buryatense* 5GB1C grown in the presence of 30 µg/mL lanthanum chloride. The mutation rates are calculated by the ratio of the number of zeocin-resistant colonies to the total number of colonies estimated by dilution plating. The mutation rate is relevant for genes involved in the lanthanide switch, with the results from each of four independent biological experiments shown (n=4).

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"M. buryatense" 5GB1C LanA ----- 0
E. coli FecA MTPLRVFRKTTPLVNTIRLSLLPLAGLSFSAFAAQVNIAPGSLDKALNQYAAHSGFTLSV 60

"M. buryatense" 5GB1C LanA -----MSLTSIIKIESNQATGSCOG-QIAPFRMCLMTVAVLAAVNANAQVDDR 49
E. coli FecA DASLTRGKQSNGLHGDYDVESGLQQLDCSGLQVKPL-----GNSWTLEP 106

"M. buryatense" 5GB1C LanA PPAVKLPPLLEITGE-----EPSQLEHIPCSEGFVIDKTTLDROGPLSTKDALRTIPGTHI 103
E. coli FecA APAPKE DALIVGDWLGDARENDFVEH-ACARDVIRREDFAKTGATTMREVLNRIQVSA 165
TonB box N-terminal plug domain

"M. buryatense" 5GB1C LanA VDEDVLCRR---FNLGIRGLDPRRSVRTQLLEDGAPIQALAPYSDPSNHYIPTN-KRIDRI 159
E. coli FecA PENNGTCSHDLAMNFGIRGLNPRLASRSTVLDGIPVFFAPYQPOLSLAPVSLGNMDEI 225

"M. buryatense" 5GB1C LanA EVLKSGSOTMYGPOTVGGAVNFVSAPIPEEFG-----GSISAAGGNGGYDTH-LRIIGG 212
E. coli FecA DVVRGGGAVRYGPOSVGGV/NFVTRAIPODFGIEAGVEGQLSPTSSQNNPKETHNLMVGG 285

"M. buryatense" 5GB1C LanA IFLDN-VGLSLDYIRQESDGNRSGHOE-----VDDLALKALIKIDDRQRLMLKGLLTHEE 266
E. coli FecA IADNGFGTALLY-----SGTRGSDWRBHSATRIDDLMLKSKYAPDEVH--TFNSLLOYD 338
β-barrel transmembrane pore domain

"M. buryatense" 5GB1C LanA ADMGEAGLTSEMYR-----RNORTNPLRNDSEFVRRYAGQALYEFDISDTMQEFTNITG 320
E. coli FecA ---GEADMPGGLSRADYDADRWQSTRPY--DRFWGRRKLASLGYQFQPDSDQHKF---NIG 391

"M. buryatense" 5GB1C LanA NHMERESTIQANDSSQMNANCANREPISADVAPTCGNEQRPRTYNVFGIEPKL--VFMHD 378
E. coli FecA --FYTQTLR-----SGYLEQ--GRITUS-----PRNYWVRGIEPRYSQIFM-- 429

"M. buryatense" 5GB1C LanA AFGLQS-ETTLGIRGHFEWADRERYVGNAGPRDITQGRENNNHGRNRY-QDNSLETOALS 436
E. coli FecA -IGPSAHEVGVGYRYLNESTHEMRY-----TATSSGQLPS--GSSPYDRDTRSGTEAHA 481

"M. buryatense" 5GB1C LanA FFAQNRFFLGDFVTVPGRVVEHY--YQDNINNDGATESLVRTEALPGVGVTYNCIDNTT 494
E. coli FecA WYLDKIDIGNWTITPGMRFEHIESYQNA--ITGTHEEVSYNAPLPALNVLYHTDSWN 539

"M. buryatense" 5GB1C LanA LFAGVHRGFAP---GRIGDFVDPTKNILSQVEPELSLNYEAGVRTSPTPGVNLEMTYFRI 551
E. coli FecA LYANTEGSFGTVQYSQICKAVCS-----GNVEPEKARTWELGTRYD-DGALTAEMLGFLI 593

"M. buryatense" 5GB1C LanA DFENQIVEDITVEDTRFVNVEEVEGVEVETGFRLDNSQLFGTDYNYLTTSTYTLDAYFA 611
E. coli FecA NFNNQYDSNQT-NDTVTAR-GKREHIGLETQARYDLGTLTPTLDNVSIIYASYAVVNA--- 648

"M. buryatense" 5GB1C LanA SNEAR-AGIVRDNRLPYAPEBLINANVGVETP---WGLDIRFGIQSVSQQYVDIENTREE 667
E. coli FecA --EIREKGDYGNLVPFSPKH--KGLGVQYKPGNWTFLNSDFQ--SSQFADNANTVKE 702

"M. buryatense" 5GB1C LanA NANGQEGIIIPGYIVFNVSANYQV---VKNNVFMNGYNLSDKKFIASRVD----GIHPGQ 720
E. coli FecA SADGSTCRIPGFMLWGARVAYDFGPOMADLNLAFGVKNIIFDQDYFIRSYDDNNKGIYAGQ 762

"M. buryatense" 5GB1C LanA --GFQMMGGVKKWTF 732
E. coli FecA PRTLYMOGSLKF-- 774

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Fig S2 Protein alignment of "M. buryatense" 5GB1C LanA and E. coli ferric citrate transporter FecA. Pairwise protein alignment of LanA and FecA, generated by EMBOSS Needle alignment software. Identical residues are shaded in black, while biochemically similar residues are shaded in grey. The Pfam-annotated TonB box, N-terminal plug, and beta-barrel protein domains for the FecA protein are indicated in red, blue, and green, respectively.

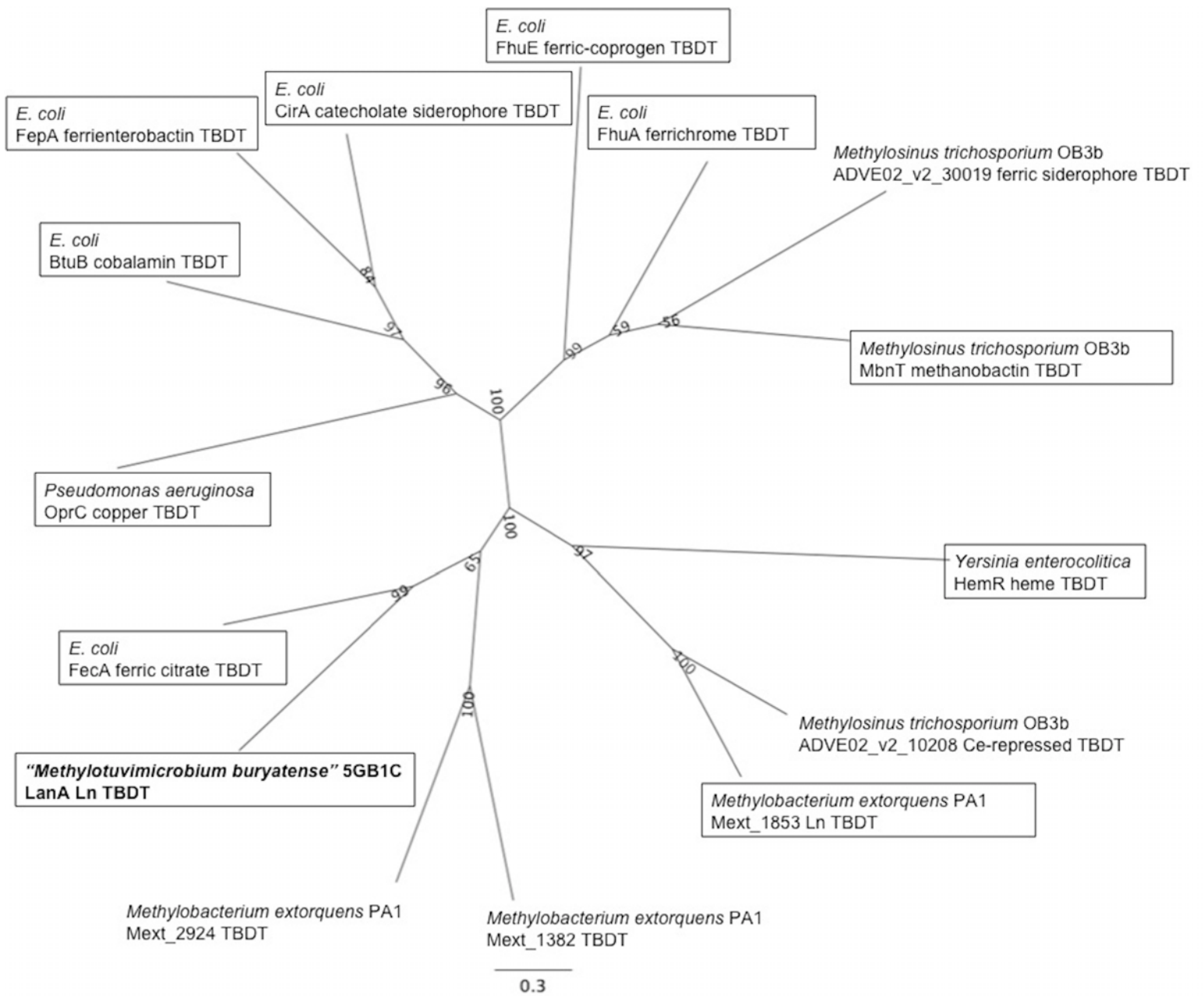


Fig S3 LanA is most closely related to the ferric citrate transporter FecA compared to known transporters and other methylophilic homologs. Maximum likelihood tree generated from a multiple sequence alignment of selected TonB-dependent transporters (TBDTs). Posterior probabilities are indicated at nodes. Protein names and known annotations were collected from NCBI and KEGG. Proteins that have been studied in peer-reviewed journals are boxed. The scale bar indicates the distance for 0.3 amino acid substitutions per site.

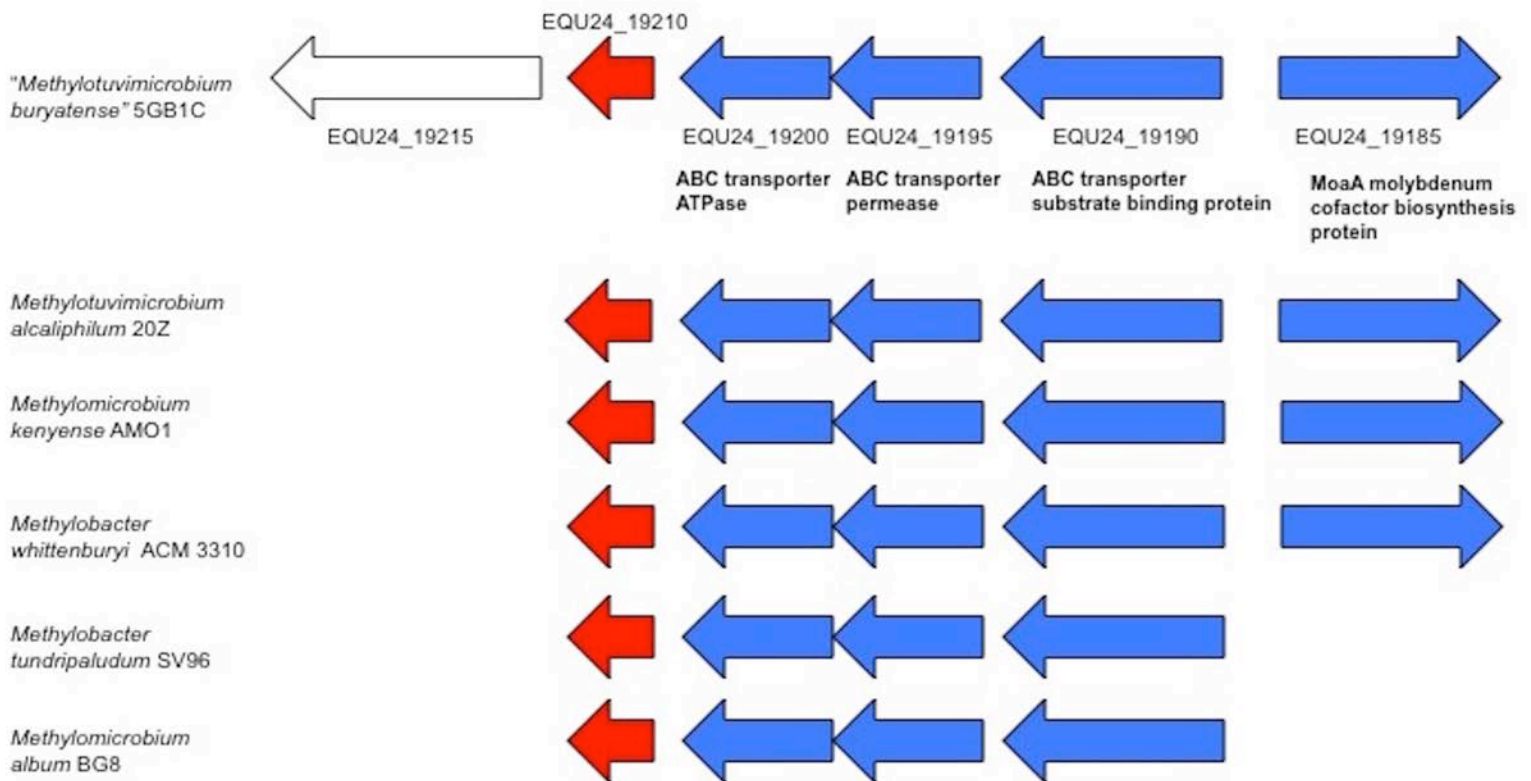


Fig S4 Genome synteny of EQU24_19210 in other Type I methanotrophs. The EQU24_19210 gene (shown in red) is regulated similarly to *xoxF*. It resides next to an ABC transporter gene cluster in many closely related methanotrophs, and in some methanotrophs resides near the *moaA* gene encoding a molybdenum cofactor biosynthesis protein (shown in blue). Open reading frames not conserved in other organisms are shown in white.

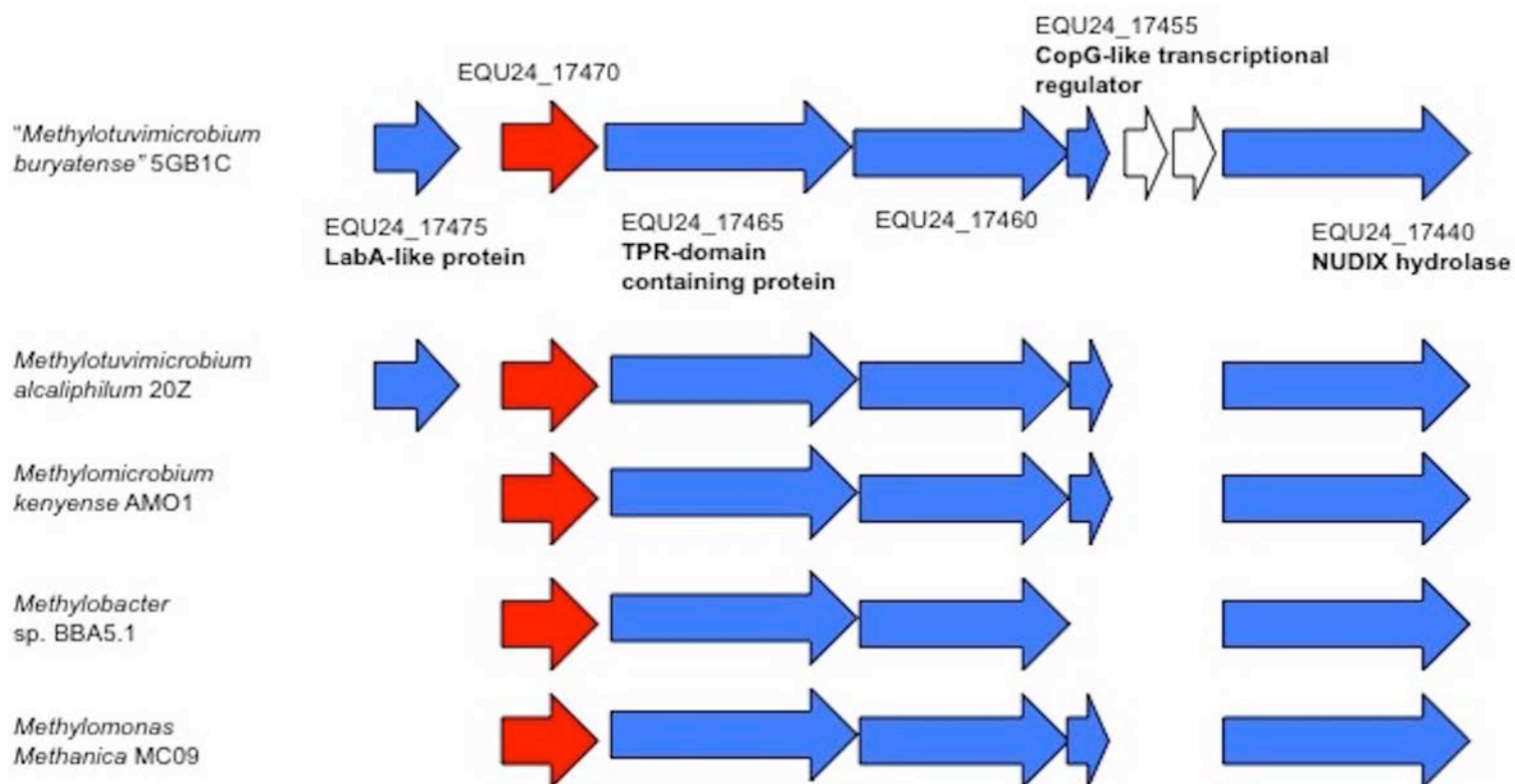


Fig S5 Genome synteny of EQU24_17470 in other Type I methanotrophs. The EQU24_17470 gene (shown in red) is induced by lanthanum. It resides next to several genes that encode conserved domains, including a NUDIX hydrolase and a TPR-domain containing protein, and in some organisms a CopG-like transcriptional regulator (shown in blue). Open reading frames not conserved in other organisms are shown in white.

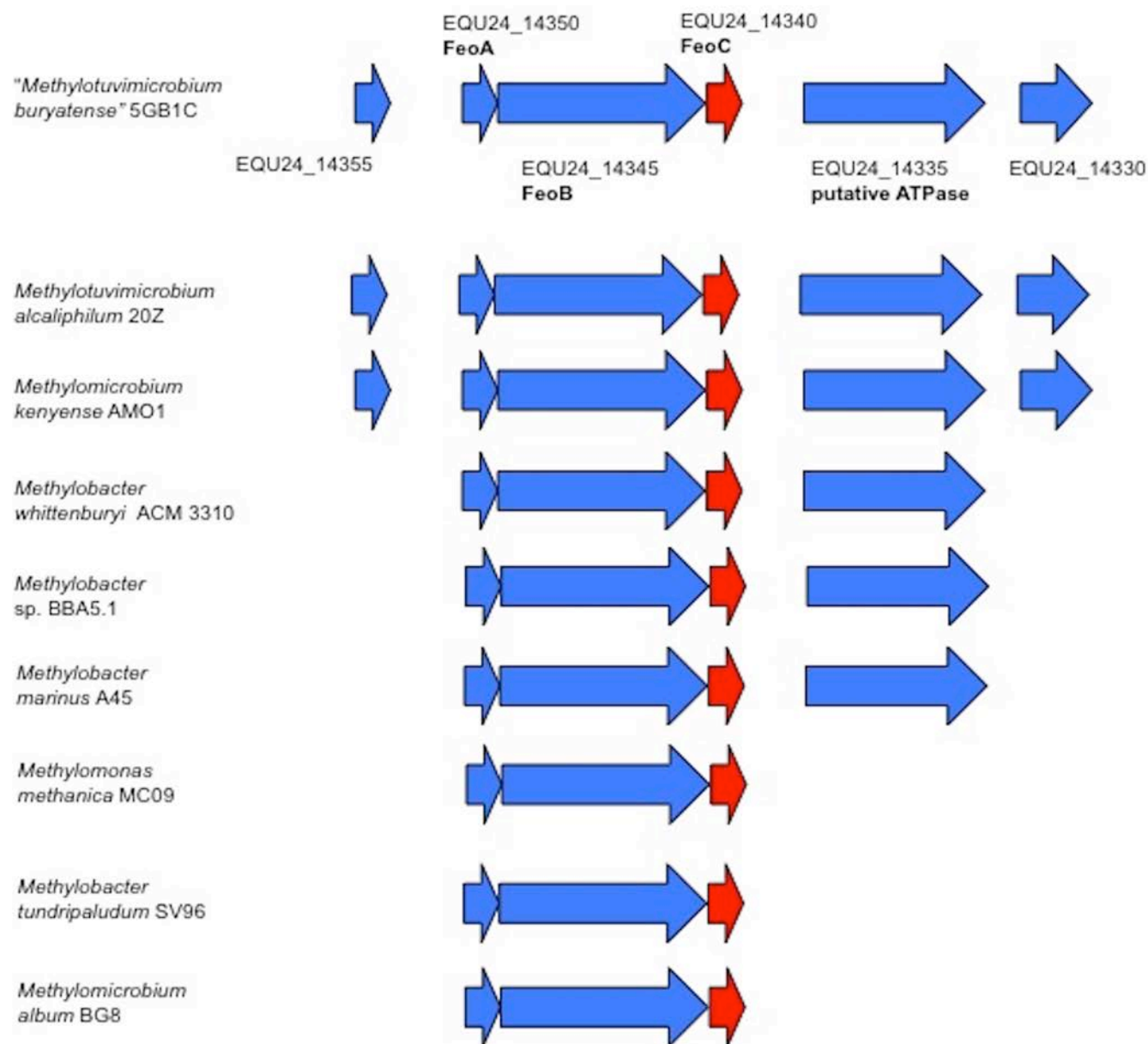


Fig S6 Genome synteny of EQU24_14340 in other Type I methanotrophs. The EQU24_14340 gene (shown in red) is induced by lanthanum. It resides next to FeoAB ferrous iron transporter gene homologs in 9 different Type I methanotrophs, and a putative ATPase in some organisms (shown in blue).

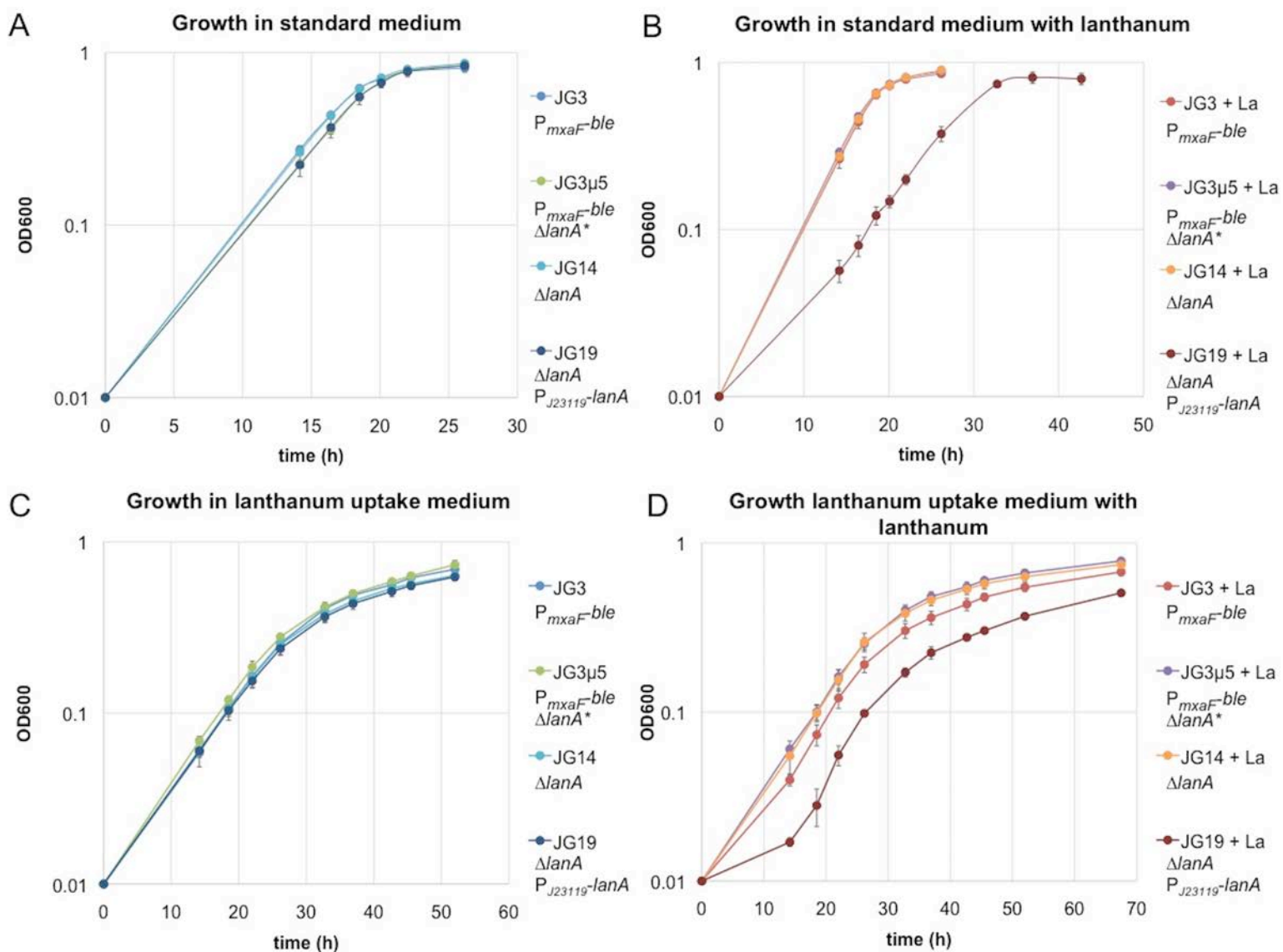


Fig S7 Deletion of *lanA* does not result in a growth phenotype, but overexpression of *lanA* leads to slower growth. “*M. buryatense*” 5GB1C derivatives were grown in either standard medium (A and B) or lanthanum uptake medium (C and D), without (A and C) or with (B and D) lanthanum. 30 μ M La was used for standard medium and 2 μ M La was used for lanthanum uptake medium, to be consistent with other experiments. n=3.

Lanthanum uptake in wild-type and mutants

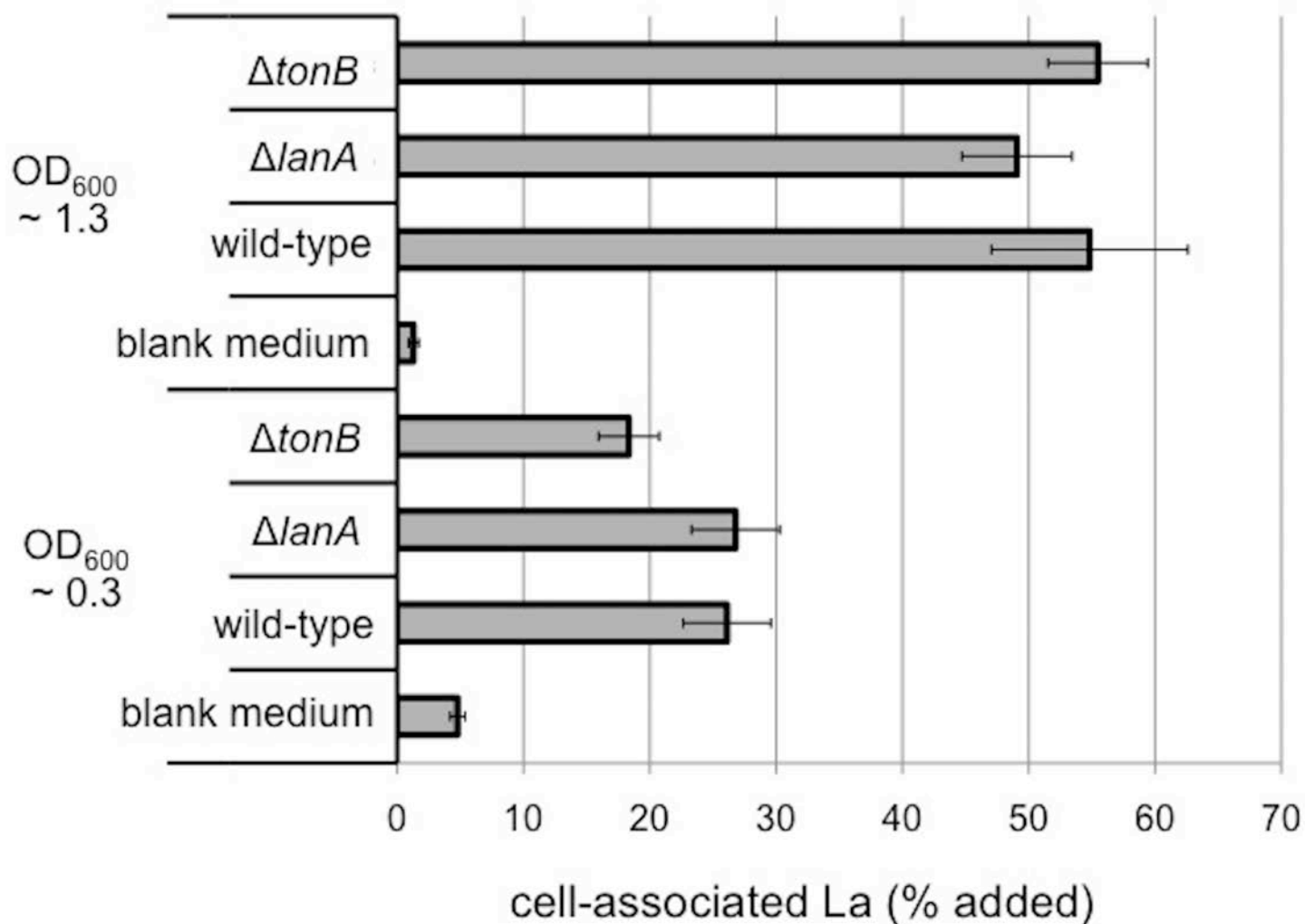


Fig S8 Lanthanum uptake is not affected in either *ΔlanA* or *ΔtonB*. Cells were inoculated into medium containing 2 μ M La. Cells were harvested at the indicated optical densities, corresponding to early exponential phase (OD₆₀₀ ~ 0.3) and early stationary phase (OD₆₀₀ ~ 1.3). Lysed cell pellets and supernatants were analyzed by ICP-MS to determine La concentration, and the % La associated with the pellets is reported. Results are the average \pm standard deviation of two biological replicates, including the results from medium not inoculated with cells (n=2).

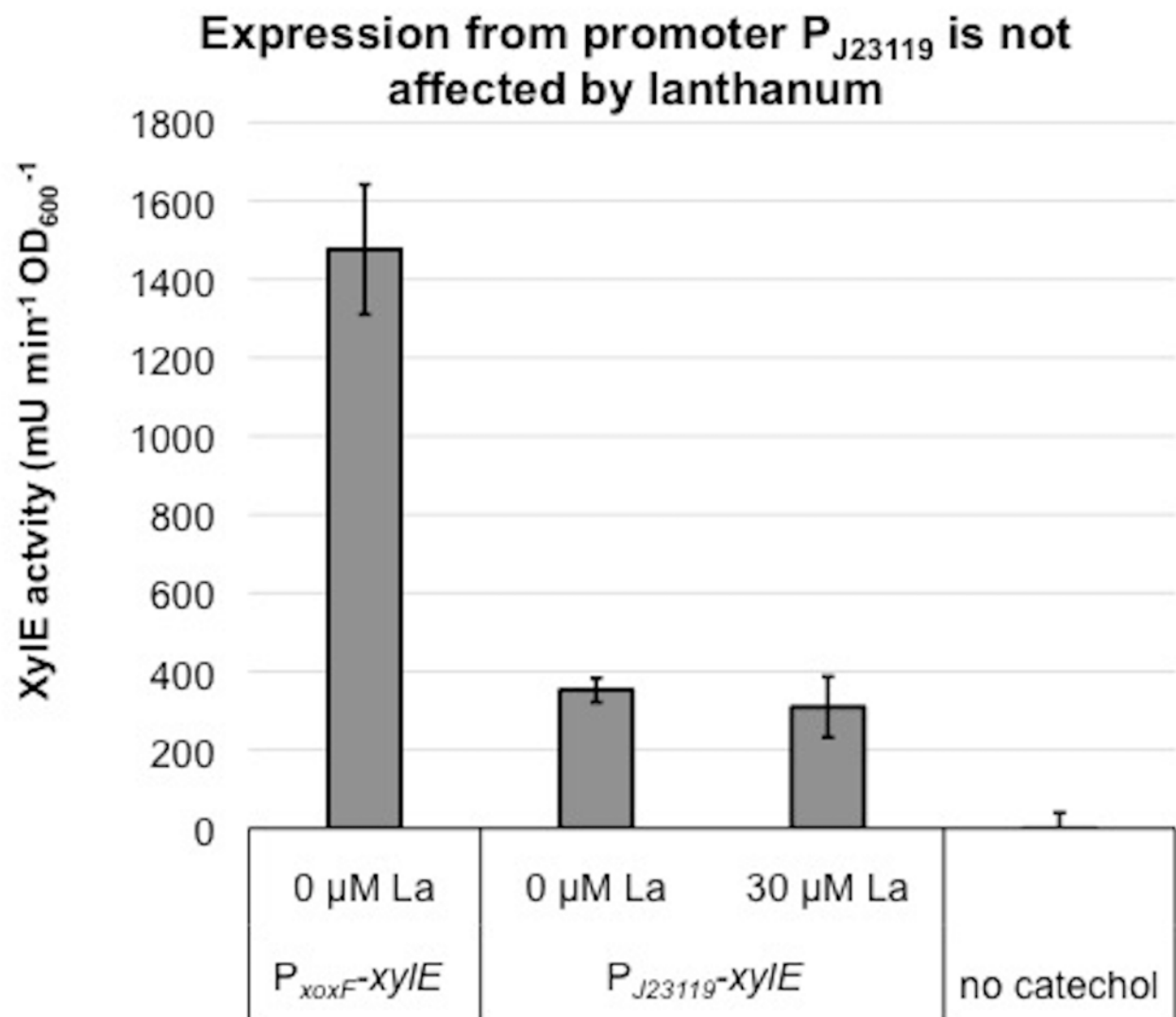


Fig S9 The P_{J23119} promoter used to express *lanA* is not affected by lanthanum. Whole cell catechol-2,3-dioxygenase reporter gene assays were performed for wild-type and mutant strains. Both P_{xoxF} -xylE and the P_{J23119} -xylE were created from the wild-type background strain. Cells were either grown without La or in the presence of 30 μM La in standard medium. Results are the average ± standard deviation of two biological replicates (n=2).

Pmx_aF-xylE reporter activity with increasing La

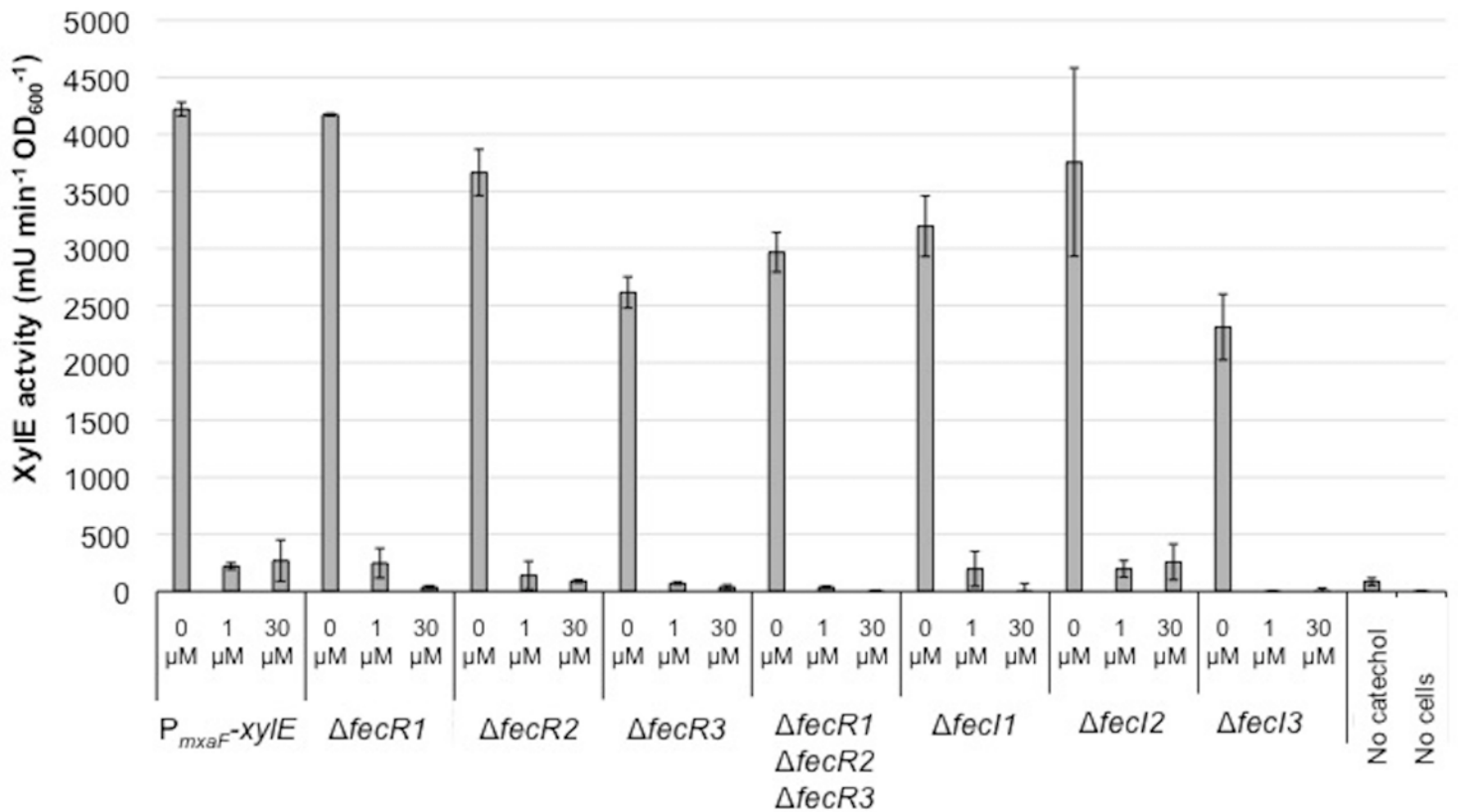


Fig S10 *fecR* and *fecI* deletions have no clear effect on *P_{mx_aF}-xylE* reporter gene expression.

Whole cell catechol-2,3-dioxygenase reporter gene assays were performed for wild-type and mutant strains. The wild-type was FC31 (*P_{mx_aF}-xylE*), and all mutants were created from this background strain. Cells were either grown without La or in the presence of 1 μM La or 30 μM La. Results are the average ± standard deviation of two biological replicates (n=2).

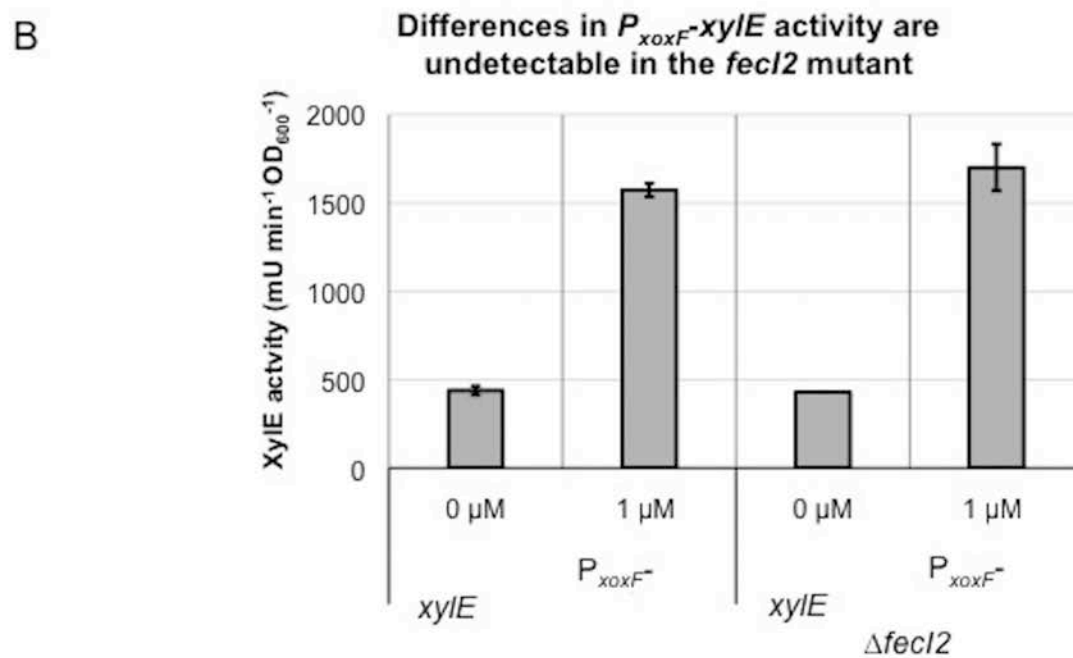
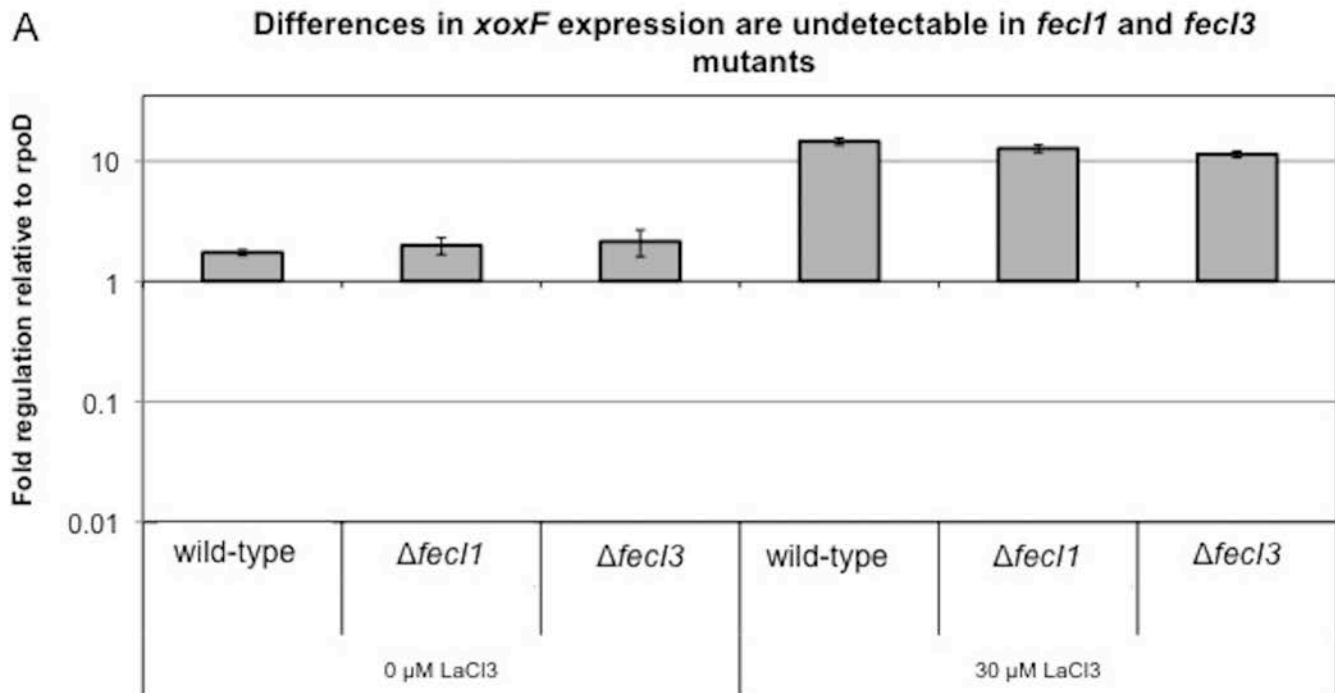


Fig S11 *fecI* deletions have no clear effect on *xoxF* gene regulation. **A)** Reverse transcriptase polymerase chain reaction (RT-PCR) was performed on RNA harvested from *M. buryatense* wild-type and mutants in the presence or absence of 30 μM lanthanum. Primers specific for *xoxF* methanol dehydrogenase genes were used to quantify the lanthanide switch. All cycle threshold values were normalized to the constitutive *rpoD* gene. Results show the average \pm standard deviation of two biological replicates ($n=2$). **B)** Whole cell catechol-2,3-dioxygenase reporter gene assays were performed for wild-type and mutant strains. The wild-type was JG41 (P_{xoxF} -*xyIE*), and the $\Delta fecI2$ mutant was created from this background strain. Cells were either grown without La or in the presence of 1 μM La. Results are the average \pm standard deviation of two biological replicates ($n=2$).

