| **TABLE S1: Primers used in this study** | |
|---|---|
| **Primer** | **Sequence (5' →3')** |
| **pJDG2z** | |
| JG39_pAWP87_backbone_fwd | TAGTTGTCGGGAAGATGCGTGATCTG |
| JG40_pAWP87_backbone_rev | TAATTCCTCCAGATTGATTTTTCGC |
| JG49_zeo_F | cattatgcgaaaaatcaatctggaggaattATGGCCAAGTTGACCAGTGCC |
| JG50_zeo_R | ggatcagatcacgcatcttcccgacaactaTCAGTCCTGCTCCTCGGCCA |
| **Knockout vector backbone primers** | |
| AP88 | TACGTACGCGTGTTAACCGG |
| JG88_backbone_rev | CTGTCAGACCAAGTTTACTCATATATACTTTAGATTG |
| **pJDG14 *lanA* knockout plasmid** | |
| JG119_screening_F | caacacatgccaactgctctt |
| JG120_up_130812del_F | CAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGcatttcgcgtccatccgcca |
| JG121_up_130812del_R | atgagtggtagcgcacggtgatttttttaaagttgcgacagc |
| JG122_dn_130812del_F | tttaaaaaatcaccgtgcgctaccactcataagccac |
| JG123_dn_130812del_R | CTCACCGGTTAACACGCGTACGTAggcatcattggcatgcacatg |
| JG124_screening_R | tcaaaaagcgccgttggttg |
| **pJDG16 *fecR1* knockout plasmid** | |
| JG169_up | CAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGgctacgaagtcgcttgggt |
| JG170_up_R | catccgatcaactatgtgatgctgtactagagtagc |
| JG171_dn_F | agtacagcatcacatagttgatcggatgggcggatgaataatg |
| JG172_dn_R | ACCGGTTAACACGCGTACGTAgcgaatggcttgttaattgctg |
| **pJDG17 *fecR2* knockout plasmid** | |
| JG173_130847_up_F | CAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGagtaaatcgataggaaattcc |
| JG174_130847_up_R | gggctaccatcgccagagagtggtttaaatactatgacgtatcac |
| JG175_130847_dn_F | atttaaaccactctctggcgatggtagcccggatg |
| JG176_130847_dn_R | TCACCGGTTAACACGCGTACGTAttcggtctgccacaaggtttg |
| **pJDG18 *fecR3* knockout plasmid** | |
| JG177_50004_up_F | CAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGcaaggtataacaactgggatcgg |
| JG178_50004_up_R | ctagccgactataacgctgtcgttattattgggtcccac |
| JG179_50004_dn_F | aataataacgacagcgttatagtcggctagtttgcatg |
| JG180_50004_dn_R | TCACCGGTTAACACGCGTACGTAgccagattgaaatgtagcttgt |
| **pJDG27 PJ23119-*lanA* complementation plasmid** | |
| JG226_lanA_23119_F | CTAAAGAGGAGAAAGGATCTatgtctttaacttctataataaaaatcgaatctaatc |
| JG227_lanA_R | GCTTACTATCTTAGCCATGTTTCCTCAATGGttagaaagtccacttcacaccg |
| JG228_J23119_lanA_R | cgatttttattatagaagttaaagacatAGATCCTTTCTCCTCTTTAGATCtATTATAC |
| JG182_F | CCATTGAGGAAACATGGCTAAGATAG |
| **pJDG34 *tonB* knockout plasmid** | |
| JG269_TonB_LF_F | CAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGggtttcgcagtcaaatcggt |
| JG270_TonB_LF_R | cgaatcgaaatcatgaagggtccggctc |
| JG271_TonB_RF_F | ccggacccttcatgatttcgattcgctgaggaaca |
| JG272_TonB_RF_R | CTCACCGGTTAACACGCGTACGTAagttatacttcccaagccatttacct |
| **pJDG49 P*lanA*-*lanA* complementation plasmid** | |
| JG35_backbone_fwd | TAGTTGTCGGGAAGATGCGTG |
| JG318_compl_bkbn_R | CAGCTCACTCAAAGGCGGTAAT |
| JG320_lanA_R | CAGATCACGCATCTTCCCGACAACTAttagaaagtccacttcacaccg |
| JG321_PlanA_F | CGTATTACCGCCTTTGAGTGAGCTGggttaatggtaagcagtgagca |
| **pJDG51 *fecI2* knockout plasmid** | |
| JG344_130846_LF_F | CAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGgttcgcatagctcgagttgc |
| JG345_130846_LF_R | ggtcaattttgaaccatgatgaaacggtccaactcgt |
| JG346_130846_RF_F | ggaccgtttcatcatggttcaaaattgacctttttctcgct |
| JG347_130846_RF_R | TCACCGGTTAACACGCGTACGTAggcatgttccaatacggttac |

| | |
|---|---|
| **pJDG52 P*xoxF-xylE* knock-in** | |
| FC431 | TCATGCGCTTCATGGTTAAACTGCCGAATTaaccgcaaatgcctggtcaatc |
| FC463 | CGGTCGCATTACACCTTTGTTCATatatttcctcctggtatcctgttacac |
| FC292_F | ATGAACAAAGGTGTAATGCGACCG |
| JG183_R | AATTCGGCAGTTTAACCATGAAG |
| **pSMF8 PJ23119-*xylE* plasmid** | |
| SF1_pDL_F | AACGAACGATTCATGACCGTGCTCACCTGACtcgagtaaggatctccagg |
| SF4_pDL014_R | CCCGGTCGCATTACACCTTTGTTCATagatcctttctcctctttagatctattatacc |
| FC292_F | ATGAACAAAGGTGTAATGCGACCG |
| JG70 | TCAGGTGAGCACGGTCATGAATC |
| **cJDG1 P*mxaF-ble* knock-in** | |
| FC351_F | ACAGTCCGAACAACGATTTCG |
| AP193 | AATTCGGCAGTTTAACCATG |
| AP235 | TCATGCGCTTCATGGTTAAACTGCCGAATTAATTAAACCGGGAATGATGTC |
| JG61_zeo_R | GCCATGCTTACTATCTTAGCCATGTTTCCTCAATGGtcagtcctgctcctcggcca |
| AP192 | CCATTGAGGAAACATGGCTA |
| FC352_R | AGTGATTCTGGTCGCGCACA |
| **cJDG3 linear *fecI1* knockout** | |
| CM33 | AAAGTACGCCATTATCGGCAG |
| CM34 | aactggtccacctacaacaaagctctcatcAGGTTCAACCGCATTCATACG |
| CM35 | gatgagagctttgttgtaggtggaccagtt |
| CM36 | tctcgagtcccgtcaagtcagcgtaatgct |
| CM37 | agcattacgctgacttgacgggactcgagaTATTTCGGTGAGTTGGGTCG |
| CM38 | GCTTGCGGACAATAAACTCG |
| **cJDG13 linear *fecI3* knockout** | |
| JG293_km_50001_LF_F | CGGAAATAGAACgatgagagctttgttgtaggtgg |
| JG294_km_50001_RF_R | GATTTACTTTCATTTTCACCtctcgagtcccgtcaagtc |
| 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_169mxaF_F | AGTTGTACGACATCAACATCACG |
| FC404_367mxaF_R | GCTTCGGTTTGAATTGCCACA |
| 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 |
|---|---|---|---|---|
| JG3μ2/JG3μ3 | (C)5→6 | coding (1356/2199 nt) | MBURv2_130812 | 21 |
| JG3μ5 | G→A | W643* (TGG→TGA) | MBURv2_130812 | 0 |
| JG3μ7/JG3μ8 | G→A | W643* (TGG→TGA) | MBURv2_130812 | 38 |
| JG3μ11 | C→T | Q657* (CAA→TAA) | MBURv2_130812 | 56 |
| JG3μ15 | C→T | Q720* (CAG→TAG) | MBURv2_130812 | 2 |
| JG3μ16 | G→A | W643* (TGG→TGA) | MBURv2_130812 | 56 |
| JG3μ1 | C→T | Q657* (CAA→TAA) | MBURv2_130812 | Not sequenced |
| JG3μ4 | C→T | Q336* (CAA→TAA) | MBURv2_130812 | Not sequenced |
| JG3μ6 | G→A | W396* (TGG→TGA) | MBURv2_130812 | Not sequenced |
| JG3μ9 | G→A | W643* (TGG→TGA) | MBURv2_130812 | Not sequenced |
| JG3μ10 | C→T | Q383* (CAG→TAG) | MBURv2_130812 | Not sequenced |
| JG3μ12 | G→A | W396* (TGG→TGA) | MBURv2_130812 | Not sequenced |
| JG3μ13 | G→A | W643* (TGG→TGA) | MBURv2_130812 | Not sequenced |

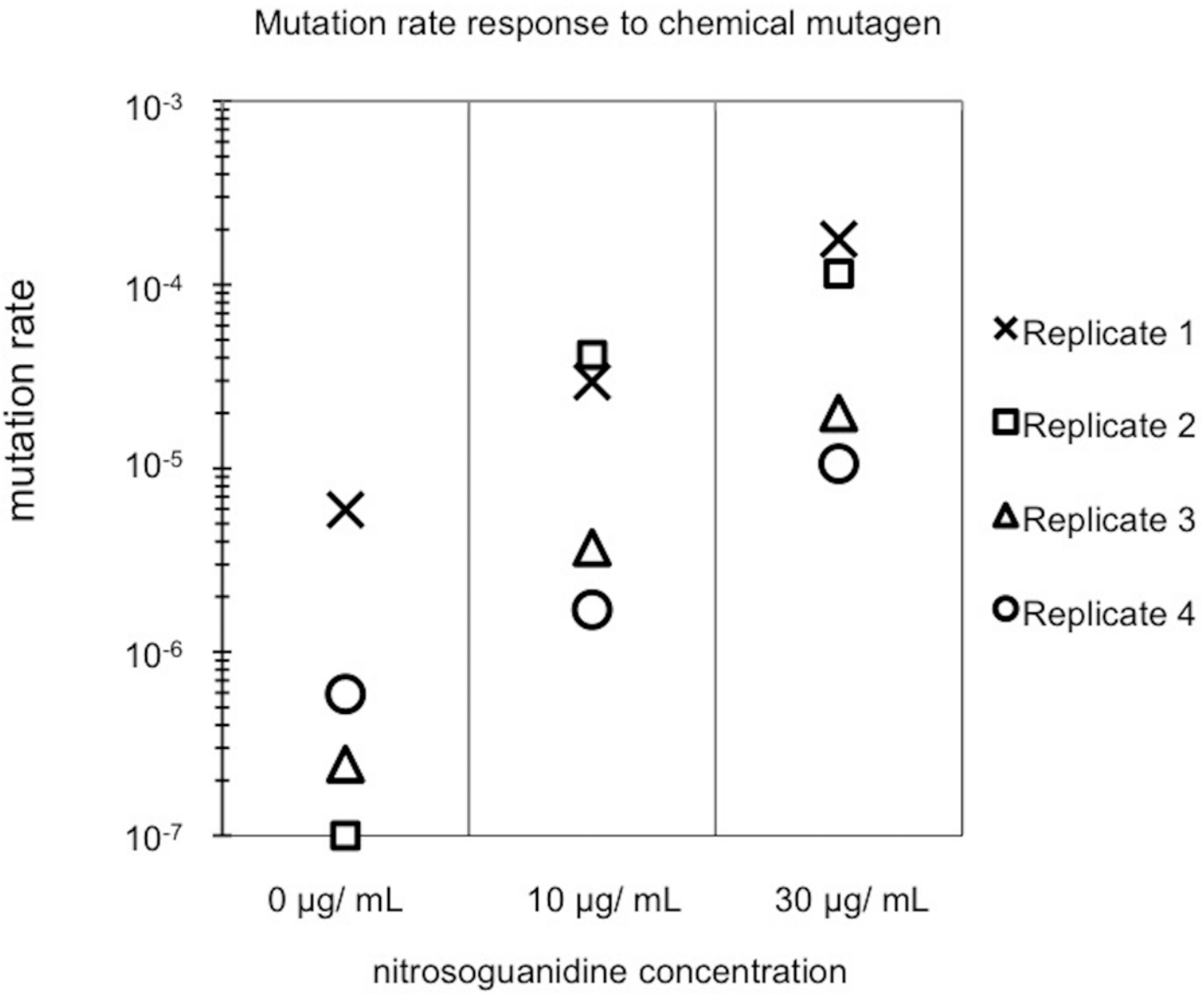


**Fig S1 Nitrosoguanidine increases the mutation rate of *Methylomicrobium buryatense* 5GB1C.** Data represent results from the mutagenized $P_{mxaF}$-*zeo* reporter strain of *M. buryatense* 5GB1C grown in the presence of 30 µg/mL lanthanum chloride. The mutation rates are calculated by the ratio of 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).

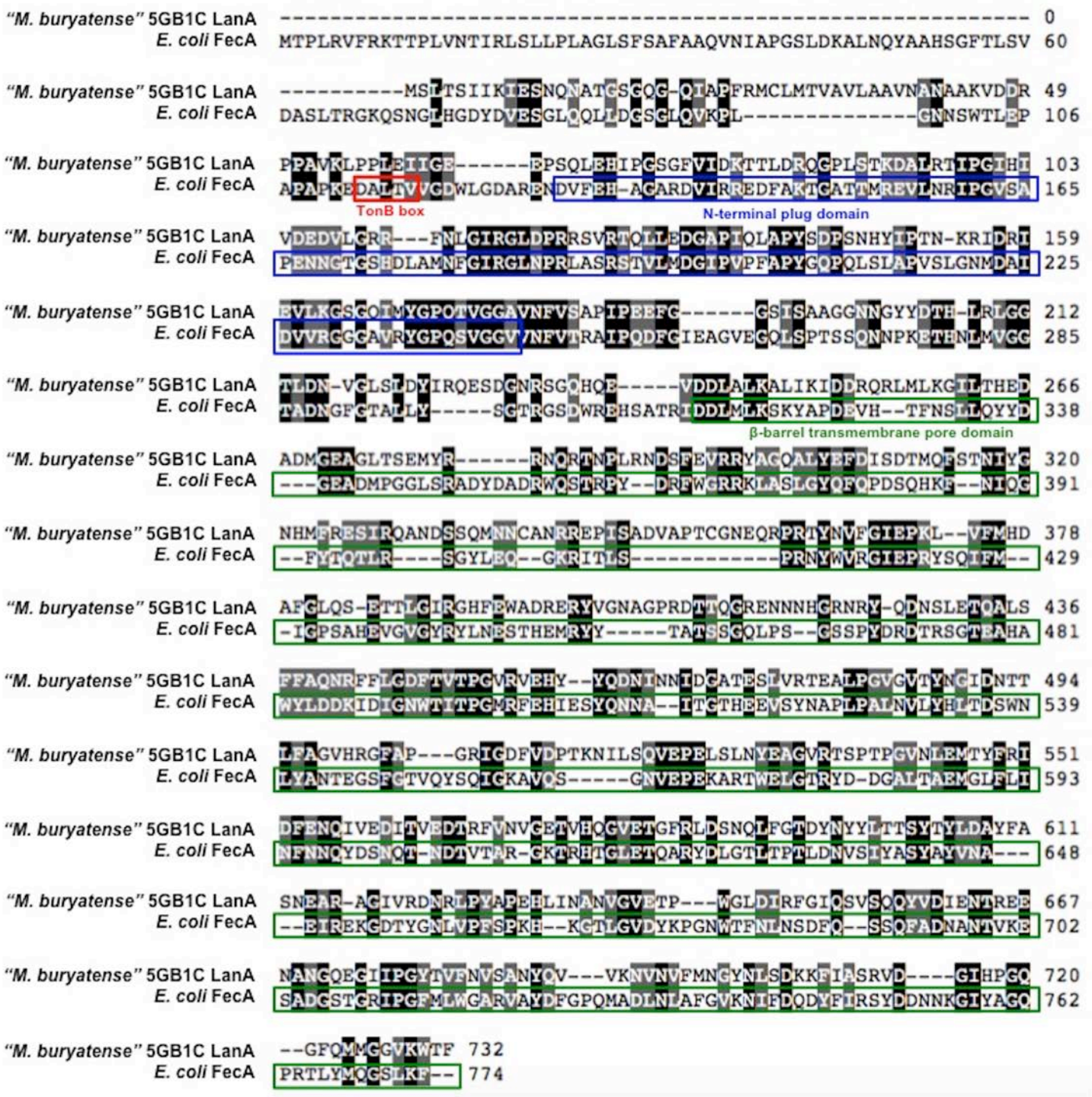


**Fig S2 Protein alignment of "*M. buryatense*" 5GB1C LanA and *E. coli* ferric citrate transporter FecA.** Pairwise protein alignment of LanA and FecA, generated by EMBOSS Needle aligment software. Identical residues are shaded in black, while biochemically similar residues are shaded in grey. The Pfam-annotated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

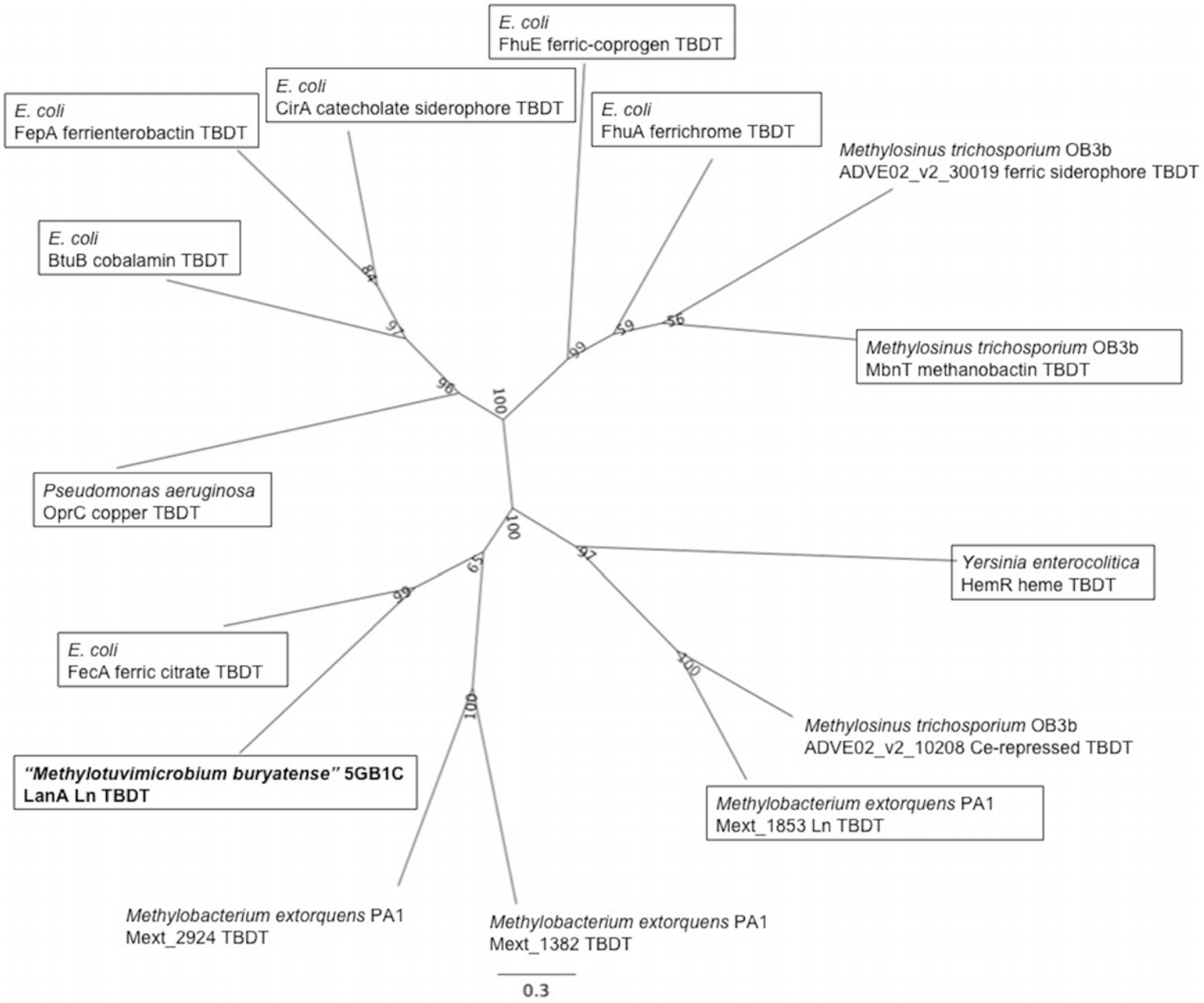


**Fig S3 LanA is most closely related to the ferric citrate transporter FecA compared to known transporters and other methylotrophic homologs.** Maximum likelihood tree generated from a multiple sequence alignment of selected TonB-dependent transporters (TBDTs). Posterior probabilities are indicated 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.

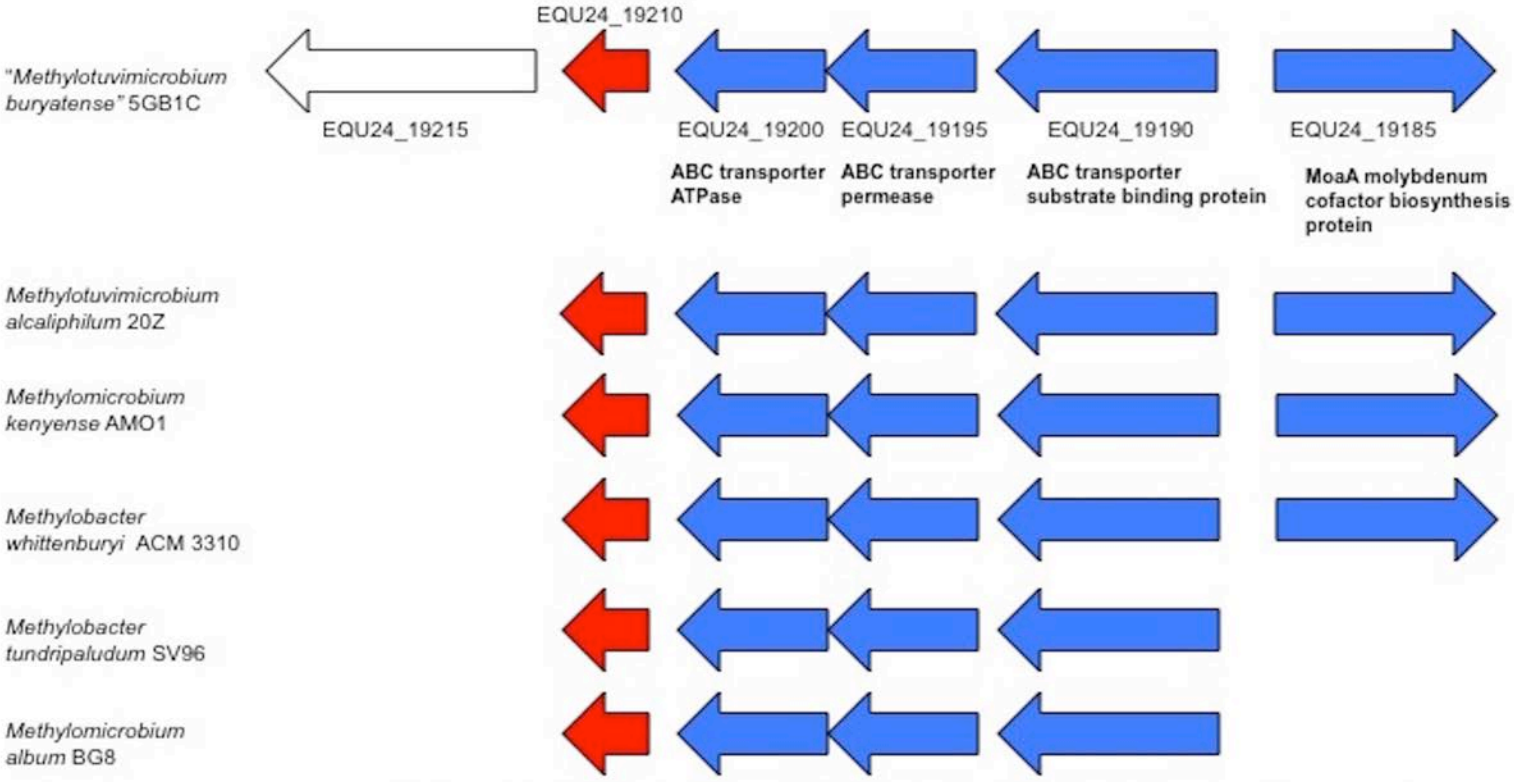


**Fig S4 Genome synteny of EQU24_19210 in other Type I methanotrophs.** The EQU24_19210 gene (shown in red) is regulated similarly to *xoxF*. It resides next to an ABC transporter gene cluster in many closely related methanotrophs, and in some methanotrophs resides near the *moaA* gene encoding a molybdenum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

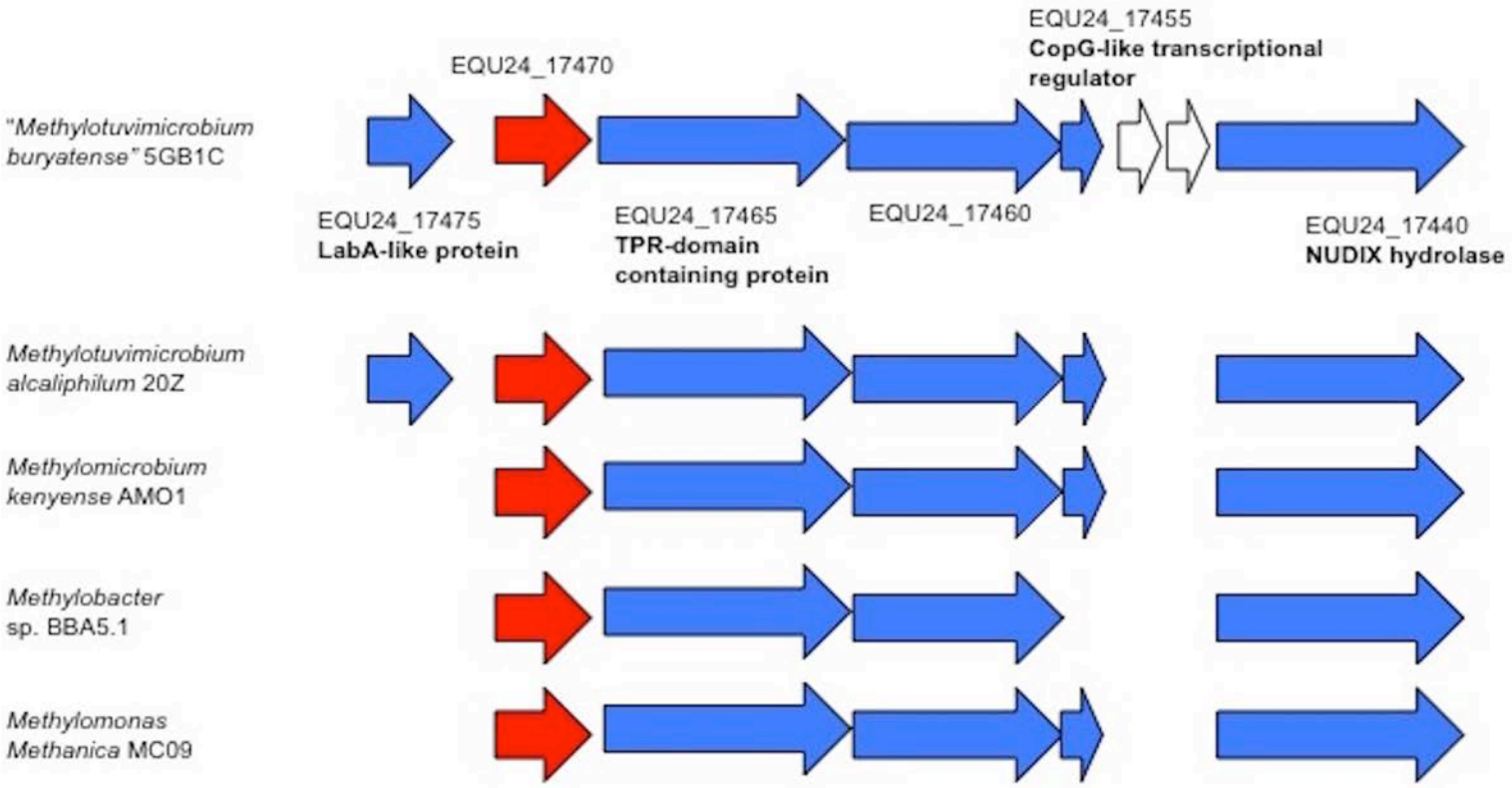


**Fig S5 Genome synteny of EQU24_17470 in other Type I methanotrophs.** The EQU24_17470 gene (shown in red) is induced by lanthanum. It resides next to several genes that encode conserved domains, including a NUDIX hydrolase and a TPR-domain containing protein, and in some organisms a CopG-like transcriptional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

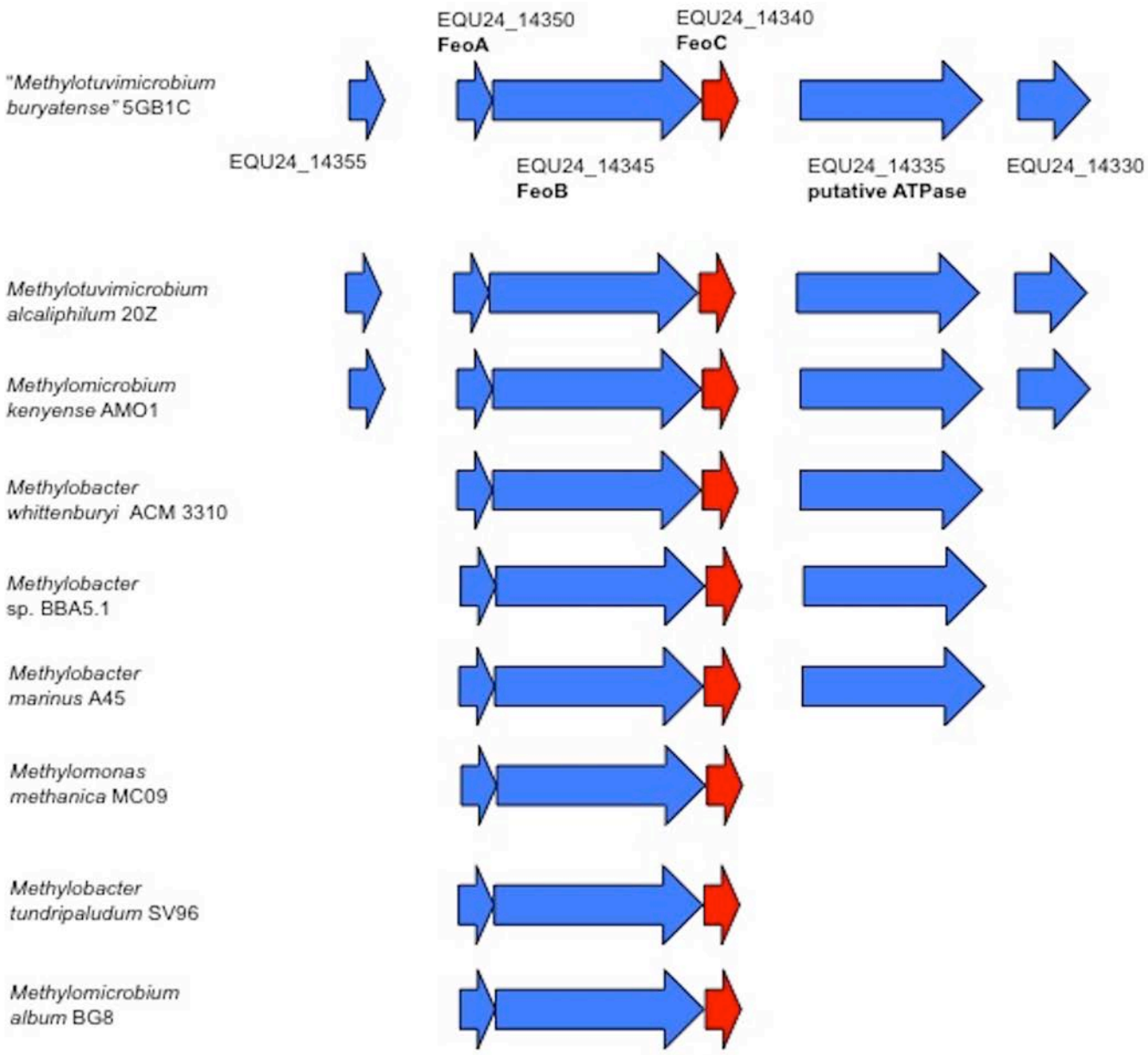


**Fig S6 Genome synteny of EQU24_14340 in other Type I methanotrophs.** The EQU24_14340 gene (shown in red) is induced by lanthanum. It resides next to FeoAB ferrous iron transporter gene homologs in 9 different Type I methanotrophs, and a putative ATPase in some organisms (shown in blue).

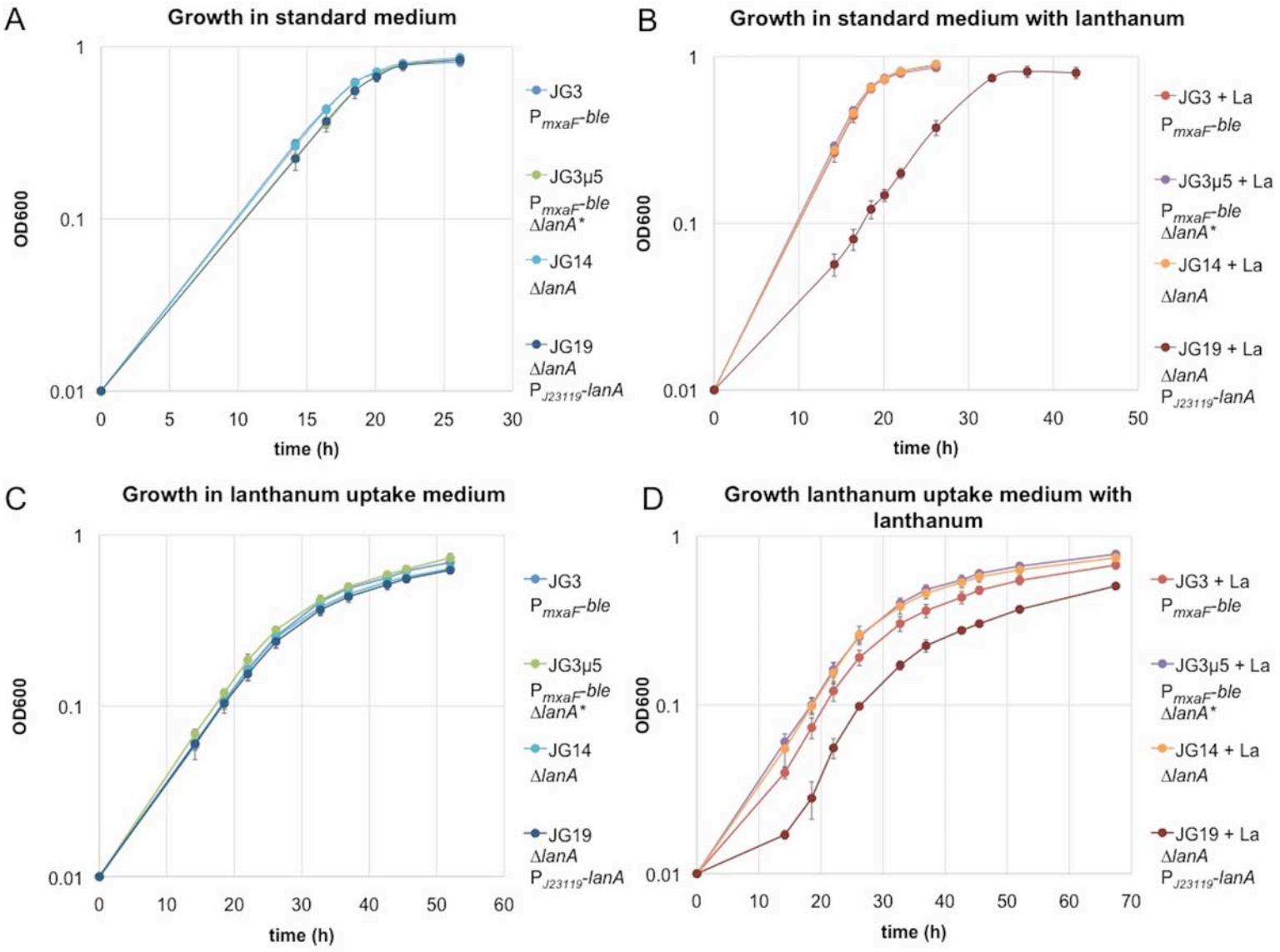


**Fig S7 Deletion of *lanA* does not result in a growth phenotype, but overexpression of *lanA* leads to slower growth.** "*M. buryatense*" 5GB1C derivatives were grown in either standard medium **(A and B)** or lanthanum uptake medium **(C and D)**, without **(A and C)** or with **(B and D)** lanthanum.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

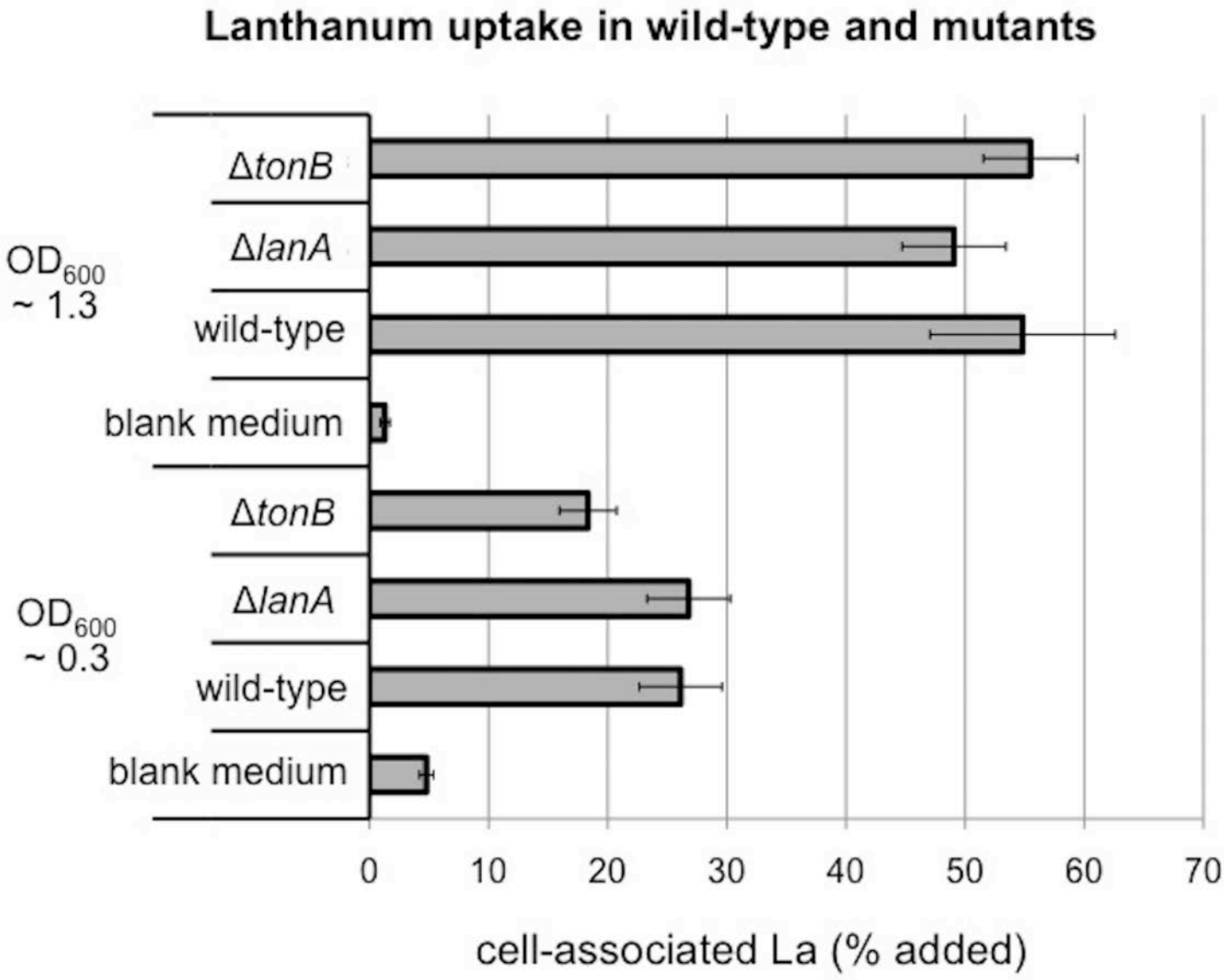


**Fig S8 Lanthanum uptake is not affected in either Δ*lanA* or Δ*tonB*.** Cells were inoculated into medium containing 2 μM La. Cells were harvested at the indicated optical densities, corresponding to early exponential phase ($OD_{600}$ ~ 0.3) and early stationary phase ($OD_{600}$ ~ 1.3). Lysed cell 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 ± standard deviation of two biological replicates, including the results from medium not inoculated with cells (n=2).

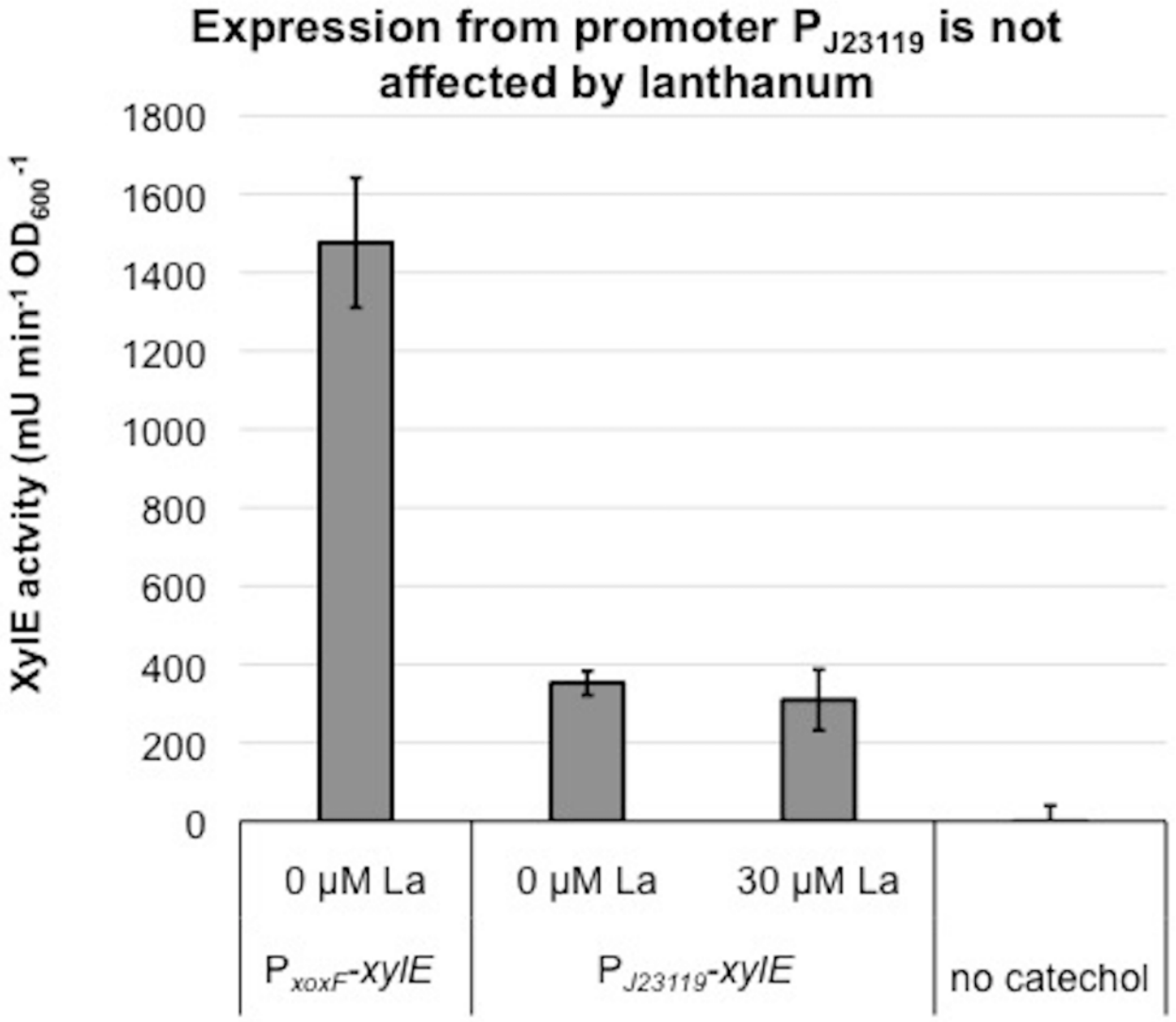


**Fig S9 The $P_{J23119}$ promoter used to express *lanA* is not affected by lanthanum.** Whole cell catechol-2,3-dioxygenase reporter gene assays were performed for wild-type and mutant strains. Both $P_{xoxF}$-*xylE* and the $P_{J23119}$-*xylE* were created from the wild-type background strain. Cells 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).

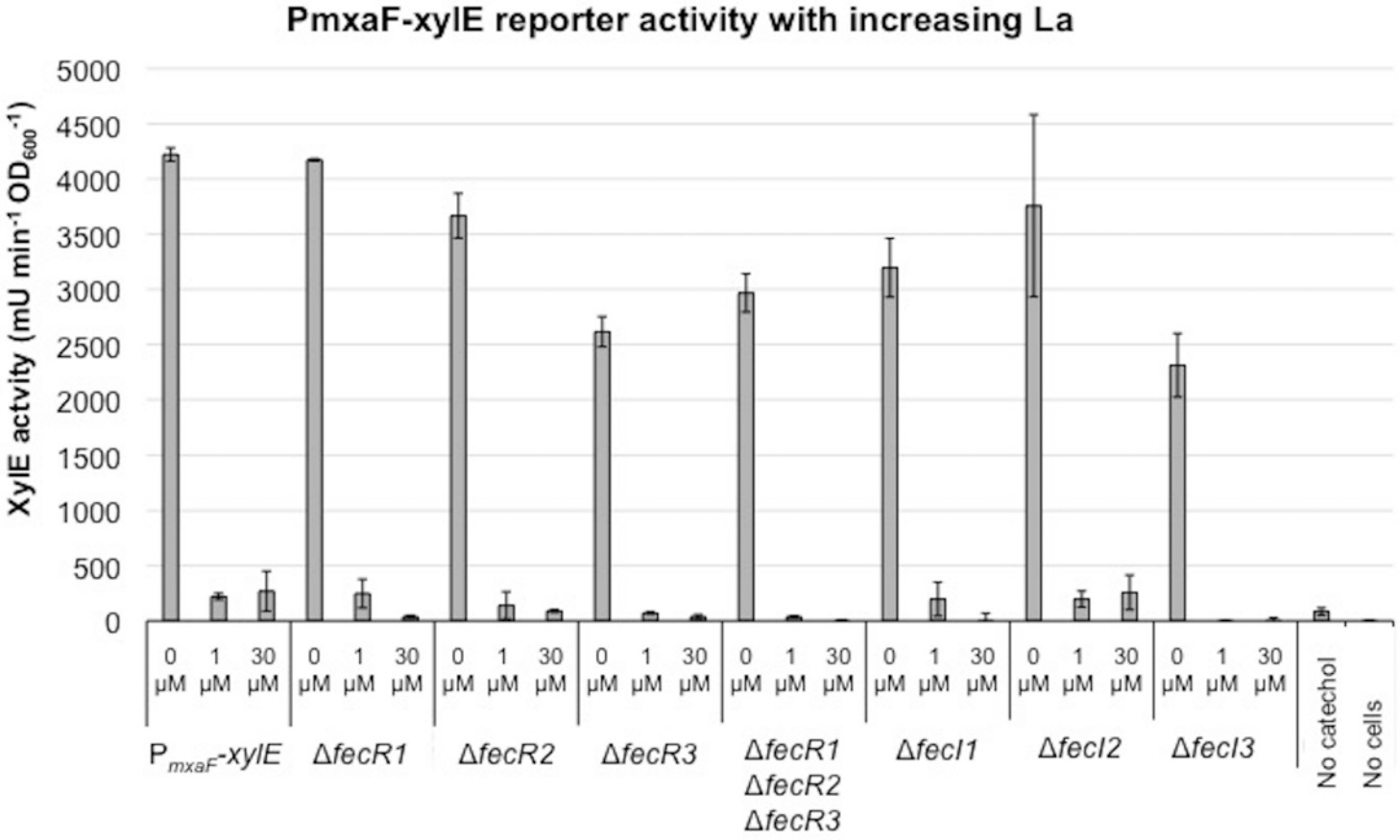


**Fig S10 *fecR* and *fecI* deletions have no clear effect on $P_{mxaF}$-*xylE* reporter gene expression.** Whole cell catechol-2,3-dioxygenase reporter gene assays were performed for wild-type and mutant strains. The wild-type was FC31 ($P_{mxaF}$-*xylE*), and all mutants were created from this background 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).

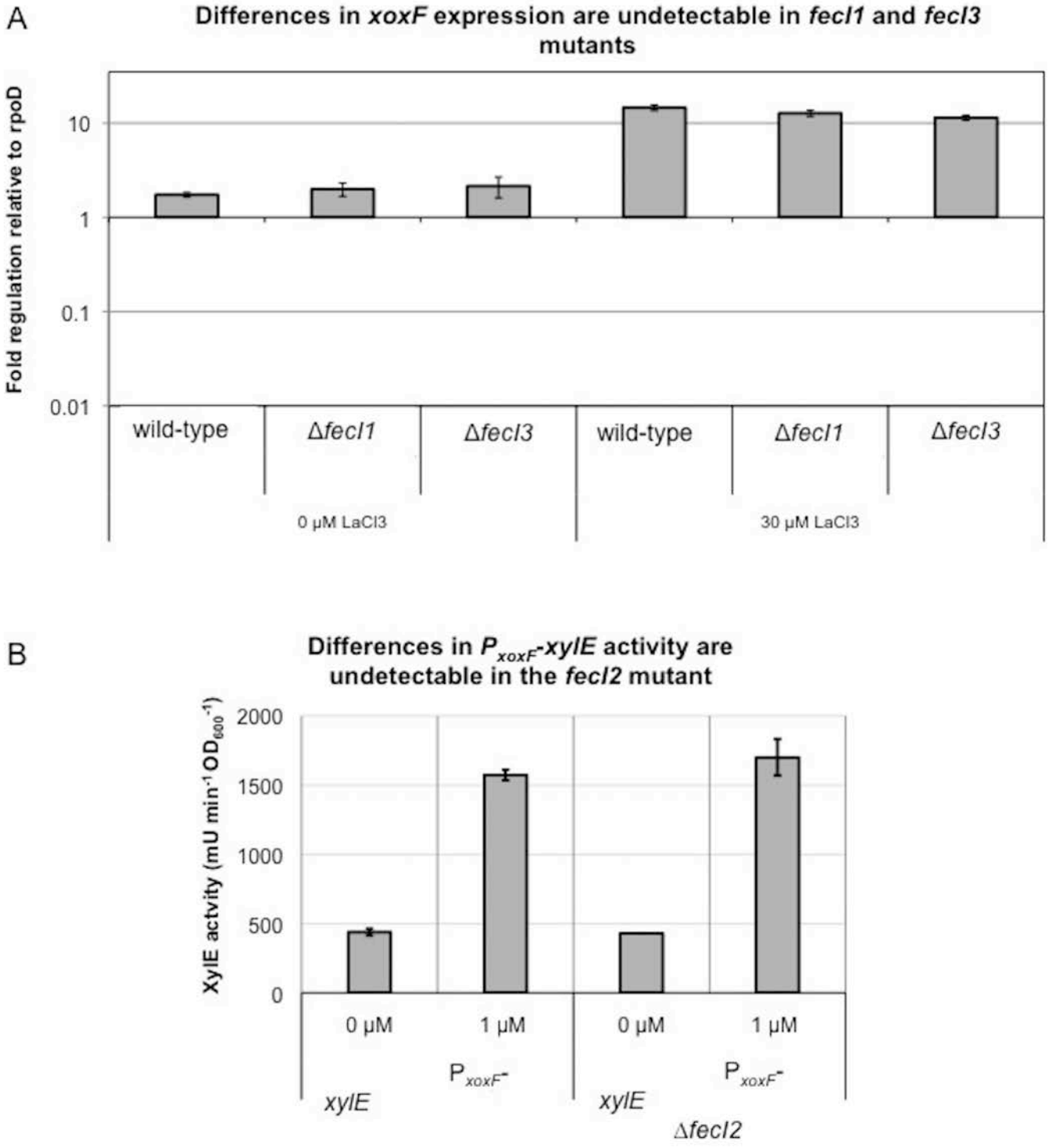


**Fig S11 *fecI* deletions have no clear effect on *xoxF* gene regulation. A)** Reverse transcriptase polymerase chain reaction (RT-PCR) was performed on RNA harvested from *M. buryatense* wild-type and mutants in the presence or absence of 30 µM lanthanum. Primers specific for *xoxF* methanol dehydrogenase genes were used to quantify the lanthanide switch. All cycle threshold values were normalized to the constitutive *rpoD* gene. Results show the average ± 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 ($P_{xoxF}$-*xylE*), and the Δ*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 ± standard deviation of two biological replicates (n=2).