TABLE S1 Sequence similarity between homologous proteins involved in Tcb metabolism[a]

		Acinetobacter baylyi ADP1[b]				
		TcuR **ACIAD_RS07100**	TclR **ACIAD_RS07105**	TcuA **ACIAD_RS07085**	TcuB **ACIAD_RS07080**	TcuC **ACIAD_RS07100**
Salmonella enterica Typhimurium LT2[b]	TcuR **STM0692**	44 (67) %	42 (65) %			
	TcuA **STM0691**			75 (85) %		
	TcuB **STM0690**				58 (73) %	
	TcuC **STM0689**					60 (74) %

[a]Values in boxes indicate the percentage of identical and (similar) amino acids in a pairwise alignment of the protein sequences indicated. Similarity was evaluated using the default parameters for the Ident and Sim program (1).

[b]Locus tags, in bold, correspond to genes in reference genomes: NC_005966 (ADP1) and NC_003197 (LT2).

TABLE S2 *Acinetobacter baylyi* strains derived from ADP1

Strain	Relevant Characteristics[a, b]	Source
ADP1[c]	Wild-type strain (BD413)	(2, 3)
ACN1373	Δ*tcuR*::*sacB*-Ω*K51373* *sacB*-ΩK from pRMJI replaces *tcuR* (**1,540,519-1,541,442**)[d] pBAC1122/AatII x ADP1	This study
ACN1376	Δ*tcuR51376* Deletion of *tcuR* (**1,540,519-1,541,442**)[d] pBAC1120/AatII x ACN1373	This study
ACN1385	Δ*pacI*::*sacB*-Ω*K51385* *sacB*-ΩK from pRMJ1 replaces *pacI* (**1,541,580-1,542,368**)[d] pBAC1142/AatII & XmnI x ADP1	This study
ACN1388	Δ*pacI51388* Deletion of *pacI* (**1,541,580-1,542,368**)[d] pBAC1137/AatII & XmnI x ACN1385	This study
ACN1419	Δ*tcuC*::*sacB*-Ω*K51419* *sacB*-ΩK from pRMJ1 replaces *tcuC* (**1,542,464-1,543,768**)[d] PCR product (pBAC1141 template with primers oST63 & M13R) x ADP1	This study
ACN1445	Δ*tcuR51376, tclR51445* Δ*tcuR* as in ACN1376, *tclR* mutations (C→T **1,544,371** and A→G **1,544,323**)[d] ACN1376-derived Tcb[+] spontaneous mutant, TclR(R200Q, L216P)	This study
ACN1456	Δ*tcuC51456* Deletion of *tcuC* (**1,542,464-1,543,768**)[d] pBAC1138/BspHI & SphI x ACN1419	This study
ACN1556	Δ*tcuR51376, tclR51556* Δ*tcuR* as in ACN1376, *tclR* (C→T **1,544,371**)[d] ACN1376-derived Tcb[+] spontaneous mutant, TclR(R200Q)	This study
ACN3122	Δ*tcuR51376*, Δ*tcuA*::*gfp*$_{sf}$-Ω*K53122* Δ*tcuR* as in ACN1376, *gfp*$_{sf}$-ΩK replaces *tcuA* (**1,539,009-1,540,412**)[d, e] pBAC2102/BamHI x ACN1376	This study
ACN3123	Δ*tcuA*::*gfp*$_{sf}$ -Ω*K53122* Δ*tcuA* fusion as in ACN3122 pBAC2109/BsaI x ADP1	This study
ACN3124	Δ*tcuR51376*, Δ*tclR*::*sacB*-Ω*K53124* *sacB*-ΩK replaces *tclR* (**1,544,043-1,544,936**)[d] pBAC2101/BsaI & SacI x ACN1376	This study
ACN3125	Δ*cltA*::*sacB*-Ω*S53125* *sacB*-ΩS replaces *cltA* (**388,259-389,574**)[d] pBAC2110/AhdI & AatII x ADP1	This study
ACN3168	Δ*tcuC51456*, Δ*cltA*::*sacB*-Ω*S53125* Δ*tcuC* as in ACN1456, *sacB*-ΩS replaces *cltA* as in ACN3125 pBAC2110/AhdI & AatII x ACN1456	This study

ACN3171	$\Delta tcuR51376$, $\Delta tclR53171$ $\Delta tcuR$ as in ACN1376, deletion of *tclR* (**1,544,043-1,544,936**)[d] pBAC2093/BsaI x ACN3124	This study
ACN3172	$\Delta tclR$::*sacB*-$\Omega K53124$ *sacB*-ΩK replaces *tclR* as in ACN3124 pBAC2101/BsaI & SacI x ADP1	This study
ACN3173	$\Delta tcuR51376$, $\Delta tclR53171$, $\Delta tcuA$::*gfp*$_{sf}$ -$\Omega K53122$ $\Delta tcuR$ as in ACN1376, $\Delta tclR$ as in ACN3171, *tcuA* fusion as in ACN3122 pBAC2102/BamHI x ACN3171	This study
ACN3178	$\Delta tclR53171$ $\Delta tclR$ as in ACN3171 pBAC2093/BsaI x ACN3172	This study
ACN3179	$\Delta tcuR51376$, *tclR51445* $\Delta tcuR$ as in ACN1376, *tclR* mutations as in ACN1445 [TclR(R200Q, L216P)] pBAC1164/BsaI x ACN3124	This study
ACN3184	$\Delta tclR53171$, $\Delta tcuA$::*gfp*$_{sf}$ -$\Omega K53122$ $\Delta tclR$ as in ACN3171, *tcuA* fusion as in ACN3122 pBAC2109/AatII & SbfI x ACN3178	This study
ACN3187	$\Delta tcuR51376$, *tclR51556* $\Delta tcuR$ as in ACN1376, *tclR* mutation as in ACN1556 [TclR(R200Q)] pBAC1863/AatII x ACN3124	This study
ACN3190	$\Delta tcuR51376$, *tclR51445*, $\Delta tcuA$::*gfp*$_{sf}$ -$\Omega K53122$ $\Delta tcuR$ as in ACN1376, *tclR* as in ACN1445 [TclR(R200Q, L216P)], *tcuA* fusion as in ACN3122 pBAC2102/BamHI x ACN3179	This study
ACN3191	$\Delta tcuR51376$, *tclR51556*, $\Delta tcuA$::*gfp*$_{sf}$ -$\Omega K53122$ $\Delta tcuR$ as in ACN1376, *tclR* as in ACN1556 [TclR(R200Q)], *tcuA* fusion as in ACN3122 pBAC2102/BamHI x ACN3187	This study
ACN3222	$\Delta tcuR51376$, *tcuC*-*gfp*$_{sf}$-$\Omega K53222$, $\Delta pacI53222$ $\Delta tcuR$ as in ACN1376, Region downstream of *tcuC* including *pacI* deleted (**1,541,578 – 1,542,444**)[d] and replaced with a *gfp*$_{sf}$ -ΩK DNA[e] pBAC2161/BsaI & NdeI x ACN1376	This study
ACN3223	$\Delta tcuR51376$, $\Delta tclR53171$, *tcuC*-*gfp*$_{sf}$-$\Omega K53222$, $\Delta pacI53222$ $\Delta tcuR$ as in ACN1376, $\Delta tclR$ as in ACN3171, *tcuC* fusion and $\Delta pacI$ as in ACN3222 pBAC2161/BsaI & NdeI x ACN3171	This study
ACN3224	$\Delta tclR53171$, *tcuC*-*gfp*$_{sf}$-$\Omega K53222$, $\Delta pacI53222$ $\Delta tclR$ as in ACN3171, *tcuC* fusion and $\Delta pacI$ as in ACN3222 pBAC2158/BsaI & NdeI x ACN3178	This study
ACN3225	*tcuC*-*gfp*$_{sf}$-$\Omega K53222$, $\Delta pacI53222$ *tcuC* fusion and $\Delta pacI$ as in ACN3222 pBAC2158/BsaI & NdeI x ADP1	This study
ACN3226	$\Delta cltA$::*sacB*-$\Omega S53125$, *tcuC*-*gfp*$_{sf}$-$\Omega K53222$, $\Delta pacI53222$ *sacB*-ΩS replaces *cltA* as in ACN3125, *tcuC* fusion and $\Delta pacI$ as in ACN3222 pBAC2158/BsaI & NdeI x ACN3125	This study

ACN3227	Δ*tcuR51376, tclR51445, tcuC-gfp~sf~-ΩK53222, ΔpacI53222* Δ*tcuR* as in ACN1376, *tclR* as in ACN1445 [TclR(R200Q, L216P)], *tcuC* fusion and Δ*pacI* as in ACN3222 pBAC2161/BsaI & NdeI x ACN3179	This study
ACN3228	Δ*tcuR51376, tclR51556, tcuC-gfp~sf~-ΩK53222, ΔpacI53222* Δ*tcuR* as in ACN1376, *tclR* as in ACN1556 [TclR(R200Q)], *tcuC* fusion and Δ*pacI* as in ACN3222 pBAC2161/BsaI & NdeI x ACN3187	This study

[a.] For strains generated by allelic replacement, transformation is indicated by the donor DNA x recipient strain. Plasmids (Table S3) used as donor DNA were linearized by restriction digestion, indicated by plasmid name/restriction enzyme(s). When PCR products were used as donor DNA, they were first treated with DpnI to degrade the methylated template DNA. Plasmids and primers described further in Tables S3 and S4.

[b.] Abbreviations: coding sequence (CDS), Kanamycin (Km), omega cassette conferring Km^R (ΩK), omega cassette conferring $Sm^R Sp^R$ (ΩS).

[c.] *A. baylyi* strains were derived from ADP1, previously known as *Acinetobacter calcoaceticus* or *Acinetobacter sp.* (2, 3).

[d.] Bold numbers correspond to positions on the ADP1 chromosome in NCBI entry NC_005966.

[e.] Sequence of the transcriptional reporter. To generate the Δ*tcuA*::*gfp~sf~* -Ω*K53122* transcriptional fusion, the the following purple text sequence (starting with ATG) exactly replaced the *tcuA* coding sequence in the orientation that the coding sequence of GFPsf (highlighted in grey) replaced that of TcuA. To generate the *tcuC-gfp~sf~*-Ω*K53222* transcriptional fusion, the entire following sequence (black and purple text) replaced the chromosomal sequence such that the *tcuC* promoter drives transcription of *gfp~sf~*. The coding sequence for GFP is highlighted in grey. The RBS used in *tcuC-gfp~sf~*-Ω*K53222* is in bold. The gene encoding Km^R (reverse complement) is underlined.

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Tccttca**ggagga**cagctATGGCTAGCAAAGGAGAAGAACTTTTCACGGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACA
AATTTTCTGTCCGTGGAGAGGGTGAAGGTGATGCTACAAACGGAAAACTCACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCC
AACACTTGTCACTACTCTGACCTATGGTGTTCAATGCTTTTCCCGTTATCCGGATCACATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAA
GGTTATGTACAGGAACGCACTATATCTTTCAAAGATGACGGGACCTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTA
TCGAGTTAAAGGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAACTCGAGTACAACTTTAACTCACACAATGTATACATCACGGCAGA
CAAACAAAAGAATGGAATCAAAGCTAACTTCAAAATTCGCCACAACGTTGAAGATGGTTCCGTTCAACTAGCAGACCATTATCAACAAAATACTCCA
ATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAATCTGTCCTTTCGAAAGATCCCAACGAAAGCGTGACCACATGGTCC
TTCTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATGAGCTCTACAAAggtggcggttctgaattcacacctaggtaaagctcggtacg
atccggtgattgattgagcaagctttatgcttgtaaaccgttttgtgaaaaaattttttaaaataaaaaagggggacctctagggtccccaattaatta
gtaatataatctattaaaggtcattcaaaaggtcatccaccggatcaattccctgctcgcgcaggctgggtgccaagctctcgggtaacatcaagg
cccgatccttggagcccttgccctcccgcacgatgatcgtgccgtgatcgaaatccagatccttgacccgcagttgcaaaccctcactgatccgtcg
accaaagcggccatcgtgcctccccactcctgcagttcggggggcatggatgcgcggatagccgctgctggtttcctggatgccgacggatttgcact
gccggtagaactccgcgaggtcgtccagcctcaggcagcagctgaaccaactcgcgagggatcgagcccggggtgggcgaagaactccagcatgag
atccccgcgctggaggatcatccagccggcgtcccggaaaacgattccgaagcccaacctttcatagaaggcggcggtggaatcgaaatctcgtgat
ggcaggttgggcgtcgcttggtcggtcatttcgaaccccagagtcccgctcagaagaactcgtcaagaaggcgatagaaggcgatgcgctgcgaatc
gggagcggcgataccgtaaagcacgaggaagcggtcagcccattcgccgccaagctcttcagcaatatcacgggtagccaacgctatgtcctgatag
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cgagatcctcgccgtcgggcatgcgcgccttgagctcggcgaacagttcggctggcgcgagcccctgatgctcttcgtccagatcatcctgatcgac
aagaccggcttccatccgagtacgtgctcgctcgatgcgatgtttcgcttggtggtcgaatgggcaggtagccggatcaagcgtatgcagccgccgc
attgcatcagccatgatggatactttctgcaggagcaaggtgagatgacaggagatcctgccccggcacttcgcccaatagcagccagtccttc
ccgcttcagtgacaacgtcgagcacagctgcgcaaggaacgcccgtcgtggccagccacgatagccgcgctgcctcgtcctgcagttcattcaggc
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tcatagccgaatagcctctccacccaagcggccggagaacctgcgtgcaatccatcttgttcaatcatgcgaaacgatcctcatcctgtctcttgat
cagatcttgatccctgcgccatcagatccttggcggcaagaaagccatccagtttactttgcagggcttcccaaccttaccagagggcgccccagc
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actatcaggtcaagtctgcttttattattttttaagcgtgcataataagccctacacaaattgggagatatatcatgaaaggctggcttttttcttgtt
atcgcaatagttggcgaagtaatcgcaacatccgcattaaaatctagcgagggctttactaagctgatccggtggatgacctttttgaatgaccttta
atagattatattactaattaattggggaccctagaggtccccttttttattttaaaaattttttcacaaaacggtttacaagcataaagcttgctca
atcaatcaccggatctaccgggc
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Table S3 Plasmids used in this study

Plasmid	Relevant Characteristics[a]	Source
pBAC1120	Ap^{R}Km^R; Δ*tcuR51376*; SOEing of two PCR products: one upstream of *tcuR* (using oMTV153 & oMTV154)[b], the other downstream of *tcuR* (using oMTV155 & oMTV152).[b] XhoI site replaces *tcuR* CDS. DNA ligated into pUC18 (SacI and PstI digested).	This study
pBAC1122	Ap^{R}Km^R; Δ*tcuR*::*sacB*-ΩK*51373*; *sacB*-ΩK from pRMJI digested with SalI and inserted in XhoI site of pBAC1120	This study
pBAC1137	Ap^R; Δ*pacI51388*; SOEing of two PCR products; one upstream of *pacI* (using oST47 & oST48)[b] and the other downstream of *pacI* (using oST45 & oST46).[b] BamHI and KasI sites replace *pacI* CDS. PCR product digested and ligated in pUC19.	This study
pBAC1138	Ap^R; Δ*tcuC51456*; SOEing of two PCR products: one upstream of *tcuC* (using oST51 & oST52)[b] and the other downstream of *tcuC* (PCR with oST49 & oST50).[b] BamHI and KasI sites replace *tcuC* CDS. DNA inserted in pUC19.	This study
pBAC1141	Ap^{R}Km^R; Δ*tcuC*::*sacB*-ΩK*51419*; *sacB*-ΩK from pRMJI digested with BamHI inserted in BamHI site of pBAC1138.	This study
pBAC1142	Ap^{R}Km^R; Δ*pacI*::*sacB*-ΩK*51385*; *sacB*-ΩK from pRMJI digested with BamHI inserted in BamHI site of pBAC1137.	This study
pBAC1164	Ap^{R}Km^R; *tclR51445*; ACN1445 sequence surrounding *tclR*, encoding TclR(R200Q, L216P), PCR product (with oNSL40 & oNSL46)[b] cloned into pCR2.1 TOPO vector	This study
pBAC1539	Ap^{R}Km^R; *sacB*-ΩK from pRMJI digested with BamHI inserted in pUC18	This study
pBAC1548	Ap^{R}Sm^{R}Sp^R; source of *sacB*-ΩS; ΩS from pUI1638 digested with Eco53KI inserted between Eco53KI and EcoRV of pBAC1539	This study
pBAC1863	Ap^{R}Km^R; *tclR51556*; ACN1556 sequence surrounding *tclR*, encoding TclR(R200Q),[b] PCR product (with oNSL40 & oNSL46)[b] cloned into pCR2.1 TOPO vector	This study
pBAC2093	Ap^R; Δ*tclR53171*; SOEing of two PCR products: one upstream of *tclR* (using oMTV158 & oMTV161)[b] and the other downstream of *tclR* (using oMTV160 & oMTV159).[b] XhoI site replaces *tclR* CDS. DNA inserted in pUC18.	This study
pBAC2094	Ap^R; *tcuA* region Δ*tcuR51376* in pUC19; *E. coli* assembly[c] of two PCR-generated fragments: (1) *tcuA* region surrounding Δ*tcuR51376* (ACN1376 template with oACB69 & oACB70)[b] and (2) vector DNA (pUC19 template with oACB71 & oACB72).[b]	This study
pBAC2095	Ap^{R}Km^R; ΩK downstream of *gfp$_{sf}$*; *E. coli* assembly[c] of three PCR-generated fragments (1) *gfp$_{sf}$* (pBTL-2_pcaU_1 template with oACB65 & oACB66)[b], (2) ΩK (pUI1637 template with oACB67 & oACB68)[b], and (3) vector (pUC19 template with oACB63 & oACB64)[b]. Used to make *gfp$_{sf}$* fusion plasmids pBAC2119, pBAC2109, and pBAC2102.	This study
pBAC2098	Ap^R; *tcuA* region in pUC19; *E. coli* assembly[c] of two PCR-generated fragments: (1) *tcuA* region (ADP1 template with oACB69 & oACB70)[b], and (2) vector DNA (pUC19 template with oACB71 & oACB72)[b]	This study
pBAC2099	Ap^R; *tcuC-tcuA* DNA in pUC19; *E. coli* assembly[c] of two PCR products: (1) *tcuC-tcuA* region (ADP1 template with oACB73 & oACB74)[b], and (2) vector DNA (pUC19 with oACB75 & oACB76)[b]	This study
pBAC2101	Ap^{R}Km^R; Δ*tclR*::*sacB*-ΩK*53124*; *sacB*-ΩK from pRMJI digested with SalI and inserted in XhoI site of pBAC2093	This study

pBAC2102	Ap^{R}Km^R; Δ*tcuA*::*gfp$_{sf}$* -ΩK*53122*; *E. coli* assembly[c] of two PCR-generated fragments: (1) *gfp$_{sf}$*-ΩK (from pBAC2095 template with <u>oACB79</u> and o<u>ACB80</u>)[b] and (2) vector with ADP1 tcu region DNA (pBAC2094 template with <u>oACB79</u> and <u>oACB80</u>)[b]	This study
pBAC2109	Ap^{R}Km^R; Δ*tcuA*::*gfp$_{sf}$*-ΩK*53122*; *E. coli* assembly[c] of two PCR-generated fragments: (1) *gfp$_{sf}$*-ΩK (from pBAC2095 template with <u>oACB79</u> & <u>oACB80</u>)[b] and (2) vector with ADP1 DNA for allelic replacement (pBAC2098 template with <u>oACB77</u> & <u>oACB78</u>)[b]	This study
pBAC2110	Ap^{R}Sm^{R}Sp^R; Δ*cltA*::*sacB*-ΩS*53125*; *E. coli* assembly[c] of four PCR-generated fragments: (1) DNA downstream of *cltA* (ADP1 template with <u>oACB54</u> & <u>oTCB104</u>)[b], (2) vector DNA (pUC19 template with <u>oACB59</u> & <u>oACB58</u>)[b], (3) DNA upstream of *cltA* (ADP1 template with <u>oTCB105</u> & <u>oACB55</u>)[b] and (4) *sacB*-ΩS (pBAC1548 template with <u>oACB56</u> & <u>oACB57</u>)[b]	This study
pBAC2119	Ap^{R}Km^R; insertion of *gfp$_{sf}$*-ΩK downstream of *tcuC* and deletion of *pacI* in the *tcuC-tcuA* region; *E, coli* assembly[c] of two PCR-generated fragments (1) *tcu* region DNA and vector backbone (pBAC2099 template with <u>oACB90</u> & <u>oACB87</u>)[b], and (2) *gfp$_{sf}$*-ΩK (pBAC2095 template with <u>oACB89</u> & <u>oACB86</u>)[b]	This study
pBAC2152	Ap^{R}Km^R; *tcuC* to *tcuA* region with *gfp$_{sf}$*-ΩK downstream of *tcuC* and *pacI* deleted in a pUC19 backbone; *E. coli* assembly[c] of PCR-generated DNA with pBAC2119 as template using <u>oACB151</u> & <u>oACB152</u> to add a ribosome binding site (RBS) for GFPsf expression.	This study
pBAC2158	Ap^{R}Km^R; *tcuC-gfp$_{sf}$*-ΩK*53222*, Δ*pacI53222*; DNA from *tcuC-tcuA* with deletion of region downstream of *tcuC* including *pacI*; the deleted DNA was replaced with *gfp$_{sf}$*-ΩK DNA (sequence shown in Table S1[e]); *E. coli* assembly[c] of two PCR products using pBAC2152 as template: (1) with o<u>ACB158</u> & <u>oTCB71</u> and (2) with <u>oACB159</u> & <u>oTCB51</u>. This assembly moves the *tcuC* and *gfp$_{sf}$* CDS sufficiently close to create a reporter for P$_{tcuC}$.	This study
pBAC2161	Ap^{R}Km^R; Δ*tcuR51376*; *tcuC-gfp$_{sf}$*-ΩK*53222*, Δ*pacI53222;* Derivative of pBAC2158 in which *tcuR* was deleted by *E. coli* assembly[c] of two PCR products made using pBAC2158 as template: (1) with <u>oACB76</u> & <u>oTCB161</u> and (2) with <u>oACB73</u> & <u>oTCB162</u>	This study
pUI1637	Ap^{R}Km^R; source of ΩK	(4)
pUI1638	Ap^{R}Sp^{R}Sm^R; source of ΩS	(4)
pBTL-2_pcaU_1	Km^R; source of *gfp$_{sf}$*	(5)
pRMJI	Ap^{R}Km^R; source of *sacB*-ΩK	(6)
pUC18/19	Ap^R; cloning vector	(7)
pCR2.1 TOPO	Ap^R, Km^R; cloning vector	Thermo Fisher Scientific

[a]Abbreviations: Ampicillin (Ap), Streptomycin (Sm), Spectinomycin (Sp), Kanamycin (Km), omega cassette conferring Km^R (ΩK) or Sm^{R}Sp^R (ΩS) (4); splicing by overlap extension PCR (SOEing) (8).

[b]Oligos (primers) used for PCR are underlined and shown in Table S4.

[c]*E. coli* assembly refers to the method of Kostylev et al. (9). PCR products for this method were typically generated with PrimeSTAR polymerase.

TABLE S4 Oligos (Primers) used for PCR in this study

Name	Sequence (5'-3')	Use
oACB54	CGGATCGTACCGGGCCTGATCATGCTGTGACTCTGTTTCG	With oTCB104 amplifies *cltA* downstream region from ADP1 template to make pBAC2110
oACB55	CTGTTGCATGGGCCTGCAAACGACTCTCCATCATTTGTCTGT	With oTCB105 amplifies *cltA* upstream region from ADP1 template to make pBAC2110
oACB56	ACAGACAAATGATGGAGAGTCGTTTGCAGGCCCATGCAACAG	With oACB57 amplifies *sacB*-ΩS from pBAC1548 template to make pBAC2110
oACB57	CGAAACAGAGTCACAGCATGATCAGGCCCGGTACGATCCG	With oACB56 amplifies *sacB*-ΩS from pBAC1548 template to make pBAC2110
oACB58	TGTAATACAAAGTCAGCGTATTGATGCTTGGCGTAATCATGGTCATAGC	With oACB59 amplifies vector DNA (pUC19) to make pBAC2110
oACB59	ATGACTATGCTGCTTTTCTTCTGCTGTGCGGTATTTCACACCG	With oACB58 amplifies vector DNA (pUC19) to make pBAC2110
oACB63	TCTACCGGGCACGGCCAGTGAATTCGAG	With oACB64 amplifies vector DNA (pUC19 template); used to make pBAC2095
oACB64	TGCTAGCCATCTCCTTACGCATCTGTGC	With oACB63 amplifies amplifies vector DNA (pUC19 template); used to make pBAC2095
oACB65	GCGTAAGGAGATGGCTAGCAAAGGAGAAG	With oACB66 amplifies *gfp*$_{sf}$ from pBTL-2_pcaU_1 template; used to make pBAC2095
oACB66	GTACCGAGCTTTACCTAGGTGTGAATTCAG	With oACB65 amplifies *gfp*$_{sf}$ from pBTL-2_pcaU_1 template; used to make pBAC2095
oACB67	ACCTAGGTAAAGCTCGGTACGATCCGGTG	With oACB68 amplifies ΩK from pUI1637 template; used to make pBAC2095
oACB68	CACTGGCCGTGCCCGGTAGATCCGGTGATTG	With oACB67 amplifies ΩK from pUI1637 template; used to make pBAC2095
oACB69	CGTAAGGAGAATCCTGACGCAGTTGTTG	With oACB70 amplifies *tcuA* region from ACN1376 or ADP1 to make pBAC2094 or pBAC2098, respectively
oACB70	CACTGGCCGTCGATGGCAAATTTGAGTAAAG	With oACB69 amplifies *tcuA* region from ACN1376 or ADP1 to make pBAC2094 or pBAC2098, respectively
oACB71	TTTGCCATCGACGGCCAGTGAATTCGAG	With oACB72 amplifies vector DNA (pUC19 template); used to make pBAC2094 and pBAC2098
oACB72	GCGTCAGGATTCTCCTTACGCATCTGTG	With oACB71 amplifies vector DNA (pUC19 template); used to make pBAC2094 and pBAC2098
oACB73	CGTAAGGAGACAGACCAAGGAGGTCTTAATG	With oACB74 amplifies *pacI* region from ADP1 to make pBAC2099; with oTCB162 amplifies *gfp*$_{sf}$-ΩK from pBAC2158 to make pBAC2161
oACB74	CACTGGCCGTAGAGCTGAACAACTGGAATTAAG	With oACB73 amplifies *pacI* region from ADP1 to make pBAC2099
oACB75	GTTCAGCTCTACGGCCAGTGAATTCGAG	With oACB76 amplifies the vector DNA (pUC19); used to make pBAC2099
oACB76	CCTTGGTCTGTCTCCTTACGCATCTGTG	With oACB75 amplifies the vector DNA (pUC19); used to make pBAC2099; with oTCB161 amplifies vector region of pBAC2158 to make pBAC2161
oACB77	TCTACCGGGCTAGTACTAAAGCCTTAATTCC	With oACB78 amplifies pUC19 backbone and genes upstream and downstream of *tcuA* from pBAC2094 to make pBAC2102 or from pBAC2098 to make pBAC2109
oACB78	TGCTAGCCATAGATTTCTTCCTTTTTTAGCTC	With oACB77 amplifies pUC19 backbone and genes upstream and downstream of *tcuA* from pBAC2094 to make pBAC2102 or from pBAC2098 to make pBAC2109
oACB79	GAAGAAATCTATGGCTAGCAAAGGAGAAGAACTTTTC	With oACB80 amplifies *gfp*$_{sf}$-ΩK from pBAC2095 to make pBAC2102 and pBAC2109

oACB80	TTTAGTACTAGCCCGGTAGATCCGGTGATTG	With oACB79 amplifies *gfp_{sf}*-ΩK from pBAC2095 to make pBAC2102 and pBAC2109
oACB86	AGAGCTTTTAGCCCGGTAGATCCGGTGATTG	With oACB89 amplifies *gfp_{sf}* -ΩK from pBAC2095 to make pBAC2119
oACB87	TCTACCGGGCTAAAAGCTCTAAAAGGCAG	With oACB90 amplifies pUC19 backbone and genes upstream of downstream of *pacI* from pBAC2099 to make pBAC2119 (
oACB89	GTGAGTTACTATGGCTAGCAAAGGAGAAGAACTTTTC	With oACB86 amplifies *gfp_{sf}* -ΩK from pBAC2095 to make pBAC2119
oACB90	TTGCTAGCCATAGTAACTCACTTGAAGGAC	With oACB87 amplifies pUC19 backbone and genes upstream of downstream of *pacI* from pBAC2099 to make pBAC2119
oACB151	TCCTCCTGAAGGACACAAGAACACAGC	With oACB152 to put a RBS ahead of *gfp_{sf}* in pBAC2119
oACB152	GTCCTTCAGGAGGACAGCTATGGCTAGCAAAGGAGAAGAACTTTTC	With oACB151 to put a RBS ahead of *gfp_{sf}* in pBAC2119
oACB158	ATAGCTGTCCTCCTGAAGGAAATGATGCTGACGTGTTAGAC	With oTCB71 amplifies backbone and flanking DNA from pBAC2152 to make pBAC2158
oACB159	GTCTAACACGTCAGCATCATTTCCTTCAGGAGGACAGCTATG	With oTCB51 amplifies *gfp_{sf}*-ΩK from pBAC2152 to make pBAC2158
oMTV152	GAGTCAGAGCTCCGCAAACACAGGTGGCA	With oMTV155 amplifies DNA downstream of *tcuR*; used to make pBAC1120; SacI site for cloning into pUC18.
oMTV153	GATGATCTGCAGTCTCAAGCCTGTATTTATGCC	With oMTV154 amplifies DNA upstream of *tcuR*; used to make pBAC1120; PstI site for cloning into pUC18.
oMTV154	GTAAAAATAAATACAAATGGCTTACTCGAGCACGGCAAATACTACATTGTC	With oMTV153 amplifies DNA upstream of *tcuR*; used to make pBAC1120; XhoI site introduced
oMTV155	GACAATGTAGTATTTGCCGTGCTCGAGTAAGCCATTTGTATTTATTTTTAC	With oMTV152 amplifies DNAdownstream of *tcuR*; used to make pBAC1120; XhoI site introduced.
oMTV158	GAGTCAGAGCTCGCGTGCTAAAAATTTCTTGGTCGT	*tclR* region; used with oMTV161 to make pBAC2093; SacI site for cloning into pUC18
oMTV159	GATGATCTGCAGTACACCAGTATTTGGGCATATCCT	*tclR* region; used with oMTV160 to make pBAC2093; PstI site for cloning into pUC18
oMTV160	GTATTTAAAAAAATTAATGACCCTTTACTCGAGGGTTGAAAAATATTTGATTTGTTTGATTTCCAT	*tclR* region; used with oMTV159 to make pBAC2093; XhoI site introduced
oMTV161	ATGGAAATCAAACAAATCAAATATTTTTCAACCCTCGAGTAAAGGGTCATTAATTTTTTTAAATAC	*tclR* region; used with oMTV158 to make pBAC2093; XhoI site introduced
oNSL40	CAGACCTTTACGACGTCCC	With oNSL46 amplifies *tclR*; put in pCR2.1 TOPO vector for pBAC1164 (*tclR51445*) and pBAC1863 (*tclR51556*)
oNSL46	TTCCTGAGTTACGTTATCTGC	With oNSL40 amplifies *tclR*; put in pCR2.1 TOPO vector for pBAC1164 (*tclR51445*) and pBAC1863 (*tclR51556*)
oST45	CATGTGGACGTCGCTGTATAGCCCATTGTGGA	*pacI* region; used with oST46 to make pBAC1137. AatII site for cloning into pUC19.
oST46	TGTCCTTCAAGTGAGTTACTATGGGATCCGGCGCCTAAAAGCTCTAAAAGGCAGCAT	*pacI* region; used with oST45 to make pBAC1137. BamHI and KasI sites introduced.
oST47	ATGCTGCCTTTTAGAGCTTTTAGGCGCCGGATCCCATAGTAACTCACTTGAAGGACA	*pacI* region; used with oST48 to make pBAC1137. BamHI and KasI sites introduced.
oST48	ATAACTGTCGACACCTATGGGTGGCTTGCT	*pacI* region; used with oST46 to make pBAC1137. SalI site for cloning into pUC19.
oST49	CATGTGGACGTCATTCGACCGGTGGAAAGCTTG	*tcuC* region; used with oST50 to make pBAC1138. AatII site for cloning into pUC19.
oST50	TTTCAGACCAAGGAGGTCTTAATGGGATCCGGCGCCTAACACGTCAGCATCATTTG	*tcuC* region; used with oST49 to make pBAC1138. BamHI and KasI sites introduced.
oST51	CAAATGATGCTGACGTGTTAGGCGCCGGATCCCATTAAGACCTCCTTGGTCTGAAA	*tcuC* region; used with oST52 to make pBAC1138. BamHI and KasI sites introduced.

oST52	`GATCTCGTCGACGGTGTATGAGATTGATGGCC`	*tcuC* region; used with oST51 to make pBAC1138. SalI site for cloning into pUC19.
oST63	`TGAAGCATTTATCAGGGTTATTGTCTCATGAGCG`	Used with primer M13R to generate DNA ofr transformation to make ACN1419
oTCB5	`CTTTAACCAAGGGCTGAATG`	In *tclR* for RACE
oTCB6	`GCTGCCTTGCATTAACTAAAG`	In *tcuR* for RACE
oTCB8	`ACAAGTACAACTGGCGCTTAGG`	In *tcuC* for RACE
oTCB44	`CCATTTTTCAGACCAAGG`	Upstream of *tcuC*; used with oTCB113 for cDNA evaluation
oTCB45	`GATAAGTCGTTTGAACTGG`	Upstream of *tcuC*; used with oTCB113 for cDNA evaluation
oTCB51	`TAAGCGCCAGTTGTACTTGT`	With oACB159 amplifies *gfp$_{sf}$*-ΩK from pBAC2152 to make pBAC2158
oTCB55	`TGCCTTTTAAAGCTACATTAG`	Upstream of *tcuA*; used with oTCB135 for cDNA evaluation
oTCB56	`GCTAAAAAAGGAAGAAATCTATGC`	Upstream of *tcuA*; used with oTCB135 for cDNA evaluation
oTCB57	`ATCAGACCTTTACGACGTC`	In *tcuA* for RACE
oTCB71	`ACAAGTACAACTGGCGCTTAGG`	With oACB158 amplifies backbone and flanking DNA from pBAC2152 to make pBAC2158
oTCB104	`GCAGAAGAAAAGCAGCATAG`	With oACB54 amplifies *cltA* downstream region from ADP1 to make pBAC2110
oTCB105	`CATCAATACGCTGACTTTGT`	With oACB55 amplifies *cltA* upstream region from ADP1 template to make pBAC2110
oTCB113	`TGACCAACCAGCTTAAAAC`	In *tcuC*; used with oTCB44, oTCB45, oTCB144, and oTCB145 for cDNA evaluation
oTCB135	`TCGCTTTTGGGATGTTTAC`	In *tcuB* for 5' RACE
oTCB137	`TGTGGATCGACATACCC`	In *pacI* for 5' RACE
oTCB144	`CCTGCCACATGATAAATGAATTG`	Upstream of *tcuC*; used with oTCB113 for cDNA evaluation
oTCB145	`ACTCCTTTTCTGTTTTTCCTCG`	Upstream of *tcuC*; used with oTCB113 for cDNA evaluation
oTCB154	`AGCTTAAGGTTTTTGCATCTAGC`	In *tcuA*; used with oTCB156, oTCB56, oTB55, and oTCB155 for cDNA evaluation
oTCB155	`ACACCCATTCAAGCTGAGC`	Upstream of *tcuA*; used with oTCB135 for cDNA evaluation
oTCB156	`GCATGATGTGATTGTCATTGG`	Upstream of *tcuA*; used with oTCB135 for cDNA evaluation
oTCB161	`GGACAATGTAGTATTTGCCGTGGAACTCTAAGCCATTTGTATTTATTTTTACTAAACACC`	With oACB76 amplifies pUC19 backbone from pBAC2158 to make pBAC2161
oTCB162	`GGTGTTTAGTAAAAATAAATACAAATGGCTTAGAGTTCCACGGCAAATACTACATTGTCC`	With oACB73 amplifies *gfp$_{sf}$*-ΩK from pBAC2158 to make pBAC2161
M13R	`AGCGGATAACAATTTCACACAGG`	Universal reverse primer for sequencing from pUC18/19 vectors; used with oST63 for constructing ACN1419

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FIG S1 Aligned sequences of PacI from ADP1 (top line), aconitate isomerase from *Pseudomonas* sp. WU-0701 (second from bottom), and similar proteins, including those corresponding to products of *pacI*-like genes, shown in pink in Fig. 2. The protein from WU-0701 has been biochemically characterized (10, 11). It differs by one amino acid from an uncharacterized protein in another *Pseudomonas* strain, marked by the red rectangle. This enzyme is presumed to be an aconitate isomerase. Identical and similar residues in 6 or more aligned sequences are highlighted in blue and grey, respectively. Locus tags are shown adjacent to the bacterial names. Database protein identifiers (from NCBI), in the order from top to bottom, are WP_004925208 (PacI from ADP1), WP_245636122, WP_012356708, ABS67519, WP_035529914, WP_159877488, BAP90747 (aconitate isomerase), and WP_059315091.
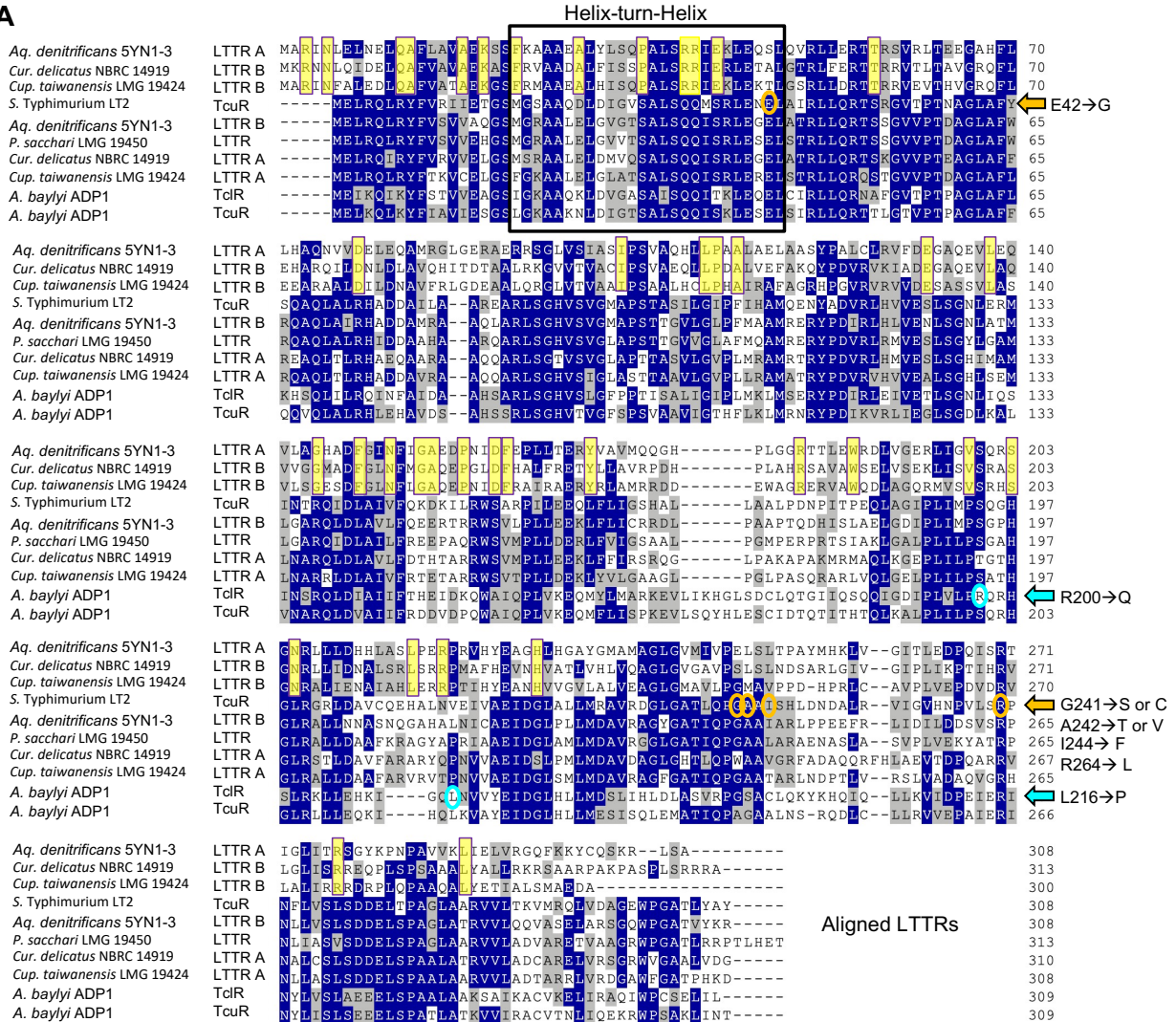
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FIG S2 Aligned sequences of LTTRs, corresponding to the genes shown in Fig. 2. (A) Identical and similar aligned residues that are in at least 5 sequences are highlighted in blue and grey, respectively. The top three sequences, which correspond to the striped genes in Fig. 2, are more like each other than to the rest of the sequences (Fig. S3). Yellow boxes highlight residues that are identical in only these three sequences. Orange circles and arrows indicate the positions of amino acid replacements in TcuR variants in LT2 that activate transcription of the *tcuABC* operon without requiring Tcb (12). Turquoise circles and arrows indicate the positions of amino acid replacements in TclR variants in ADP1 that are more responsive to Tcb than TclR at both P_{tcuC} and P_{tcuA}. (B) The helix-turn-helix (HTH) region of the proteins, identified by alignments with known structures, are similar for TcuR and TclR of ADP1. Protein identifiers, in the NCBI

database, follow, with bold numbers corresponding to those in the phylogram shown in Fig. S3 below: *A. baylyi* ADP1 TcuR (WP_004925211.1, **10**) and TclR (WP_004925203.1, **9**); *Aquitalea denitrificans* LTTR A (WP_159877486.1, **1**) and LTTR B (WP_159877492.1, **5**), *Cupriavidus taiwanensis* LTTR A (WP_012356032.1, **8**) and LTTR B (WP_012356709.1, **3**), *Curvibacter delicatus* LTTR A (WP_066705128.1, **7**) and LTTR B (WP_066705121.1, **2**), *Paraburkholderia sacchari* LTTR (WP_035529905.1, **6**), *S. enterica* LT2 TcuR (NP_459677.1, **4**).
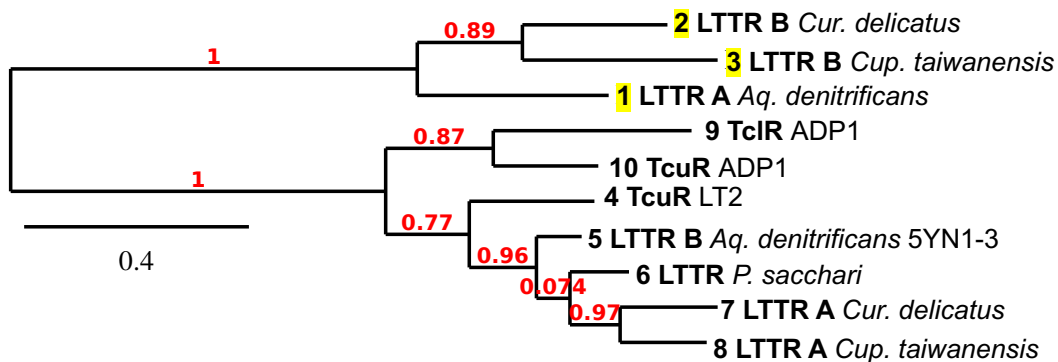

FIG S3 Phylogenetic tree (phylogram) generated from the sequences of LTTRs shown in Fig. S2. The labeled leaves (tips) indicate proteins that correspond to LTTR proteins in Fig. 2 and Fig. S2. Numbers 1-10 represent sequences from top to bottom in the alignment of Fig. S2. The tree was generated with default parameters at www.phylogeny.fr for the "one click" analysis (13). The scale marks the distance corresponding to a 0.4 (40%) genetic variation. Red text displays the branch support values. This phylogram supports the conclusion that protein sequences 1-3 (highlighted in yellow) group together by sequence similarity and are more closely related to each other than to the other regulators. There are two main branches: one leading to sequences (1-3) and the other for the remaining sequences (4-10). These two main branches have support values of 1 (100%).
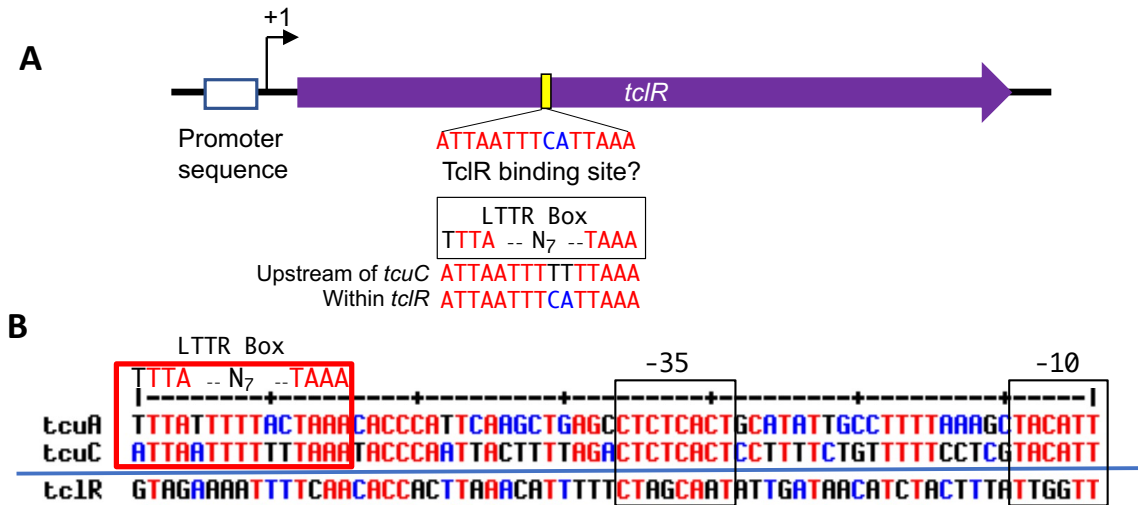
FIG S4 Transcription of *tclR*. (A) Diagram, drawn to scale, of *tclR* chromosomal region. The position of the transcriptional initiation site (+1) is indicated relative to the position of the *tclR* coding sequence (purple arrow). The yellow rectangle indicates the position of a sequence that matches the LTTR box, which is predicted to bind TclR upstream of *tcuC*. The rectangle upstream of the +1 site indicates the position of the promoter sequence shown in the lower panel (bottom line). (B) Alignment of three promoter regions for *tcuA*, *tcuC*, and *tclR* upstream of the known +1 sites for each gene. There is similarity among all three sequences in the vicinity of the promoter (-35 and -10 regions). The LTTR Box sequence that is conserved in both *tcuA* and *tcuC* (TTA-N7-TAAA), located at position approximately -63, is not observed for *tclR*.

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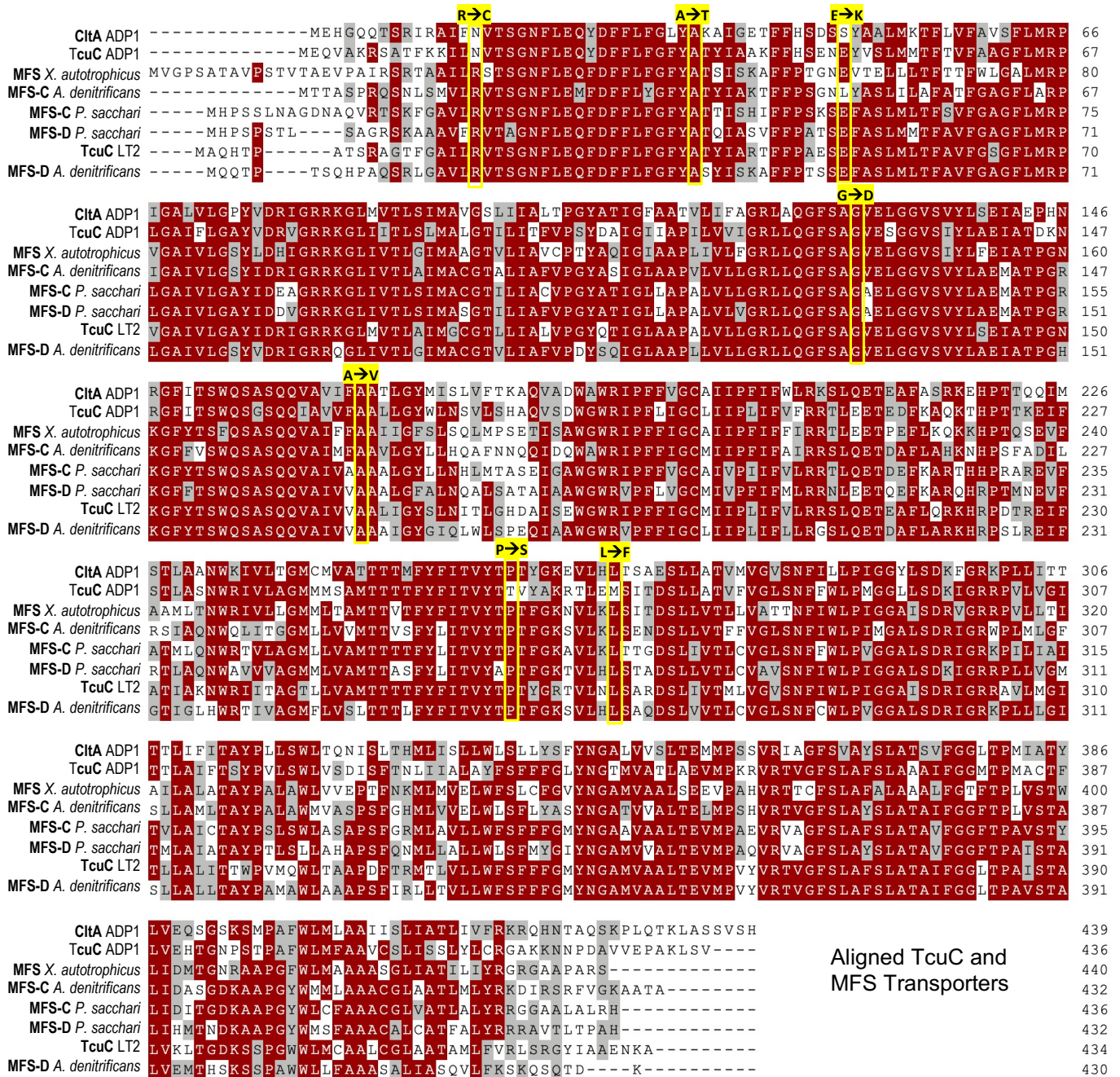

FIG S5 Alignment of MFS member transporters. Proteins from top to bottom in the alignment correspond to (NCBI protein identifier): **CltA**, locus tag ACIAD_RS01830, from ADP1 (WP_004920407.1); **TcuC**, locus tag ACIAD_RS07100, from ADP1 (WP_004925205); **MFS** *X. autotrophicus* (WP_012114294.1); **MFS-C** *A. denitrificans* (WP_159877490.1); **MFS-C** *P. sacchari* (WP_035529909.1); **MFS-D** *P. sacchari* (WP_035529912.1); **TcuC**, locus tag STM0689, from LT2 (WP_000057014.1); and **MFS-D** *A. denitrificans* (WP_159877498.1). TcuC of LT2 was originally designated CitA because of its ability to transport citrate. The seven amino acid replacements indicated above in TcuC (previously called CitA) enable growth on isocitrate presumably because of altered specificity of transport (14).
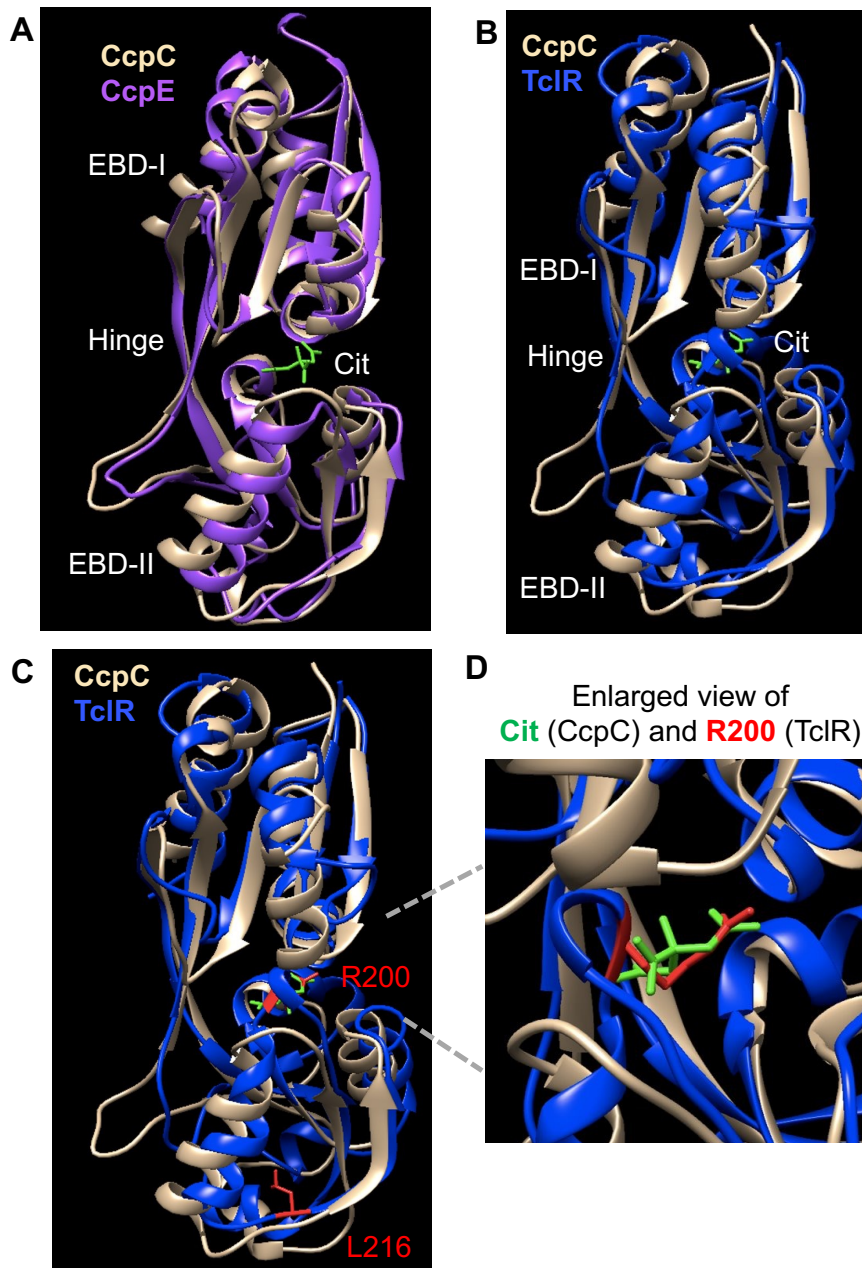
FIG S6 Comparisons of the structures of the Effector-Binding Domains (EBDs) of two citrate (Cit)-responsive LTTRs: CcpE (15) and CcpC (16). (A) structures of CcpC-EBD (tan, PDB identifier 7DMW) and CcpE (purple, PDB identifier 4QBA) are superimposed. The effector, Cit, is in a typical effector-binding pocket between the two subdomains (EBD-I and EBD-II). When the effector binds, it brings these subdomains closer together with movement accommodated by two beta-strands that form a hinge-like connector. (B) Model of the TclR-EBD, generated using the Phyre2 prediction software (17), aligned with the Cit-bound CcpC structure. (C) Alignment as in panel B, with two residues of TclR highlighted in red, R200 and L216. These residues when replaced with Q and P, respectively, resulted in TclR variants with increased responsiveness to Tcb. (D) Enlarged view of the effector binding-site of panel C showing that R200 of TclR is predicted to reside in the same location as Cit in the CcpC structure.
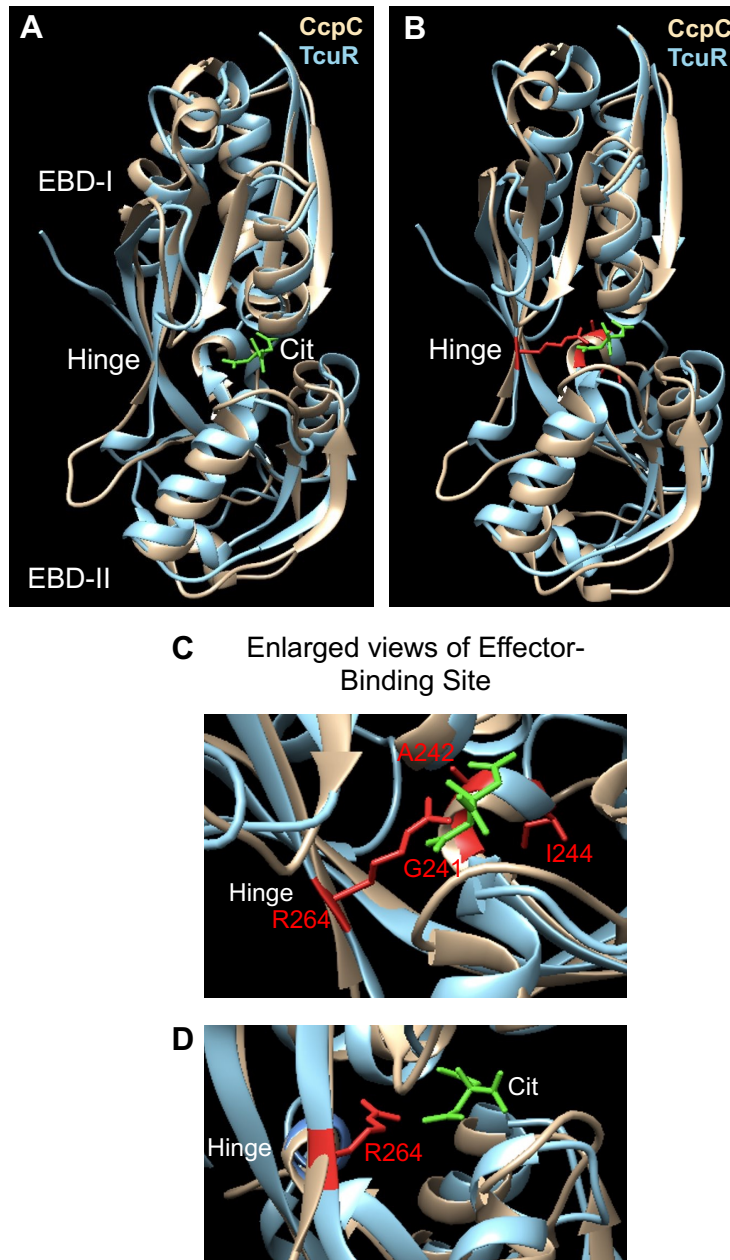
FIG S7 Comparisons of (A) the structure of the Effector-Binding Domains (EBDs) of a citrate (Cit)-responsive LTTR, CcpC (tan, PDB identifier 7DMW) (16), and a model of the TcuR-EBD of LT2, from the AlphaFold protein structure database (identifier AF-Q8ZQX2-F1) (18, 19). (B) One residue of TcuR, R264, is highlighted in red. Its replacement by L, resulted in constitutive transcription of the *tcuABC* operon in LT2 (12). In the wild-type protein, this residue is situated in the center of one of the two beta-strands that form the hinge-like connection between the EBD subunits. Enlarged views of the effector-binding pockets in (C) and (D) show that the location of the effector in the Cit-bound CcpC structure is in the vicinity of R264 and other residues highlighted in red (G241, A242, and I244). These are positions where individual amino acid replacements resulted in constitutively active TcuR variants (12).

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