Regulation of tricarboxylate transport and metabolism in Acinetobacter baylyi ADP1

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
	TcuR					
Salmonella enterica Typhimurium LT2 ^b	STM0692	44 (67) %	42 (65) %			
	TcuA					
	STM0691			75 (85) %		
	TcuB					
	STM0690				58 (73) %	
	TcuC					
	STM0689					60 (74) %

^aValues 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).

^bLocus tags, in bold, correspond to genes in reference genomes: NC_005966 (ADP1) and NC_003197 (LT2).

Strain	Relevant Characteristics ^{a, b}	Source
ADP1 ^c	Wild-type strain (BD413)	(2, 3)
ACN1373	Δ <i>tcuR::sacB</i> -ΩK <i>51373</i> sacB-ΩK from pRMJI replaces <i>tcuR</i> (1,540,519-1,541,442) ^d pBAC1122/AatII x ADP1	This study
ACN1376	$\Delta t cuR51376$ Deletion of $t cuR$ (1,540,519-1,541,442) ^d pBAC1120/AatII x ACN1373	This study
ACN1385	Δ <i>pacI::sacB</i> -ΩK51385 sacB-ΩK from pRMJ1 replaces <i>pacI</i> (1,541,580-1,542,368) ^d pBAC1142/AatII & XmnI x ADP1	This study
ACN1388	Δ <i>pacI51388</i> Deletion of <i>pacI</i> (1,541,580-1,542,368) ^d pBAC1137/AatII & XmnI x ACN1385	This study
ACN1419	$\Delta tcuC$::sacB- Ω K51419 sacB- Ω K from pRMJ1 replaces tcuC (1,542,464-1,543,768) ^d PCR product (pBAC1141 template with primers oST63 & M13R) x ADP1	This study
ACN1445	$\Delta tcuR51376$, tclR51445 $\Delta tcuR$ as in ACN1376, tclR mutations (C \rightarrow T 1,544,371 and A \rightarrow G 1,544,323) ^d ACN1376-derived Tcb ⁺ spontaneous mutant, TclR(R200Q, L216P)	This study
ACN1456	$\Delta tcuC51456$ Deletion of <i>tcuC</i> (1,542,464-1,543,768) ^d pBAC1138/BspHI & SphI x ACN1419	This study
ACN1556	$\Delta tcuR51376$, tclR51556 $\Delta tcuR$ as in ACN1376, tclR (C \rightarrow T 1,544,371) ^d ACN1376-derived Tcb ⁺ spontaneous mutant, TclR(R200Q)	This study
ACN3122	Δ <i>tcuR51376</i> , Δ <i>tcuA</i> :: <i>gfp</i> _{sf} -ΩK53122 Δ <i>tcuR</i> as in ACN1376, <i>gfp</i> _{sf} -ΩK replaces <i>tcuA</i> (1,539,009-1,540,412) ^{d, e} pBAC2102/BamHI x ACN1376	This study
ACN3123	$\Delta tcuA::gfp_{sf}-\Omega K53122$ $\Delta tcuA$ fusion as in ACN3122 pBAC2109/BsaI x ADP1	This study
ACN3124	Δ <i>tcuR51376, ΔtclR::sacB-</i> ΩK <i>53124</i> sacB-ΩK replaces tclR (1,544,043-1,544,936) ^d pBAC2101/BsaI & SacI x ACN1376	This study
ACN3125	Δ <i>cltA</i> :: <i>sacB</i> -ΩS <i>53125</i> <i>sacB</i> -ΩS replaces <i>cltA</i> (388,259-389,574) ^d pBAC2110/AhdI & AatII x ADP1	This study
ACN3168	$\Delta tcuC51456$, $\Delta cltA$::sacB- Ω S53125 $\Delta tcuC$ as in ACN1456, sacB- Ω S replaces cltA as in ACN3125 pBAC2110/AhdI & AatII x ACN1456	This study

TABLE S2 Acinetobacter baylyi strains derived from ADP1

ACN3171	$\Delta tcuR51376$, $\Delta tclR53171$ $\Delta tcuR$ as in ACN1376, deletion of <i>tclR</i> (1,544,043-1,544,936) ^d pBAC2093/BsaI x ACN3124	This study
ACN3172	Δ <i>tclR</i> :: <i>sacB</i> -ΩK53124 sacB-ΩK replaces <i>tclR</i> as in ACN3124 pBAC2101/BsaI & SacI x ADP1	This study
ACN3173	$\Delta tcuR51376$, $\Delta tclR53171$, $\Delta tcuA$::gfp _{sf} - Ω K53122 $\Delta tcuR$ as in ACN1376, $\Delta tclR$ as in ACN3171, tcuA fusion as in ACN3122 pBAC2102/BamHI x ACN3171	This study
ACN3178	Δ <i>tclR53171</i> Δ <i>tclR</i> as in ACN3171 pBAC2093/BsaI x ACN3172	This study
ACN3179	Δ <i>tcuR51376, tclR51445</i> Δ <i>tcuR</i> as in ACN1376, <i>tclR</i> mutations as in ACN1445 [TclR(R200Q, L216P)] pBAC1164/BsaI x ACN3124	This study
ACN3184	$\Delta tclR53171$, $\Delta tcuA$::gfp _{sf} - Ω K53122 $\Delta tclR$ as in ACN3171, tcuA fusion as in ACN3122 pBAC2109/AatII & SbfI x ACN3178	This study
ACN3187	Δ <i>tcuR51376, tclR51556</i> Δ <i>tcuR</i> as in ACN1376, <i>tclR</i> 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} - Ω 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} - Ω K53222, ΔpacI53222 tcuC fusion and ΔpacI 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

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ACN3227	$\Delta tcuR51376$, tclR51445, tcuC-gfp _{sf} - Ω K53222, $\Delta pacI53222$ $\Delta tcuR$ as in ACN1376, tclR as in ACN1445 [TclR(R200Q, L216P)], tcuC fusion and $\Delta pacI$ as in ACN3222 pBAC2161/BsaI & NdeI x ACN3179	This study
ACN3228	$\Delta tcuR51376$, $tclR51556$, $tcuC$ - gfp_{sf} - $\Omega K53222$, $\Delta pacI53222$ $\Delta tcuR$ as in ACN1376, $tclR$ as in ACN1556 [TclR(R200Q)], $tcuC$ fusion and $\Delta pacI$ 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 (Ω K), omega cassette conferring Sm^RSp^R (Ω S).

^{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 $\Delta tcuA$:: gfp_{sf} - $\Omega K53122$ 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} - $\Omega K53222$ 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} - $\Omega K53222$ is in bold. The gene encoding Km^R (reverse complement) is underlined.

${\tt Tccttca} {\tt ggagga} {\tt cagct} {\tt ATGGCTAGCAAAAGGAGAAGAACTTTTCACGGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAACTTTTCACGGCAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAACTTTTCACGGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAACTTTTCACGGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAACTTTTCACGGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAACTTTTCACGGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAACTTTTCACGGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAACTTTTCACGGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAACTTTTCACGGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTGTGATGTTAATGGGCACAACTTTTCACGGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTGATGTTAATGGGCACAACTTTTCACGGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTGTGTG$
AATTTTCTGTCCGTGGAGAGGGTGAAGGTGATGCTACAAACGGAAAACTCACCCTTAAATTTATTT
AACACTTGTCACTACTCTGACCTATGGTGTTCAATGCTTTTCCCGTTATCCGGATCACATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAA
ggttatgtacaggaacgcactatatctttcaaagatgacgggacctacaagacgcgtgctgaagtcaagtttgaaggtgatacccttgttaatcgta
${\tt TCGAGTTAAAGGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAACTCGAGTACAACTTTAACTCACACAATGTATACATCACGGCAGA$
caaacaaaaaaatggaatcaaagctaacttcaaaattcgccacaacgttgaagatggttccgttcaactagcagaccattatcaacaaaatactcca
ATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAATCTGTCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCC
${\tt TTCTTGAGTTTGTAACTGCTGCGGATTACACATGGCATGGATGAGCTCTACAAAggtggcggttctgaattcaccacctaggtaaagctcggtacg$
a the constant of the const
qtaatataatctattaaaggtcattcaaaaggtcatccaccggatcaattcccctgctcgcgcaggctgggtgccaagctctcgggtaacatcaagg
cccgatccttggagcccttgccctcccgcacgatgatcgtgccgtgatcgaaatccagatccttgacccgcagttgcaaaccctcactgatccgtcg
accaaagcggccatcgtgcctccccactcctgcagttcgggggcatggatgcgcggatagccgctgctggtttcctggatgccgacggatttgcact
gccggtagaactccgcgaggtcgtccagcctcaggcagcagctgaaccaactcgcgaggggatcgagccggggtgggcgaagaactccagcatgag
atcccccgcgctggaggatcatccagccggcgtcccggaaaacgattccgaagcccaacctttcatagaaggcggcggtggaatcgaaatctcgtgat
ggcaggttgggcgtcgcttggtcggtcatttcgaaccccagagtcccgctcagaagaactcgtcaagaaggcgatagaaggcgatgcgctgcgaatc
gggagcggcgataccgtaaagcaccgaggaagcggtcagcccattcgccgccaagctcttcagcaatatcacgggtagccaacgctatgtcctgatag
cggtccgccacaccccagccggccacagtcgatgaatccagaaaagcggccattttccaccatgatattcggcaagcaggcatcgccatgggtcacga
cgagatcctcgccgtcgggcatgcgcgccttgagcctggcgaacagttcggctggcgcgagcccctgatgctcttcgtccagatcatcctgatcgaccagatcatcatcctgatcgaccagatcatcatcatcctgatcgaccagatcatcatcatcatcatcatcatcatcatcatcatcatca
aagaccggcttccatccgagtacgtgctcgctcgatgcgatgtttcgcttggtggtcgaatgggcaggtagccggatcaagcgtatgcagccgccgc
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ccgcttcagtgacaacgtcgagcacagctgcgcaaggaacgcccgtcgtggccagcca
accggacaggtcggtcttgacaaaaagaaccgggcgcccctgcgctgacagccggaacacggcggcatcagagcagccgattgtctgttgtgcccag
t catage cga at a get c t c t c c a c c c a a geg g c c g g a g a a c c t g c t c a t c c t g t t c a a t c a t c c t g t c c t c t c t c t c t c t c
cagatettgateccetgegecateagateettggeggeaagaaagecateeagtttaetttgeagggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagagggegeeceeagettaettgategeggetteeeaacettaeceagettaettgategeggegeeceeagettaettgategeggetteeeaacettaeceagettaettgategeggegeeceeagettaettgategeggegetteeeaacettaeceagettaeceagettaettgategeggeggetteeeaacettaeceagettgategeggetteeeaacettaeceagett
${\tt tggcaattccggttcgcttgctgtccataaaaccgcccagtctagctatcgccatgtaagcccactgcaagctacctgctttctctttgcgcttgcg$
$\tt ttttcccttgtccagatagcccagtagctgacattcatccggggtcagcaccgtttctgcggactggctttctacgtgttccgcttcctttagcagcagcagcagcagcagcagcagcagcagcagcagca$
$\verb ccttgcgccctgagtgcttgcggcagcgtgaagctcgcgcagatcagttggaagaatttgtccactacgtgaaaggcgagatcaccaaggtagtcgg $
${\tt caaataatgtctaacaattcgttcaagccgacgccgcttcgcggcgcggcttaactcaagcgttagatgcactaagcacataattgctcacagccaa$
actatcaggtcaagtctgcttttattatttttaagcgtgcataataagccctacacaaattggggagatatatcatgaaaggctggct
atcgcaatagttggcgaagtaatcgcaacatccgcattaaaatctagcgagggctttactaagctgatccggtggatgaccttttgaatgaccttta
atagattatattactaattaattggggaccctagaggtccccttttttattttaaaaattttttcacaaaacggtttacaagcataaagcttgctca
atcaatcaccggatctaccgggc

Table S3 Plasmids used in this study

Plasmid	Relevant Characteristics ^a	Source
pBAC1120	Ap ^R Km ^R ; $\Delta tcuR51376$; SOEing of two PCR products: one upstream of $tcuR$ (using <u>oMTV153</u> & <u>oMTV154</u>) ^b , the other downstream of $tcuR$ (using <u>oMTV155</u> & <u>oMTV152</u>). ^b XhoI site replaces $tcuR$ CDS. DNA ligated into pUC18 (SacI and PstI digested).	This study
pBAC1122	Ap ^R Km ^R ; $\Delta tcuR$:: <i>sacB</i> - Ω K <i>51373</i> ; <i>sacB</i> - Ω K from pRMJI digested with SalI and inserted in XhoI site of pBAC1120	This study
pBAC1137	Ap ^R ; $\Delta pacI51388$; SOEing of two PCR products; one upstream of <i>pacI</i> (using <u>oST47</u> & <u>oST48</u>) ^b and the other downstream of <i>pacI</i> (using <u>oST45</u> & <u>oST46</u>). ^b BamHI and KasI sites replace <i>pacI</i> CDS. PCR product digested and ligated in pUC19.	This study
pBAC1138	Ap ^R ; $\Delta tcuC51456$; SOEing of two PCR products: one upstream of $tcuC$ (using <u>oST51</u> & <u>oST52</u>) ^b and the other downstream of $tcuC$ (PCR with <u>oST49</u> & <u>oST50</u>). ^b BamHI and KasI sites replace $tcuC$ CDS. DNA inserted in pUC19.	This study
pBAC1141	Ap ^R Km ^R ; $\Delta tcuC$:: <i>sacB</i> - Ω K <i>51419</i> ; <i>sacB</i> - Ω K from pRMJI digested with BamHI inserted in BamHI site of pBAC1138.	This study
pBAC1142	Ap ^R Km ^R ; $\Delta pacI::sacB-\Omega K51385$; sacB- Ω K from pRMJI digested with BamHI inserted in BamHI site of pBAC1137.	This study
pBAC1164	Ap ^R Km ^R ; <i>tclR51445</i> ; ACN1445 sequence surrounding <i>tclR</i> , encoding TclR(R200Q, L216P), PCR product (with <u>oNSL40</u> & <u>oNSL46</u>) ^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 <i>sacB</i> -ΩS; ΩS from pUI1638 digested with Eco53KI inserted between Eco53KI and EcoRV of pBAC1539	This study
pBAC1863	Ap ^R Km ^R ; <i>tclR51556</i> ; ACN1556 sequence surrounding <i>tclR</i> , encoding TclR(R200Q), ^b PCR product (with oNSL40 & oNSL46) ^b cloned into pCR2.1 TOPO vector	This study
pBAC2093	Ap ^R ; $\Delta tclR53171$; SOEing of two PCR products: one upstream of $tclR$ (using <u>oMTV158</u> & <u>oMTV161</u>) ^b and the other downstream of $tclR$ (using <u>oMTV160</u> & <u>oMTV159</u>). ^b XhoI site replaces $tclR$ CDS. DNA inserted in pUC18.	This study
pBAC2094	Ap ^R ; <i>tcuA</i> region $\Delta tcuR51376$ in pUC19; <i>E. coli</i> assembly ^c of two PCR-generated fragments: (1) <i>tcuA</i> region surrounding $\Delta tcuR51376$ (ACN1376 template with <u>oACB69</u> & <u>oACB70</u>) ^b and (2) vector DNA (pUC19 template with oACB71 & oACB72). ^b	This study
pBAC2095	Ap ^R Km ^R ; Ω K downstream of <i>gfp_{sf}</i> , <i>E. coli</i> assembly ^c of three PCR-generated fragments (1) <i>gfp_{sf}</i> (pBTL-2_pcaU_1 template with <u>oACB65</u> & <u>oACB66</u>) ^b , (2) Ω K (pUI1637 template with <u>oACB67</u> & <u>oACB68</u>) ^b , and (3) vector (pUC19 template with <u>oACB63</u> & <u>oACB64</u>) ^b . Used to make <i>gfp_{sf}</i> fusion plasmids pBAC2119, pBAC2109, and pBAC2102.	This study
pBAC2098	Ap ^R ; <i>tcuA</i> region in pUC19; <i>E. coli</i> assembly ^c of two PCR-generated fragments: (1) <i>tcuA</i> region (ADP1 template with <u>oACB69</u> & <u>oACB70</u>) ^b , and (2) vector DNA (pUC19 template with <u>oACB71</u> & <u>oACB72</u>) ^b	This study
pBAC2099	Ap ^R ; <i>tcuC-tcuA</i> DNA in pUC19; <i>E. coli</i> assembly ^c of two PCR products: (1) <i>tcuC-tcuA</i> region (ADP1 template with <u>oACB73</u> & <u>oACB74</u>) ^b , and (2) vector DNA (pUC19 with <u>oACB75</u> & <u>oACB76</u>) ^b	This study
pBAC2101	Ap ^R Km ^R ; Δ <i>tclR</i> :: <i>sacB</i> -ΩK53124; <i>sacB</i> -ΩK from pRMJI digested with SalI and inserted in XhoI site of pBAC2093	This study

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pBAC2102	Ap ^R Km ^R ; $\Delta tcuA$::gfp _{sf} - Ω K53122; E. coli assembly ^c of two PCR-generated fragments: (1) gfp _{sf} - Ω K (from pBAC2095 template with <u>oACB79</u> and <u>oACB80</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 ; $\Delta tcuA$::gfp _{sf} - Ω K53122; E. coli assembly ^c of two PCR-generated fragments: (1) gfp _{sf} - Ω K (from pBAC2095 template with <u>oACB79</u> & o <u>ACB80</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 ; $\Delta cltA$::sacB- Ω S53125; E. coli assembly ^c of four PCR-generated fragments: (1) DNA downstream of <i>cltA</i> (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 <i>cltA</i> (ADP1 template with <u>oTCB105</u> & <u>oACB55</u>) ^b and (4) sacB- Ω S (pBAC1548 template with <u>oACB56</u> & <u>oACB57</u>) ^b	This study
pBAC2119	Ap ^R Km ^R ; insertion of gfp_{sf} - Ω K downstream of <i>tcuC</i> and deletion of <i>pacI</i> in the <i>tcuC-tcuA</i> region; <i>E</i> , <i>coli</i> assembly ^c of two PCR-generated fragments (1) <i>tcu</i> region DNA and vector backbone (pBAC2099 template with <u>oACB90</u> & <u>oACB87</u>) ^b , and (2) gfp_{sf} - Ω K (pBAC2095 template with <u>oACB89</u> & <u>oACB86</u>) ^b	This study
pBAC2152	Ap ^R Km ^R ; <i>tcuC</i> to <i>tcuA</i> region with gfp_{sf} - Ω K downstream of <i>tcuC</i> and <i>pacI</i> deleted in a pUC19 backbone; <i>E. coli</i> 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 ; <i>tcuC-gfp_{sf}</i> - Ω K53222, Δ pac153222; DNA from <i>tcuC-tcuA</i> with deletion of region downstream of <i>tcuC</i> including <i>pacI</i> ; the deleted DNA was replaced with <i>gfp_{sf}</i> - Ω K DNA (sequence shown in Table S1 ^e); <i>E. coli</i> assembly ^e of two PCR products using pBAC2152 as template: (1) with o <u>ACB158</u> & o <u>TCB71</u> and (2) with o <u>ACB159</u> & o <u>TCB51</u> . This assembly moves the <i>tcuC</i> and <i>gfp_{sf}</i> -CDS sufficiently close to create a reporter for P _{tcuC} .	This study
pBAC2161	Ap ^R Km ^R ; $\Delta tcuR51376$; $tcuC$ -gfp _{sf} - Ω K53222, $\Delta pac153222$; Derivative of pBAC2158 in which $tcuR$ was deleted by <i>E. coli</i> assembly ^c of two PCR products made using pBAC2158 as template: (1) with $\underline{OACB76} \& \underline{OTCB161}$ and (2) with $\underline{OACB73} \& \underline{OTCB162}$	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 <i>sacB</i> - ΩK	(6)
pUC18/19	Ap ^R ; cloning vector	(7)
pCR2.1 TOPO	Ap ^R , Km ^R ; cloning vector	Thermo Fisher Scientific

^aAbbreviations: Ampicillin (Ap), Streptomycin (Sm), Spectinomycin (Sp), Kanamycin (Km), omega cassette conferring Km^{R} (Ω K) or Sm^{R} Sp^R (Ω S) (4); splicing by overlap extension PCR (SOEing) (8).

^bOligos (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.

Name Sequence (5'-3') Use CGGATCGTACCGGGCCTGATCATGCTGT oACB54 With oTCB104 amplifies *cltA* downstream region from ADP1 GACTCTGTTTCG template to make pBAC2110 CTGTTGCATGGGCCTGCAAACGACTCTC oACB55 With oTCB105 amplifies *cltA* upstream region from ADP1 CATCATTTGTCTGT template to make pBAC2110 ACAGACAAATGATGGAGAGTCGTTTGCA With oACB57 amplifies *sacB*- Ω S from pBAC1548 template to oACB56 GGCCCATGCAACAG make pBAC2110 CGAAACAGAGTCACAGCATGATCAGGCC With oACB56 amplifies $sacB-\Omega S$ from pBAC1548 template to oACB57 CGGTACGATCCG make pBAC2110 TGTAATACAAAGTCAGCGTATTGATGCT With oACB59 amplifies vector DNA (pUC19) to make oACB58 TGGCGTAATCATGGTCATAGC pBAC2110 ATGACTATGCTGCTTTTCTTCTGCTGTG With oACB58 amplifies vector DNA (pUC19) to make oACB59 CGGTATTTCACACCG pBAC2110 TCTACCGGGCACGGCCAGTGAATTCGAG oACB63 With oACB64 amplifies vector DNA (pUC19 template); used to make pBAC2095 TGCTAGCCATCTCCTTACGCATCTGTGC With oACB63 amplifies amplifies vector DNA (pUC19 oACB64 template); used to make pBAC2095 oACB65 GCGTAAGGAGATGGCTAGCAAAGGAGAA With oACB66 amplifies *gfp*_{sf} from pBTL-2 pcaU 1 template; used to make pBAC2095 GTACCGAGCTTTACCTAGGTGTGAATTC With oACB65 amplifies gfp_{sf} from pBTL-2 pcaU 1 template; oACB66 AG used to make pBAC2095 ACCTAGGTAAAGCTCGGTACGATCCGGT With oACB68 amplifies ΩK from pUI1637 template; used to oACB67 G make pBAC2095 CACTGGCCGTGCCCGGTAGATCCGGTGA With oACB67 amplifies ΩK from pUI1637 template; used to oACB68 TTG make pBAC2095 CGTAAGGAGAATCCTGACGCAGTTGTTG oACB69 With oACB70 amplifies *tcuA* region from ACN1376 or ADP1 to make pBAC2094 or pBAC2098, respectively CACTGGCCGTCGATGGCAAATTTGAGTA oACB70 With oACB69 amplifies tcuA region from ACN1376 or ADP1 to AAG make pBAC2094 or pBAC2098, respectively TTTGCCATCGACGGCCAGTGAATTCGAG oACB71 With oACB72 amplifies vector DNA (pUC19 template); used to make pBAC2094 and pBAC2098 GCGTCAGGATTCTCCTTACGCATCTGTG With oACB71 amplifies vector DNA (pUC19 template); used to oACB72 make pBAC2094 and pBAC2098 CGTAAGGAGACAGACCAAGGAGGTCTTA oACB73 With oACB74 amplifies pacI region from ADP1 to make ATG pBAC2099; with oTCB162 amplifies *gfpst*-ΩK from pBAC2158 to make pBAC2161 CACTGGCCGTAGAGCTGAACAACTGGAA oACB74 With oACB73 amplifies pacI region from ADP1 to make TTAAG pBAC2099 GTTCAGCTCTACGGCCAGTGAATTCGAG oACB75 With oACB76 amplifies the vector DNA (pUC19); used to make pBAC2099 CCTTGGTCTGTCTCCTTACGCATCTGTG oACB76 With oACB75 amplifies the vector DNA (pUC19); used to make pBAC2099; with oTCB161 amplifies vector region of pBAC2158 to make pBAC2161 TCTACCGGGCTAGTACTAAAGCCTTAAT With oACB78 amplifies pUC19 backbone and genes upstream oACB77 TCC and downstream of *tcuA* from pBAC2094 to make pBAC2102 or from pBAC2098 to make pBAC2109 TGCTAGCCATAGATTTCTTCCTTTTTTA With oACB77 amplifies pUC19 backbone and genes upstream oACB78 GCTC and downstream of *tcuA* from pBAC2094 to make pBAC2102 or from pBAC2098 to make pBAC2109 GAAGAAATCTATGGCTAGCAAAGGAGAA oACB79 With oACB80 amplifies gfp_{sf} - ΩK from pBAC2095 to make GAACTTTTC pBAC2102 and pBAC2109

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

oACB80	TTTAGTACTAGCCCGGTAGATCCGGTGA TTG	With oACB79 amplifies gfp_{sf} ΩK from pBAC2095 to make
		$\frac{1}{10000000000000000000000000000000000$
oACB86	CCGGTGATTG	with oACB89 amplifies gfp_{sf} - ΩK from pBAC2095 to make pBAC2119
oACB87	TCTACCGGGCTAAAAGCTCTAAAAGGCA	With oACB90 amplifies pUC19 backbone and genes upstream of
операт	G	downstream of <i>pacI</i> from pBAC2099 to make pBAC2119 (
oACB89	GTGAGTTACTATGGCTAGCAAAGGAGAA	With oACB86 amplifies gfp_{sf} - ΩK from pBAC2095 to make
		pBAC2119
oACB90	TTGCTAGCCATAGTAACTCACTTGAAGG	With oACB87 amplifies pUC19 backbone and genes upstream of
	AC	downstream of <i>pacI</i> from pBAC2099 to make pBAC2119
oACB151	TCCTCCTGAAGGACACAAGAACACAGC	With oACB152 to put a RBS ahead of <i>gfn</i> f in pBAC2119
$\alpha \Delta CB152$	GTCCTTCAGGAGGACAGCTATGGCTAGC	With $\alpha A CB151$ to put a RBS ahead of <i>afn</i> cin pBAC2119
0/10/10/2	AAAGGAGAAGAACTTTTC	
oACB158		With oTCB/I amplifies backbone and flanking DNA from
	IGACGIGIIAGAC	pBAC2152 to make pBAC2158
oACB159	GTCTAACACGTCAGCATCATTTCCTTCA	With oTCB51 amplifies gfp_{sf} - ΩK from pBAC2152 to make
	GGAGGACAGCTATG	nBAC2158
oMTV152	GAGTCAGAGCTCCGCAAACACAGGTGGC	With aMTV155 amplified DNA downstream of touP: used to
01111132	A	1 DACI100 Q I ' C 1 ' UCI0
		make pBAC1120; Sacl site for cloning into pUC18.
oMTV153	GATGATCTGCAGTCTCAAGCCTGTATTT	With oMTV154 amplifies DNA upstream of <i>tcuR</i> ; used to make
	ATGUU	pBAC1120; PstI site for cloning into pUC18.
oMTV154	GTAAAAATAAATACAAATGGCTTACTCG	With oMTV153 amplifies DNA upstream of <i>tcuR</i> : used to make
011111101	AGCACGGCAAATACTACATTGTC	nBAC1120: Xhol site introduced
- MTV155	GACAATGTAGTATTTGCCGTGCTCGAGT	With MTV152 and if an DNA damage of the Draw day
OM 1 V 155	AAGCCATTTGTATTTATTTTTAC	with oMTV152 amplifies DNAdownstream of <i>tcuR</i> ; used to
		make pBAC1120; Xhol site introduced.
oMTV158	GAGTCAGAGCTCGCGTGCTAAAAATTTC	<i>tclR</i> region; used with oMTV161 to make pBAC2093; SacI site
	TTGGTCGT	for cloning into pUC18
oMTV159	GATGATCTGCAGTACACCAGTATTTGGG	tclR region: used with oMTV160 to make pBAC2093. PstI site
011111109	CATATCCT	for cloping into nUC18
oMTV160	GTATTTAAAAAATTAATGACCCTTTAC	to Program used with aMTV150 to make nDAC2002. Vhol site
01011 v 100	TCGAGGGTTGAAAAATATTTGATTTGTT	icir region, used with own v 159 to make pBAC2095; Anoi site
	TGATTTCCAT	introduced
oMTV161	ATGGAAATCAAACAAATCAAATATTTTT	<i>tclR</i> region: used with oMTV158 to make pBAC2093; Xhol site
	CAACCCTCGAGTAAAGGGTCATTAATTT	introduced
NGL 40		
oNSL40	CAGACCITIACGACGICCC	With oNSL46 amplifies <i>tclR</i> ; put in pCR2.1 TOPO vector for
		pBAC1164 (<i>tclR51445</i>) and pBAC1863 (<i>tclR51556</i>)
oNSL46	TTCCTGAGTTACGTTATCTGC	With oNSL40 amplifies <i>tclR</i> ; put in pCR2.1 TOPO vector for
		pBAC1164 (tclR51445) and pBAC1863 (tclR51556)
oST45	CATGTGGACGTCGCTGTATAGCCCATTG	<i>pacI</i> region: used with oST46 to make nBAC1137 AatII site for
00110	TGGA	cloning into nUC10
- CT4(Noning into pool 7.
05146	CGGCGCCTAAAAGCTCTAAAAGGCAGCA	<i>paci</i> region; used with oS145 to make pBAC113/. BamHI and
	Т	Kasl sites introduced.
oST47	ATGCTGCCTTTTAGAGCTTTTAGGCGCC	<i>pacI</i> region: used with oST48 to make nBAC1137 BamHI and
00117	<mark>GGATCC</mark> CATAGTAACTCACTTGAAGGAC	Kasl sites introduced
	A	Kasi sites introduced.
oST48	ATAACTGTCGACACCTATGGGTGGCTTG	<i>pacI</i> region; used with oST46 to make pBAC1137. Sall site for
	UT	cloning into pUC19.
oST49	CATGTGGACGTCATTCGACCGGTGGAAA	tcuC region: used with oST50 to make nBAC1138 AatII site for
55117	GCTTG	cloning into nUC19
OT 50		$\frac{1}{1} = \frac{1}{1} = \frac{1}$
05150	CCGGCGCCTAACACGTCAGCATCATTG	<i>icuc</i> region; used with oS149 to make pBAC1138. BamHI and
		Kasl sites introduced.
oST51	CAAATGATGCTGACGTGTTAGGCGCC <mark>GG</mark>	<i>tcuC</i> region; used with oST52 to make pBAC1138. BamHI and
	ATCCCATTAAGACCTCCTTGGTCTGAAA	KasI sites introduced.

oST52	GATCTCGTCGACGGTGTATGAGATTGAT	<i>tcuC</i> region: used with oST51 to make pBAC1138. Sall site for
	GGCC	cloning into pUC19.
oST63	TGAAGCATTTATCAGGGTTATTGTCTCA	Used with primer M13R to generate DNA of transformation to
	TGAGCG	make ACN1419
oTCB5	CTTTAACCAAGGGCTGAATG	In <i>tclR</i> for RACE
oTCB6	GCTGCCTTGCATTAACTAAAG	In <i>tcuR</i> for RACE
oTCB8	ACAAGTACAACTGGCGCTTAGG	In <i>tcuC</i> for RACE
oTCB44	CCATTTTTCAGACCAAGG	Upstream of <i>tcuC</i> ; used with oTCB113 for cDNA evaluation
oTCB45	GATAAGTCGTTTGAACTGG	Upstream of <i>tcuC</i> ; used with oTCB113 for cDNA evaluation
oTCB51	TAAGCGCCAGTTGTACTTGT	With oACB159 amplifies gfp_{sf} - ΩK from pBAC2152 to make
		pBAC2158
oTCB55	TGCCTTTTAAAGCTACATTAG	Upstream of <i>tcuA</i> ; used with oTCB135 for cDNA evaluation
oTCB56	GCTAAAAAAGGAAGAAATCTATGC	Upstream of <i>tcuA</i> ; used with oTCB135 for cDNA evaluation
oTCB57	ATCAGACCTTTACGACGTC	In <i>tcuA</i> for RACE
oTCB71	ACAAGTACAACTGGCGCTTAGG	With oACB158 amplifies backbone and flanking DNA from
		pBAC2152 to make pBAC2158
oTCB104	GCAGAAGAAAAGCAGCATAG	With oACB54 amplifies <i>cltA</i> downstream region from ADP1 to
		make pBAC2110
oTCB105	CATCAATACGCTGACTTTGT	With oACB55 amplifies <i>cltA</i> upstream region from ADP1
		template to make pBAC2110
oTCB113	TGACCAACCAGCTTAAAAC	In <i>tcuC</i> ; used with oTCB44, oTCB45, oTCB144, and oTCB145
		for cDNA evaluation
oTCB135	TCGCTTTTGGGATGTTTAC	In <i>tcuB</i> for 5' RACE
oTCB137	TGTGGATCGACATACCC	In <i>pacI</i> for 5' RACE
oTCB144	CCTGCCACATGATAAATGAATTG	Upstream of <i>tcuC</i> ; used with oTCB113 for cDNA evaluation
oTCB145	ACTCCTTTTCTGTTTTTCCTCG	Upstream of <i>tcuC</i> ; used with oTCB113 for cDNA evaluation
oTCB154	AGCTTAAGGTTTTTGCATCTAGC	In <i>tcuA</i> ; used with oTCB156, oTCB56, oTB55, and oTCB155 for
		cDNA evaluation
oTCB155	ACACCCATTCAAGCTGAGC	Upstream of <i>tcuA</i> ; used with oTCB135 for cDNA evaluation
oTCB156	GCATGATGTGATTGTCATTGG	Upstream of <i>tcuA</i> ; used with oTCB135 for cDNA evaluation
oTCB161		With oACB76 amplifies pUC19 backbone from pBAC2158 to
	CACC	make pBAC2161
oTCB162	GGTGTTTAGTAAAAATAAATACAAATGG	With oACB73 amplifies gfp_{sf} - ΩK from pBAC2158 to make
	CTTAGAGTTCCACGGCAAATACTACATT GTCC	pBAC2161
M13R	AGCGGATAACAATTTCACACAGG	Universal reverse primer for sequencing from pUC18/19 vectors
		used with oST63 for constructing ACN1419
	1	



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.

Regulation of tricarboxylate transport and metabolism in Acinetobacter baylyi ADP1

Α	Helix-turn-Helix	
Aq. denitrificans 5YN1-3LTTR ACur. delicatus NBRC 14919LTTR BCup. taiwanensis LMG 19424LTTR BS. Typhimurium LT2TcuRAq. denitrificans 5YN1-3LTTR BP. sacchari LMG 19450LTTR ACup. taiwanensis LMG 19424LTTR AA. baylyi ADP1TcuR	MARINLEINELOAFLAVAEKSS KAAABALYISOPALSRIIEKLEOSLOVRLLERTTRSVRITEEGAHFL MKRNNLOIDELOAFVAVAEKASPRVAADALFISSPALSRIERLETALGTRIFERTTRRVTLTAVGROFL MARINFALEDLOAFVATAEKGSPRMAABALHISOPALSRIERLEKTLGSRLERTTRRVEVTHVGROFL MELROLRYFVRIIETGSMGSAADDIDIGVSALSOOMSRLENBUATRLLORTSRGVTPTNAGLAFW MELROLRYFVSVVAGGMGRAALELGVVTSALSOOISRLEGELATRLLORTSRGVVPTDAGLAFW MELROLRYFVSVVBHGSMGRAALELGVVTSALSOOISRLEGELATRLLORTSRGVVPTDAGLAFW MELROLRYFVSVVEHGSMGRAALELGVVTSALSOOISRLEGELATRLLORTSRGVVPTDAGLAFW MELROLRYFVSVVEHGSMGRAALELGVVTSALSOOISRLEGELATRLLORTSRGVVPTDAGLAFW MELROLRYFVSVVEHGSMGRAALELGVVTSALSOOISRLEGELATRLLORTSRGVVPTDAGLAFW MELROLRYFVSVVEHGSMGRAALELGVVTSALSOOISRLEGELATRLLORTSRGVVPTDAGLAFW MELROLRYFVSVVEHGSMGRAALELGVATSALSOOISRLEGELATRLLORTSRGVVPTDAGLAFW MELROLRYFTKVELGSFGKAALELGVATSALSOOISRLEGELATRLLORTSRGVVPTDAGLAFW	70 70 E42→G 65 65 65 65 65 65 65
Aq. denitrificans 5YN1-3 Cur. delicatus NBRC 14919 Dup. taiwanensis LMG 19424 S. Typhimurium LT2 Aq. denitrificans 5YN1-3 Cur. delicatus NBRC 14919 Cur. Abaylyi ADP1 A. baylyi ADP1 Cur. delicatus NBRC 14919 Cur. delicatus NBRC 14919 	LHAQNVV <mark>DE LE CAMRGLGERAERSGLVSIASI PSVAQHI LPAALAELAASYPALCLRVFDE GAQEVLEQ</mark> EHARQILDNAVFRIGDEAALCRGLVTVACIPSVAEQLLPAALVEFAKQYPDVRVKIADE GAQEVLEQ EBARATLILDNAVFRIGDEAALCRGLVTVAALPSAALHCLPAALRAFAGHPGVRVVVDESASSVLAS SQAQLALRHADDAILAAREALSGHVSVGMAPSTGVLGLPAALRAFAGHPGVRVVVDESASSVLAS RQAQLALRHADDAILAARCARLSGHVSVGMAPSTGVLGLPFMAMRERYPDVRLHVESLSGNLERM RQAQLALRHADDAARAAQLARLSGHVSVGMAPSTGVLGLPFMAMRERYPDVRLHVESLSGNLERM RQAQLALRHADDAARAAQLARLSGHVSVGLAPSTTGVLGLPFMAMRERYPDVRLHVESLSGVLGM RBAQLTLRHADDAARAAQARLSGHVSVGLAPSTTGVLGLPFMAMRERYPDVRLHMVESLSGVLGM RBAQLTLRHADDAARAAQARLSGTVSVGLAPSTTGVLGLPFMAMREYPDVRLHMVESLSGHIAAM RGAQLTLRHADDAARAAQARLSGTVSVGLAPSTTGVLGLPLMRAMRTYPDVRLHMVESLSGHIAAM RGAQLTLRHADDAARAAQARLSGTVSVGLAPSTTAVLGVPLRAMARTYPDVRLHMVESLSGLIAM KHSQLILRQINFAIDAARARSGHVSIGLAFTAAVLGVPLRAMARTYPDVRLHMVESLSGLISG KHSQLILRQINFAIDAARSARLSGHVSLGFPPTISALIGIPLMKIMSERYPDIRLEIVETLSGNLIQS QVQLALRHLEHAVDSAHSSRLSGHVTVGFSPSVAAVIGTHFLKLMRNRYPDIKVRLIEGLSGDLKAL	140 140 133 133 133 133 133 133 133 133 133
Aq. denitrificans 5YN1-3 Cur. delicatus NBRC 14919 Cup. taiwanensis LMG 19424 S. Typhimurium LT2 Aq. denitrificans 5YN1-3 P. sacchari LMG 19450 Cur. delicatus NBRC 14919 Cup. taiwanensis LMG 19424 Cup. taiwanensis LMG 19444 Cup. taiwanensis LMG 19444 Cup	VLASHAD SINT CAEDEN IDFEPLITER YVAVMQQGHPLGETTLEWRDLVGERLIGVSQRS VVG MADFELNEMGA DEPGLDFHALFRETYLLAVRPDHPLAHRSAVAWSELVSEKLISVSRAS VLSESDFELNTCADEN IDFRATRAERYRLAWRDDEWAGHERVAWDDLAGCRMVSVSRHS INTROIDLAIVFOKDKILRWSARFILEFCIFLIGSHALPAAPTQDHISLAELGDIPLIMPSQGH LGARQIDLAVLGCERTRRWSVIPLLEFKLFICRRDLPAAPTQDHISLAELGDIPLIMPSGFH LGARQIDLAVLGCERTRRWSVMPLLDBKLFFICSRALPAAPTQDHISLAELGDIPLIMPSGFH LNARQIDLAVLGTHTARWSVMPLLEFKLFFICSRALPGMPERPRTSIAKLGAIPLILPSGAH LNARQIDLAVLGTHTARWSVMPLLEFKLFFIRSRQGPGLPASQRARLVQLGEPPLIPTGTH INSRCDIAIFHEIDRQWAIOPLYREQMIYMLWBAKEVIJKHGLSDCLGTGIQSQOIGDIPLVFSAH VNARQIDVAIIFRDUVDPCWAIOPLVREOMYLMARKEVIJKHGLSDCLGTGIQSQOIGDIPLVFAR	203 203 197 197 197 197 197 197 203 R200→Q
Aq. denitrificans 5YN1-3LTTR ACur. delicatus NBRC 14919LTTR BCup. taiwanensis LMG 19424LTTR BS. Typhimurium LT2TcuRAq. denitrificans 5YN1-3LTTR BP. sacchari LMG 19450LTTR ACup. taiwanensis LMG 19424LTTR AA. baylyi ADP1TcuRA. baylyi ADP1TcuR	GNRILLDHHLAS.PERPRUHYEACHLHGAYGMAMAGIGVMIVPELSITPAYMHKUV-GITIEDEOISRT GMRILLDHALSRI, SRRPMAFHEVNHVATLVHLVOAGIGVGAVPSISINDSARLGIV-GITIEDEOISRT GMRILLDNALSRI, SRRPMAFHEVNHVATLVHLVOAGIGVGAVPSISINDSARLGIV-GITIEDEOISRT GMRILLDNALHLERRPITHYEANHVVGVLALWEAGIGMAVLPGMAVPPD-HPRCC-AVPLVEDVDRV GLRGRLDAVCQHALNVEIVAEIDGIALLMRAVRDGIGATLOHGAISHLDNALR-VIGVHNVVSV GLRALLNASNCGAHALNICEIIGEPLLMDAVRAGYGAIOPGAAIARLPPEFF-LIDIIDSVSRP GLRALLDAAFKRAGYAPRIAAEIDGIAMLMDAVRGGIGATIOPGAAIARAENASIA-SVPLVEKYATRP GLRSTLDAVFARARVORVVAEIDSLEMLMDAVRAGIGATIOPGAAIARAENASIA-SVPLVEKYATRP GLRSTLDAVFARARVORVVAEIDGIEMLMDAVRAGFGATIOPGAAIARLNDPTUV-RSIVADAQVGRH SIRKLLEHKIGUNVVEIDGHLLMDSNIHLDIASVRAGFGATOQCAATARLNDPT SIRKLEHKIGUNVVEIDGHLLMDSIHLDDSIVPGAALARAENASIA-COVFUKVOIGEIERI GLRSILDAVFARARVORVIGEIDGISMLMDAVRAGFGATIOPGAAIARLNDPTUV-RSIVADAQVGRH	271 270 G241→S or C 265 A242→T or V 265 I244→ F 267 R264→ L 265 L216→P 266
Aq. denitrificans 5YN1-3 Cur. delicatus NBRC 14919LTTR B LTTR BCup. taiwanensis IMG 19424LTTR BS. Typhimurium IT2 Rq. denitrificans 5YN1-3 Cur. delicatus NBRC 14919 Cup. taiwanensis IMG 19424LTTR B LTTR B LTTR AA. baylyi ADP1TcuR	IGUITRSGYKPNPAVVK.LELVRGQFKKYCQSKRLSA LGLISRREQPLSPSAALVALURKRSAARPAKPASPLSRRA IALIRKDRPHCPAQALVETIALSMAEDA NFLVSLSDDELTPAGLAARVVLQVASELARSCQMPGATVAK NLLVSLSDDELSPAGLAARVVLQVASELARSCQMPGATVAK NLLSSLSDDELSPAGLAARVVLQVASELARSCQMPGATVAK NLLSSLSDDELSPAGLAARVVLQVASELARSCQMPGATVAK NLLSSLSDDELSPAALARVVLQVASELVASCRWVGAALVDG NLLSSLSDDELSPAALARVVLQTAREVASCRWVGAALVDG NLLASLSDDELSPAALARVVLDATTAREVROCMVGAALVDG NLLASLSDDELSPAALARVVLDATTAREVROCMVGAALVDG NLLASLSDDELSPAALARVVLDATTAREVROCMVGAALVDG NLLASLSDELSPAALARVVLDATTAREVROCMVGAALVDG	308 313 300 308 308 313 310 308 309 309
B helix	turn helix	



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 $P_{\underline{tcuC}}$ and $P_{\underline{tcuA}}$. (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

Regulation of tricarboxylate transport and metabolism in Acinetobacter baylyi ADP1

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 <u>www.phylogeny.fr</u> 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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		Γ→K	
	MEHGOOTSRIRAIFNVTSGNFLEOYDFFLFGLYAKAIGE	TFFHSDSSYAALMKTFLVFAVSFLMRP	66
	MEOVAKRSATFKKIINVTSGNFLEOYDFFLFGFYATYIAA	KFFHSEN <mark>EYVSLMMTFTVFAAGFLMRP</mark>	67
MES X autotrophicus	MVGPSATAVPSTVTAEVPAIRSRTAAIIRSTSGNFLEOFDFFLFGFYATSISK	AFFPTGNEVTELLLTFTTFWLGALMRP	80
MFS-C A denitrificans	MTTASPROSNLSMVLRVTSGNFLEMFDFFLYGFYATYIAK	TFFPSGNLYASLILAFATFGAGFLARP	67
MES-C P sacchari	MHPSSLNAGDNAOVRTSKEGAVIRVTSGNELEOFDEELEGEVATTISH	I F F P S K S E F A S L M L T F S V F G A G F L M R P	75
MFS-D P sacchari	MHPSPSTLSAGRSKAAAVERVTAGNELEOFDEELEGEVATOLAS	VEEPATSEEASI.MMTEAVEGAGEI.MRP	71
		TEEDAESEEASIMITEAVECSCELMED	70
MES-D A denitrificans		AFEDTSSFFASIMUTEAVEGAGELMRI	71
			11
	TCATUL CRYNDRT CRRVCT MUTLETMAUCET TTAL TROUVATTORAATUUT TRAC		116
			147
		THE OCEAN CARL CONSTITUTE TA TONN	160
MES C A donitrificono	VGATVLGSTEDETGRAKGLTVTLGTMAAGTVLTAVCFTTAQTGTAAFLTVLFG		1 4 7
MES C D acachari		RLLQGFSAGVELGGVSVILAEMAIFGR	14/
MFS-C P. Sacchan	LGAIVLGAYIDEAGRRKGLIVTLSIMACGTILIAGVPGYATIGLLAPALVLLG	RLLQGF SAGAELGGVSVYLAEMATPGR	155
WIFS-D P. sacchan	LGAIVLGAYIDDVGRRKGLIVTLSIMASGTILIAFVPGYATIGLLAPALVLVG	RLLQGFSAGAELGGVSVYLAEMATPGR	151
	VGAIVLGAYIDRIGRRKGLMVTLAIMGCGTLLIALVPGYQTIGLAAPALVLLG	RLLQGFSAGVELGGVSVYL <mark>S</mark> EIATPGN	150
WIFS-D A. Uerillinicans	LGAIVLGSYNDRIGRRQGLIVTLGIMACGTNLIAFVPDYSQIGLAAPLLVLLG	RLLQGFSA <mark>G</mark> VELGGVSVYLAEIATPG <mark>H</mark>	151
	A→V		
CitA ADP1	RGFITSWQSASQQVAVIFAATLGYMISLVFTKAQVADWAWRIPFFVGCAIIPF	IFWLRKSLQETEAFASRKEHPTTQQIM	226
TcuC ADP1	RGFITSWQSGSQQIAVVFAALLGY <mark>WLNSVLSHAQVSDWGWRIPFLIGCLIIPL</mark>	IFVFRRTLEETEDFKAQKTHPTTKEIF	227
MFS X. autotrophicus	KGFYTSFQSASQQVAIFFAAIIGFSLSQLMPSETISAWGWRIPFFIGCAIIPF	`IFFIRRTLEETPEFLKQKKHPTQSEVF	240
MFS-C A. denitrificans	KGFFVSWQSASQQVAIMFAAVLGYLLHQAFNNQQIDQWAWRIPFFIGCMIIPF	IFAIRRSLQETDAFLAHKNHPSFADIL	227
MFS-C P. sacchari	KGFYTSWQSASQQVAIV <mark>A</mark> AAALGY <mark>L</mark> LNHLMTASEIGAWGWRIPFFVGCAIVPI	IFVLRRTLQETDEFKARTHHPRAREVF	235
MFS-D P. sacchari	K G F F T S W Q S A S Q Q V A I V V A A A L G F A L N Q A L S A T A I A A W G W R V P F L V G C M I V P F	IFMLRRNLEETQEFKARQHRPTMNEVF	231
TcuC LT2	KGFYTSWQSASQQVAIV <mark>V</mark> AALIGY <mark>SLNITLGHDAISEWGWRIPFFIGCM</mark> IIPL	IF <mark>VLRRSLQETE</mark> AFL <mark>Q</mark> RKH <mark>RPDTR</mark> EIF	230
MFS-D A. denitrificans	KGFYTSWQSASQQVAIV <mark>V</mark> A <mark>A</mark> AIGY <mark>GIQLWLSPEQIA</mark> AWGWR <mark>V</mark> PFFIGCLIIPL	IFL <mark>LR</mark> GSLQET <mark>D</mark> AFLARKH <mark>RP</mark> SL <mark>R</mark> EIF	231
	<u>P→S</u> L→F		
CItA ADP1	STLAANWKIVLTGMCMVATTTTMFYFITVYTPTYGKEVLHLTSAESLLATVMV	'GVSNFILLPIGGYLSDKFGRKPLLITT	306
TcuC ADP1	STLASNWRIVLAGMMMSAMTTTTFYFITVYT <mark>TVYA</mark> KRTLEMSITDSLLATVFV	'GLSNFFWLPMGG <mark>LLSD</mark> KIGRRPVLVGI	307
MFS X. autotrophicus	AAMLTNWRIVLLGMMLTAMTTVTFYFITVYTPTFGKNVLKLSITDSLLVTLLV	ATTNFIWLPIGGAISDRVGRRPVLLTI	320
MFS-C A. denitrificans	RSIAQNWQLITGGMLLVVMTTVSFYLITVYTPTFGKSVLKL <mark>S</mark> ENDSLLVTFFV	'GLSNFIWLPI <mark>M</mark> GALSDRIGR <mark>W</mark> PL <mark>MLG</mark> F	307
MFS-C P. sacchari	ATMLQNWRTVLAGMLLVAMTTTTFYLITVYTPTFGKAVL <mark>KL</mark> TTGDSLIVTLCV	GLSNFFWLPVGGALSDRIGRKPILIAI	315
MFS-D P. sacchari	RTLAQNWAVVVAGMMLVAMTTASFYLITVYAPTFGKTVLHLSTADSLLVTLCV	AVSNFIWLPIGGALSD <mark>K</mark> IGRRPLL <mark>V</mark> GM	311
TcuC LT2	ATIA <mark>KNWRIITAGTLLVAMTTTTFYFITVYT<mark>P</mark>TYGRTVLN<mark>LS</mark>ARDSLIVTMLV</mark>	'G <mark>V</mark> SNFIWLPIGGA <mark>I</mark> SDRIGRR <mark>AV</mark> L <mark>M</mark> GI	310
MFS-D A. denitrificans	GTIGLHWRTIVAGMFLVSLTTTLFYFITVYT <mark>P</mark> TFGK <mark>SVLHLS</mark> AQDSLVVTLCV	GLSNF <mark>C</mark> WLP <mark>V</mark> GGALSDRIGR <mark>K</mark> PLL <mark>L</mark> GI	311
CItA ADP1	TTLIFITAYPLLSWLTQNISLTHMLISLLWLSLLYSFYNGALVVSLTEMMPSS	VRIAGFSVAYSLATSVFGGLTPMIATY	386
TcuC ADP1	T T L A I F T S Y P V L S W L V S D I S F T N L I I A L A Y F S F F F G L Y N G T M V A T L A E V M P K R	VRTVGFSLAFSLA <mark>A</mark> AIFGG <mark>M</mark> TP <mark>MAC</mark> TF	387
MFS X. autotrophicus	AILALATAYPALAWLVVEPTFNKMLMVELWFSLCFGVYNGAMVAALSEEVPAH	VRTTCFSLAF <mark>A</mark> LA <mark>AAL</mark> FGTFTPLVSTW	400
MFS-C A. denitrificans	SLLAMLTAYPALAWMVASPSFGHMLVVELWLSFLYASYNGATVVALTELMPSH	VRTVGFSLA <mark>Y</mark> SLATAIFGGFTP <mark>L</mark> VSTA	387
MFS-C P. sacchari	TVLAICTAYP <mark>SLSWLASAPSFGRMLA</mark> VLLWFSFFFG <mark>M</mark> YNGAAVAALTEVMPAE	VR <mark>VA</mark> GFSLAFSLATA <mark>V</mark> FGGFTPAVST <mark>Y</mark>	395
MFS-D P. sacchari	TMLAIATAYPTLSLLAHAPSFQNMLLALLWLSFMYGIYNGAMVVALTEVMPAQ	VR <mark>VA</mark> GFSLA <mark>Y</mark> SLATA <mark>V</mark> FGGFTPAISTA	391
TcuC LT2	TLLALITTWPVMQWLTAAPDFTRMTLVLLWFSFFFGMYNGAMVAALTEVMPVY	VRTVGFSLAFSLATAIFGG <mark>L</mark> TPA <mark>I</mark> STA	390
MFS-D A. denitrificans	SLLALLTAYPAMAWLAAAPSFIRLLTVLLWFSFFFGMYNGAMVAALTEVMPVY	VRTVGFSLAFSLATAIFGG <mark>L</mark> TPAVSTA	391
CItA ADP1	LVEQS <mark>GSKS</mark> MPAFWLML <mark>AA</mark> IIS <mark>LIATL</mark> IVF <mark>R</mark> KRQHNTAQSKPLQTKLASSVSH		439
TcuC ADP1	LVEHTGNPSTPAFWLMFAAVCSLISSLYLCRGAKKNNPDAVVEPAKLSV		436
MFS X. autotrophicus	LIDM <mark>TG</mark> NR <mark>AAPGFWLM</mark> AAAA <mark>SGLIAT</mark> ILIYRGRGAAPARS		440
MFS-C A. denitrificans	LIDAS <mark>GDKAAPG</mark> YW <mark>M</mark> ML <mark>AAACGLAATL</mark> MLYRKDIRSRFVGKAATA	MFS Transporters	432
MFS-C P. sacchari	LIDI <mark>TGDKAAPG</mark> YWL <mark>CFAAACGLVATL</mark> ALYRRGGAALALRH		436
MFS-D P. sacchari	LIHMTNDKAAPGYWMSFAAACALCATFALYRRRAVTLTPAH		432
TcuC LT2	LVKLTGDKSSPGWWLMCAALCGLAATAMLFVRLSRGYIAAENKA		434
MFS-D A. denitrificans	LVEMTHSKSSPAWWLLFAAASALIASQVLFKSKQSQTDK		430

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 TcIR-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 TcIR highlighted in red, R200 and L216. These residues when replaced with Q and P, respectively, resulted in TcIR variants with increased responsiveness to Tcb. (D) Enlarged view of the effector binding-site of panel C showing that R200 of TcIR is predicted to reside in the same location as Cit in the CcpC structure.



C Enlarged views of Effector-Binding Site



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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