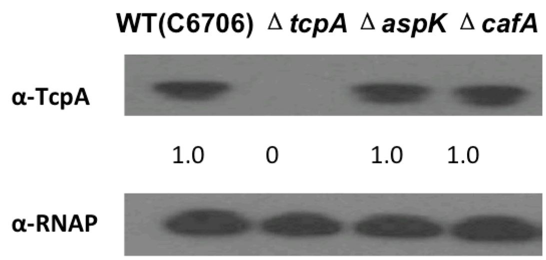


Figure S1

Fig. S1 *aspK* and *cafA* deletion mutants show similar levels of TcpA compared to the wt in AKI conditions. TcpA expression detected by immunoblot in the indicated strains cultivated in AKI conditons; numbers correspond to densitometry measurements normalized to the value of the wt strain. Levels of RNAP alpha serve as a loading control. Densitometry measurements derived from triplicate experiments are shown.

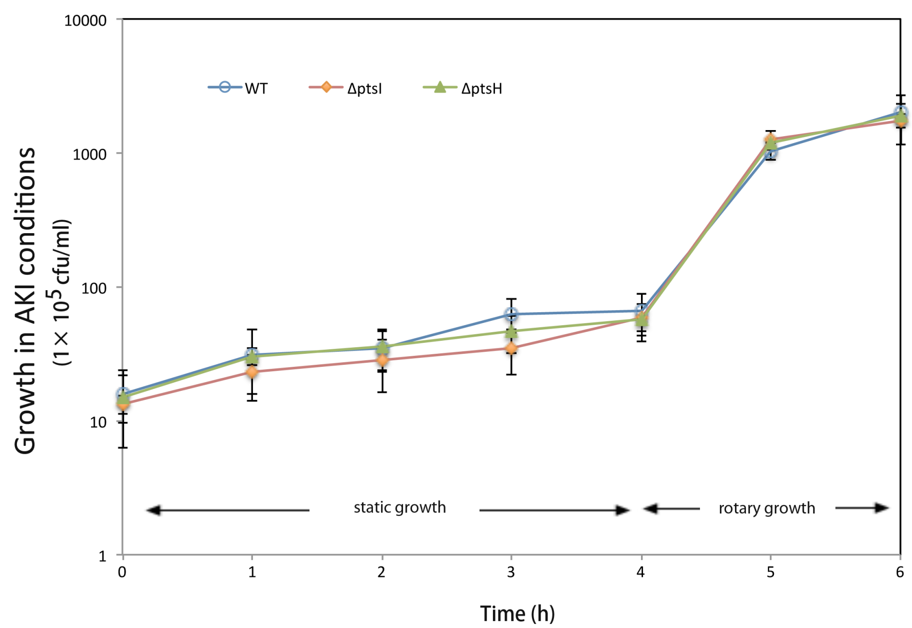


Figure S2

Fig. S2 Growth curve of *V. cholerae* strains grown in AKI induction conditions. The cultures were sampled at indicated time points and plated on the LB agar plates with Streptomycin (200μg/ml) after serial dilutions. Three independent experiments were performed in triplicate.

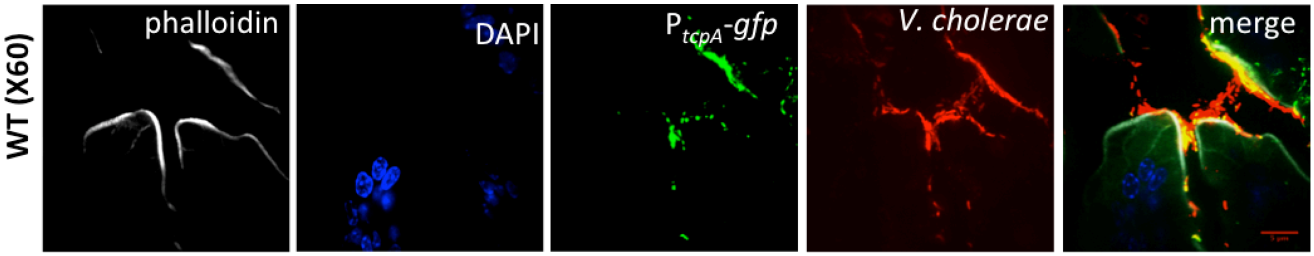


Figure S3

Fig. S3 Heterogeneous expression of *tcpA* in wt *V. cholerae* is seen during infection of suckling mice. *V. cholerae* cells (red) in the small intestine were detected by immunofluorescence, and *tcpA* expression (green) was detected from the chromosomal P_{tcpA}-*gfp* reporter. Tissue sections were counterstained with phalloidin (gray) and DAPI (blue) to visualize actin and nuclei, respectively, and observed under 60× objectives.

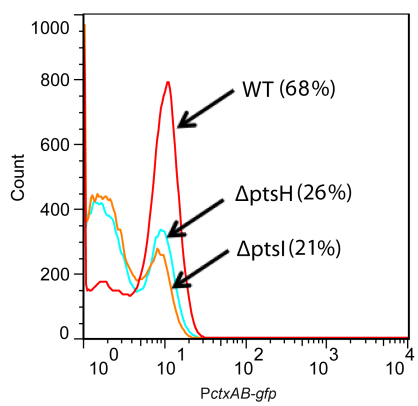
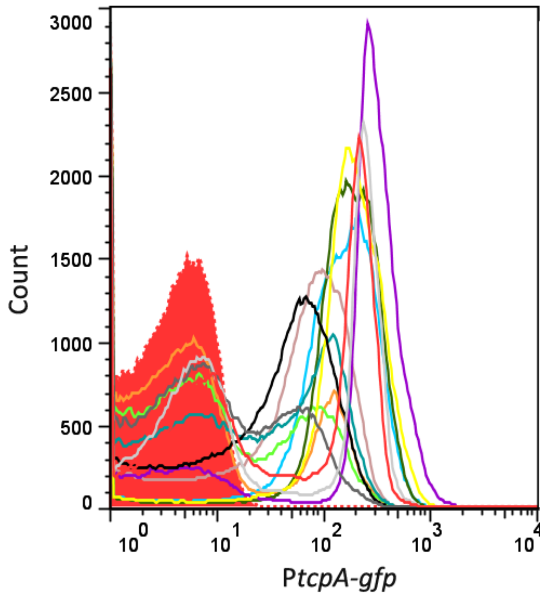


Figure S4

Fig. S4 GFP expression from a chromosomal P_{ctxAB}-*gfp* in the indicated strains after growth in AKI conditions and monitored by flow cytometery



Key	strain	% of positive
	Δcrp ΔptsI	52
	Δcrp ΔptsH	47
	ΔcpdA ΔptsI	27
	ΔcpdA ΔptsH	34
	ΔcyaA ΔptsH	83
	ΔcyaA ΔptsI	73
	Δcrp	74
	ΔcpdA	56
	ΔcyaA	85
	ΔptsI	23
	ΔptsH	19
	WT	80
	WT(control)	0

Figure S5

Fig. S5 Flow cytometry assay of TcpA expression after the indicated strains were grown in AKI conditions. In the flow cytometry experiments, each strain contains a chromosomal P_{tcpA}-GFP reporter, except for a wt (control) strain that was used to establish the background level of fluorescence. The percentage of GFP positive cells (% of positive) are shown.

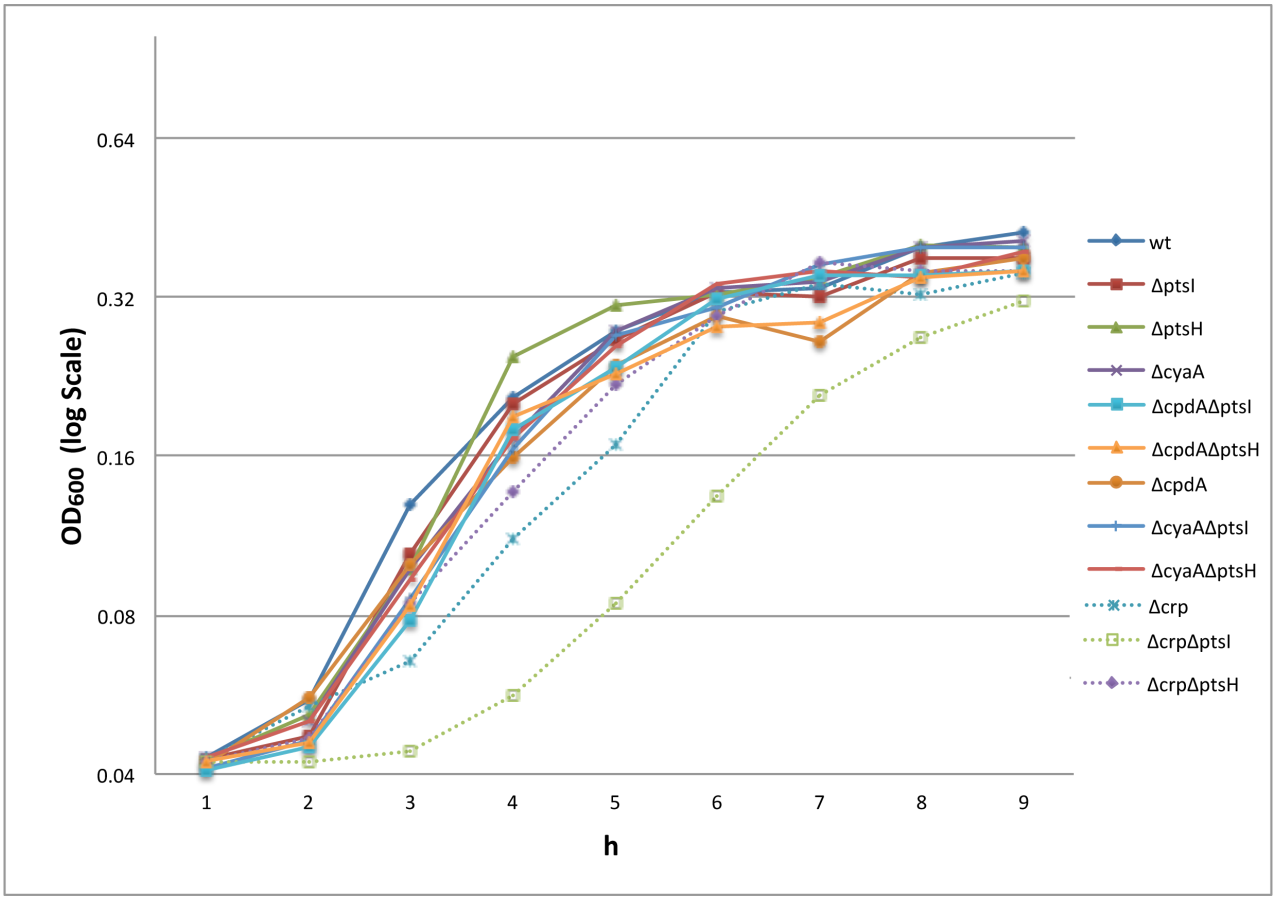


Figure S6

Fig. S6 Growth curves of the wt and PTS related mutants incubated in LB. The bacterial cultures were sampled at indicated time points and OD_{600} were assayed. Data represent three independent assays and values are reported as the mean.

Table S1 Primers used for this study

Construct	primer name	sequence (5'-3')*
P_{tcpA}-bla	PtcpAF1	tggtcgctcggcaatgaatcggggattggtaccgcgGTCTTATCATGAGCCGCCTA
	PtcpAr1	GGAATAAGGGCGACACGGAAATGTTGAATACTCATATTTATATAACTCCACCATTTGTGTTTTAA
	blaF1	ATGAGTATTCAACATTTCCGTGTC
	blar1	tcgtccactgatacatggcgtgatgattggtaccatGCGGCATCAGAGCAGATTG
P_{tcpA}-GFP	PtcpAF2	tggtcgctcggcaatgaatcggggattggtaccgcgGTCTTATCATGAGCCGCCTA
	PtcpAr2	ACAACTCCAGTGAAAAGTTCTTCTCCTTTACTCATATTTATATAACTCCACCATTTGTGTTTTAA
	gfpF1	ATGAGTAAAGGAGAAGAACTTTTCACTG
	gpfr1	tcgtccactgatacatggcgtgatgattggtaccatTATTTGTATAGTTCATCCATGCCATG
P_{ctxAB}-GFP	PctxF1	tggtcgctcggcaatgaatcggggattggtaccgcgATGTTCTCTTATTCGTTTTACGGC
	PctxR1	ACAACTCCAGTGAAAAGTTCTTCTCCTTTACTCATATAATGCTCCCTTTGTTTAACAGAAA
	gfpF1	ATGAGTAAAGGAGAAGAACTTTTCACTG
	gpfr1	tcgtccactgatacatggcgtgatgattggtaccatTATTTGTATAGTTCATCCATGCCATG
ΔVC0547	VC0547_UP_R	ACATTTGCAGGGTCTAACATTAGCATAGATCAGTATTCACCTTCCCAAGACCAAAGTCT
	VC0547_UP_F	aggtatatgtgatgggttaaaaaggatcgatcctGCGGGTGTTAACGTACTGGT
	VC0547_DOWN_F	GAGACTTTGGTCTTGGGAAGGTGAATACTGATCTATGCTAATGTTAGACCCTGCAAATGT
	VC0547_DOWN_R	ccgggagagctcgatatcgcatgcggtacctctagTCGGCAAAACAACTCAACCG
	QY05476Dedo-R	TCTTGCGGAGGCACATATTACGATTTA
	QY0547Deup-F	CTGCTGGAGAAAGAGATCCAACAGT
ΔVC0419	QY04196Dedo-R	AAGAGATCATCGAGCTGTTTGACTA
	QY0419Deup-f	CGCCATTGGCATGTGAAATGACAATA
	VC0419_UP_R	TCACGCGCGTAATTTCGCGCAAAATCTCAAGAGCGCACCTCAATGATTAAAATGATTAGA
	VC0419_UP_F	aggtatatgtgatgggttaaaaaggatcgatcctATCCCCTAGACGTCGTGAAC
	VC0419_DOWN_F	TCTAATCATTTTAATCATTGAGGTGCGCTCTTGAGATTTTGCGCGAAATTACGCG
	VC0419_DOWN_R	ccgggagagctcgatatcgcatgcggtacctctagTCGCCCGTAAATCAAGGACT
pCVD442 primer	CVD442F	ccagccctcctgtttgaagatg
	CVD442R	ACTGAGAAGCCCTTAGAGCC
ΔVC0965	0965DELUPF	aggtatatgtgatgggttaaaaaggatcgatcctGCAGACATCACTGTGACTTCTAACG
	0965DELUPR	TGGTATCAACCGATAATTACATTTTTTCTTTTTTGATGAGGGCTAAAATC
	0965DELDOF	CATCAAAAAAGAAAAAATGTAATTATCGGTTGATACCAAGGAGATAGG
	0965DELDOR	ccgggagagctcgatatcgcatgcggtacctctagCCTACGGTCACTGAACCAGAAAG
	0965delTupf	TGTACGAGAAGCAAGTAGAAATCACT
	0965delTdor	TTACTTGGTCACGCGTAGGATC
	0965delTinF	GCAACAGATATCCGTGATATCGG
	0965delTinR	AGATGCCGCTCATTGAGAACT
ΔVC0966	0966DELUPF	aggtatatgtgatgggttaaaaaggatcgatcctCGGTTGAACCGGCTGAATCA
	0966DELUPR	TTGAAAGGAAGACCGGATTACATGTTTTATACCCCAATGAGTTTATTTTTTG
	0966DELDOF	TCATTGGGGTATAAAACATGTAATCCGGTCTTCCTTTCAAAGCC
	0966DELDOR	ccgggagagctcgatatcgcatgcggtacctctagTTCGCTCAGCGATACGATGTG
	0966delTupf	AAGGCACTCAAGGTAAAGCTATTCT
	0966delTdor	TAGGCAACAAGGACAACTTCTTGAT
	0966delTinF	ACGAGAAGCAAGTAGAAATCACTGC

	0966delTinR	GTGAAGTTGGTCCATCAGAGCA
ptsI complement	VC0965pbadcomf	agcgaattcgagctcggtacccggggatccTTGACCAGTTTAAGGTAAGGCTATGAT
	VC0965pbadcomr	aacagccaagcttgcatgcctgcaggtcgCTATCTCCTTGGTATCAACCGATAATTACG
ptsH complement	VC0966pbadcomf	gcgaattcgagctcggtacccggggatccACAAACTTACAAAAAATAAACTCATTGGGG
	VC0966pbadcomr	aaacagccaagcttgcatgcctgcaggtcgCATTTTGCGTGAACAAAATGGCTTTG
Δ*cyaA*	cyaAdelTOutF	AATCACATCGCCGCGCGTGA
	cyaAdelToutR	ACTTTGTTTTGCATCATCAGGCATATACA
	cyaAdelTinF	GCCAATTTCTTCATCATGAATGAAGAGCGT
	CyaAdelTinR	CAGTTGATAAACCAAGTTACGCATCACG
	cyaAdelUPF	aggtatatgtgatgggttaaaaaggatcgatcctCCATAGTCACCAACTCCACCTGCA
	CyaAdelupR	ACGTCAACGAGTTACAAGTTTGCTTCCCTGATATGACAGATAAAA
	CyaAdeldoF	ATCAGGGAAGCAAACTTGTAACTCGTTGACGTCTCAGGTTTTCTA
	CyaAdelDOR	cgggagagctcgatatcgcatgcggtacctctagGTGGCCCAGTTTGCCCGCAT
Δ*crp*	crpdelToutF:	TTTCAGCGTGTTCCTACGGTCGA
	CrpdelToutR:	AAGGTTTTCATTAGTGAGCTGAAAGTGGA
	CrpdelTinF:	TAAACCTCAAACCGATCCAACACTAGA
	CrpdelTinR:	ATCAGGTTCTGCTCTTCCAGCAT
	crpdelupF:	aggtatatgtgatgggttaaaaaggatcgatcctTACTTCTAACCCACTCAATGCCAAAG
	CrpdelupR:	TTATCGGGGCACTTAcatAATAATCTCACTTCCTCTGCAGGGTAC
	CrpdeldoF:	CAGAGGAAGTGAGATTATTatgTAAGTGCCCCGATAACCCGTCTC
	CrpdeldoR:	cgggagagctcgatatcgcatgcggtacctctagAAGAGTGTTCACCTGAGCTGCAAG
Δ*cpdA*	cpdAdelToutF	GTGATGAGTCGGACTGAAGCG
	cpdAdelToutR	CCCTCTTCCACGGTCAGTTTAG
	cpdAdelTinF	TCGATCAAGCTTATTCAGATCACGG
	cpdAdelTinR	AAATCAGGTAAAAATCGGCCTTGTT
	cpdAdelupF	aggtatatgtgatgggttaaaaaggatcgatcctTATCAATTGGTCAAAGATGGGCGAA
	cpdAdelUPR	CGAGCAGTCATATCACAAAATCGGTAAACCTAACTCTGTTATTTA
	cpdAdeldoF	GGTTTACCGATTTTGTGATATGACTGCTCGTCCTGCT
	cpdAdeldoR	ccgggagagctcgatatcgcatgcggtacctctagCCGCTTGGTACTTCTCAACCG
ΔVC0964	0964delTUPF	AACTGCGCACCGAAGGTCAT
	0964delTdor	TATTAAAGTGTTGCAACACGGTGCTT
	0964DELUPF	aggtatatgtgatgggttaaaaaggatcgatcctGCTTTCGATGAAAACATCGAAATCGG
	0964DELUPR	AATCGAACCAAGCGATTACATTGTGTCATGCTCCTAACGTTTTA
	0964DELDOF	GGAGCATGACACAATGTAATCGCTTGGTTCGATTTAAAACGT
	0964DELDOR	ccgggagagctcgatatcgcatgcggtacctctagTCCCATTAACGCGCCGTCAT
ΔVC0672	0672delTUPF	CGGCTAAATGTCGGGATTGTGA
	0672delTdor	AACCAATCATGCCCGATATCGC
	0672DELUPF	aggtatatgtgatgggttaaaaaggatcgatcctTTTGTAACAACCATGGTCAGGTTTT
	0672DELUPR	TTCACATTTGGTTCCTACATGCTTAACCTCTGTGAGTGTT
	0672DELDOF	AGAGGTTAAGCATGTAGGAACCAAATGTGAATATTGAACTCTTAG
	0672DELDOR	ccgggagagctcgatatcgcatgcggtacctctagAATCGCAAAACCACAGACCGA

ΔVCA0518	A0518delTUPF	GCGAGTTCTGTTCAAGTAATTTTGCC
	A0518delTdor	ATTCATGCTGCGCAGTTGGGCA
	A0518DELUPF	aggtatatgtgatgggttaaaaaggatcgatcctAAACGCGCATAACTGGTGTTTTC
	A0518DELUPR	ATGTGATGCCCCTTACATTCTTAACTCCTGTCTGCCTCTA
	A0518DELDOF	CAGGAGTTAAGAATGTAAGGGGCATCACATGACAAAAAAA
	A0518DELDOR	ccgggagagctcgatatcgcatgcggtacctctagTGAGATACCTTGTGGCAAACTGC
ΔVC1820	Vc1820delTUPF	GGCAGGATCTGGATCAACTGTTAC
	Vc1820delTdor	CCGTCATACCACCAAGAATCGC
	Vc1820DELUPF	aggtatatgtgatgggttaaaaaggatcgatcctATGCGGCCTTTGGTGGCTATAA
	Vc1820DELUPR	TAATTTCACGCCTTACATTTATTGGCTCATAAAGTATTTAACTGATGA
	Vc1820DELDOF	TGAGCCAATAAATGTAAGGCGTGAAATTATGAGTACCCTAAC
	Vc1820DELDOR	ccgggagagctcgatatcgcatgcggtacctctagACCTAACACCACTTTAGAAGCGCC
ΔVC2013	Vc2013delTUPF	TCCGACAAGCAACCGATCTTAAAG
	Vc2013delTdor	ATGGCCAGCTGTTTGGTGGAA
	Vc2013DELUPF	aggtatatgtgatgggttaaaaaggatcgatcctAGGTCGTGAAAGCCTTACCTCT
	Vc2013DELUPR	GGGGAAGAATGAGATTACACCCAGTGAAAATGTAACCACTT
	Vc2013DELDOF	ACATTTTCACTGGGTGTAATCTCATTCTTCCCCCTAGAGAAAAG
	Vc2013DELDOR	ccgggagagctcgatatcgcatgcggtacctctagCTGGTATTGATGACTTGGCTATTGAAGA
ΔVC1822	Vc1822delTUPF	AGACAGCGGTTGGGCAACATG
	Vc1822delTdor	ATCGCACTAGGCTCACTCACCA
	Vc1822DELUPF	aggtatatgtgatgggttaaaaaggatcgatcctGACGCTGGCATTCACATCATTCA
	Vc1822DELUPR	TGATTCAGAATGTTACACAATTGTTTTACCTTACTGTTGTTTGAA
	Vc1822DELDOF	AAGGTAAAACAATTGTGTAACATTCTGAATCACTACACTTGAGATAGA
	Vc1822DELDOR	ccgggagagctcgatatcgcatgcggtacctctagCTGCACTCATCGTTGGCTTCTG
ΔVC0995	Vc0995delTUPF	GTGTAGATTTTGCAGTTCGTTAGCGC
	Vc0995delTdor	CCTTTTTGAATCAGCTCAATCGCGTATT
	Vc0995DELUPF	aggtatatgtgatgggttaaaaaggatcgatcctATACATAATCAGCTAATCCTCTTGTCGAT
	Vc0995DELUPR	GTTTAAACTCGATTACACCTTAAGTTCCCCCTATAGGATTT
	Vc0995DELDOF	GGGGAACTTAAGGTGTAATCGAGTTTAAACCCTAGCCTGAATC
	Vc0995DELDOR	ccgggagagctcgatatcgcatgcggtacctctagCATACAACTTGTCAAAATAGTCAGAGGAGT
ΔVCA1045	Vca1045delTUPF	AACAACCTTCATCTGTACTATGTGTAGGT
	Vca1045delTdor	TGCAACGGTGGCACAATGCGA
	Vca1045DELUPF	aggtatatgtgatgggttaaaaaggatcgatcctCTTCCTACTTACGTATAGTGTACGTTCAT
	Vca1045DELUPR	GGAGCAAAAACGTTACATCGCGTCCCCCGTTGGAT
	Vca1045DELDOF	CGGGGGACGCGATGTAACGTTTTTGCTCCTGAGGCAA
	Vca1045DELDOR	ccgggagagctcgatatcgcatgcggtacctctagTCGACTGCGGAATCCACAAAGC
ΔVC0911	Vc0911delToutF	TGATTGGTTCGACGCTGTTTGG
	Vc0911delToutR	CTATAGGGGTGACTCAATCAGGATGATG
	Vc0911delupF	aggtatatgtgatgggttaaaaaggatcgatcctCTTCATGTATGCTCCTCTCGTGATTAC
	Vc0911delupR	AGACACCTTGTTGCTcatACTACTCTCACTTACTCAAAAAAATTGACT
	Vc0911delDOF	AAGTGAGAGTAGTatgAGCAACAAGGTGTCTCTGAAAGTG

	Vc0911delDOR	ccgggagagctcgatatcgcatgcggtacctctagCGTGTTTCGTATGGACGTAACACT
ΔVC0910	Vc0910delToutF	CGCCTATCTCAATTGGTGTGTTGA
	Vc0910delToutR	CACCACCGTTAACCGGCTTACG
	Vc0910delupF	aggtatatgtgatgggttaaaaaggatcgatcctGCATGAGATTGTGGCGAATTACCA
	Vc0910delupR	AATCGGGTAGAGAttaCACCCTATTGAATTATTATCATTGCAGCA
	Vc0910delDOF	TTCAATAGGGTGtaaTCTCTACCCGATTGAGGAGTGG
	Vc0910delDOR	ccgggagagctcgatatcgcatgcggtacctctagCCAAATATAGTAATCACGGTAAGGGCTAT
ΔVCA0245	Vca0245delToutF	GTTACGCGCCGAAGAAGATGC
	Vca0245delToutR	CGAGTGCCATTGCGGAGATCTC
	Vca0245delupF	aggtatatgtgatgggttaaaaaggatcgatcctACAAGGTAGTATCACGGTCAGTGA
	Vca0245delupR	TACATACTCCCTCctaCATGGTTCTATCTCCAGAGTAGAGCT
	Vca0245delDOF	TGGAGATAGAACCATGtagGAGGGAGTATGTACAGCGAACT
	Vca0245delDOR	ccgggagagctcgatatcgcatgcggtacctctagTGTGCACCGCATCTTCGGCATT
qRT-PCR	toxTf	GCGTTGGGCAGATATTTGTGG
	toxTR	GAAACGCTAGCAAACCCAGAC
	tcpAf	CTACCGCAAACGCAAATGCT
	tcpAR	GGTCAAGCCACCGACTGTAA
	ctxAf	TTGGAGCATTCCCACAACCC
	ctxAR	GCTCCAGCAGCAGATGGTTA
	ToxRf	GGTTTGGCTCGGGTTAGTGA
	toxrR	CAGCCAGCCAATGTTGTGAC
	toxSf	AGTGACGTCTACCCGACTGA
	toxSr	CTGCTGCTCAGTAGCTGGTT
	gyrBf	GTGGTTTCGGTGAAAGTGCC
	gyrBr	GTTTTTCGCTTCGCTTGGGT
	AphAf	CGATGCAACCGGGTACGATA
	AphAr	TGGTTTGCCTTCCTGTGGTT
	AphBf	CTTGGCTGTTGGTCAGTTGC
	AphBr	GGTTGCCAGCCCTCAATACT
	cyaAf	TCATCCTCTGATCCCCGGTT
	cyaAr	ACAAGCCTAAAATCGCCGGA
	Crpf	TCTTGATGCGCCTTTCAGGT
	crpR	TCTGGCTGACGTGCAAGATT
	cpdAf	CATTACTGTGCTTGGCGACG
	cpdAr	GCAAACTGAGTGAGCAGCAG
	tcpPf	GGGGTATGTCCGCGTGATTT
	tcpPr	GCTTGGTGTACCAATCAGCC
	TCPHF	TAACGATCATCGCACTCCCG
	TCPHR	GTGGATCGGTCTGGGTAAGC
PtsI(H189A)	VC0965H189AUPR	AGCCATGATAGAAGTTGCAGAGGTACGGCCGCCAA
	VC0965H189ADOF	GGCGGCCGTACCTCTGCAACTTCTATCATGGCTCGCTCTCT

PtsH(H15A)	VC0966H15AUPR	TGCAGCAGGACGAGTTGCAAGGCCGTTTTCTGCAGT
	VC0966H15ADOF	GCAGAAAACGGCCTTGCAACTCGTCCTGCTGCACAGT
VC0964(H91D)	VC0964H91Dupr	CGTGTCGATACCGAAGTCAACAAACAGCTCAACGCCGTCA
	Vc0964H91Ddof	GTTGAGCTGTTTGTTGACTTCGGTATCGACACGGTTGA

*Lowercase nucleotides indicate the overhangs used for isothermal assembly in the construction of the plasmids.

Table S2 Genes found by TIS to be required for expression and Carb resistance from P*tcpA-bla*

Classificationn	Locus	Gene name	MannU (*p*-value)*	TIGR annotation	Reference
Carb-response related genes	VC2635	*pbp1A*	1.0×10^{-10}	penicillin-binding protein 1A	1
	VC1887	*csiV*	0.00026	hypothetical protein	2
	VC2300	*ampG*	0.00004	AmpG protein, putative	3
	VC0581	*lpoA*	1.0×10^{-10}	lipoprotein, putative	1
Established TCP regulators	VC0838	*tcpN*/*toxT*	0.00001	TCP pilus virulence regulatory protein	4
	VC1049	*aphB*	1.0×10^{-10}	transcriptional regulator, LysR family	5
	VC2647	*aphA*	1.0×10^{-10}	PadR family transcriptional regulator	6
	VC0826	*tcpP*	1.0×10^{-10}	toxin co-regulated pilus biosynthesis protein P	7
	VC0827	*tcpH*	1.0×10^{-10}	toxin co-regulated pilus biosynthesis protein H	8
	VC0984	*toxR*	0.00006	regulatory protein ToxR	9
	VC1021	*luxO*	1.0×10^{-10}	LuxO repressor protein	10
	VC0347	*hfq*	0.00073	host factor-I, putative	11
	VC2529	*rpoN*	1.0×10^{-10}	RNA polymerase sigma-54 factor	12
	VC0034	*tcpG*/*dsbA*	0.00025	thiol:disulfide interchange protein	13
Established factors involved in *in vivo* colonization	VC0965	*ptsI*	1.0×10^{-10}	phosphoenolpyruvate-protein phosphotransferase, EI	14
	VC2433	*cpdA*	0.00419	cyclic AMP phosphodiesterase	14
	VC1097	*pta*	0.00014	phosphate acetyltransferase	14
	VC0724	*pstC-1*	1.0×10^{-10}	phosphate ABC transporter, permease protein	14
	VC0725	*pstA-1*	0.00014	phosphate ABC transporter, permease protein	14
	VC1839	*tolQ*	0.00074	TolQ protein	14,15
	VC1716	*mukF*	0.00094	MukF protein	14
	VC1714	*mukB*	1.0×10^{-10}	cell division protein MukB	14
Other putative TCP regulators	VC0966	*ptsH*	0.00009	Phosphocarrier protein HPr	
	VC0547	*aspK*	0.00103	aspartokinase, alpha and beta subunits	
	VC0419	*cafA*	1.0×10^{-10}	ribonuclease G/cytoplasmic axial filament protein	
	VC2736	*hslO*/*hsp33*	0.00005	33 kDa chaperonin (Heat shock protein 33) (HSP33)	
	VC2153	VC2153	0.00128	D,D carboxypeptidase-related protein	
	VC0346	*miaA*	0.00026	tRNA delta(2)-isopentenylpyrophosphate transferase	
	VC2775	*mnmG/gidA*	0.00372	tRNA uridine 5-carboxymethylaminomethyl modification enzyme	
	VC0556	*gshA*	0.00001	glutamate--cysteine ligase	
	VC2033	*adhE*	1.0×10^{-10}	alcohol dehydrogenase/acetaldehyde dehydrogenase	
	VC1866	*pflB*	0.00012	formate acetyltransferase	
	VC0003	*mnmE*/*trmE*/*thdF*	0.0008	thiophene and furan oxidation protein ThdF	
	VCA0511	*nrdD*	1.0×10^{-10}	anaerobic ribonucleoside-triphosphate reductase	

*Limit of detection and cutoff are p-value $<1.0\times10^{-10}$ and p-value <0.005, respectively, with the Mann Whitney U-based analysis of the TIS data.

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