Supplemental Materials

1) Table S1-S3

2) Figure S1, S2 with figure legends

3) References

Table S1. Bacterial strains used in this study.

Strain	Relevant genotype	Reference/resource
E. coli		
DH5α λpir	*sup E44, ΔlacU169 (ΦlacZΔM15), recA1, endA1, hsdR17, thi-1, gyrA96, relA1, λpir*	Lab collections
SM10λpir	Km^R, *thi-1, thr, leu, tonA, lacY, supE, recA::RP4-2-Tc::Mu, λpir*	Lab collections
XL1-Blue	*end*A1 *gyr*A96 *thi*-1 *hsd*R17 *sup*E44 *rel*A1 *lac*	Lab collections
BTH101	*F-, cya-99, araD139, galE15, galK16, rpsL1 (Strr), hsdR2, mcrA1, mcrB1*	(1)
BTH101 *ΔdsbA*	BTH101 Δ*dsbA*	(2)
V. cholerae		
C6706	wild type, El Tor C6706, Sm^r	(3)
LZV441	C6706 Δ*toxRS,* Δ*tcpPH,*	This study
LZV1122	C6706 Δ*dsbA* Δ*toxRS,* Δ*tcpPH,* Sm^r	This study

Table S2. Plasmids used in this study.

Plasmid	Relevant property	Reference
pWM91	*oriR6KmobRP4*; Ap^r	(4)
pSRKtc	*lac* promoter and *lacI*^{q}, Tet^r	(5)
pACYC117	Multicopy number cloning vector, Km^r	NEB
pUT18	*lac* promoter and the T18 fragment for N-terminal heterologous protein fusion. Amp^r	(1)
pUT18C	pUT18-derived vector, designed to create C-terminal heterologous protein fusion, Amp^r	(1)
pKT25	*lac* promoter and the T25 fragment for C-terminal heterologous protein fusion. Km^r	(1)
pKNT25	pKT25-derived vector, designed to create N-terminal heterologous protein fusion. Km^r	(1)
pUT18C-tcpPH	pUT18C-derived, *tcpPH*, Amp^r	(2)
pKT25- tcpPH	pKT25-derived, *tcpPH*, Amp^r	(2)
pUT18C-tcpPH(C207S)	pUT18C-derived, *tcpP (C207S)tcpH*, Amp^r	(2)
pUT18C-tcpPH(C218S)	pUT18C-derived, *tcpP(C218S)tcpH*, Amp^r	(2)
pUT18C-C_P-tcpH	pUT18C-derived, *tcpP*(1-142 aa), *tcpH*, Amp^r	(2)
pUT18C-C_P-T_P-tcpH	pUT18C-derived, *tcpP*(1-176 aa),*tcpH,*Amp^r	(2)
pUT18C-T_P-P_P-tcpH	pUT18C-derived, *tcpP*(143-223 aa),*tcpH,*Amp^r	(2)
pACYC-tcpPH(C207S)	pACYC117-derived, *tcpP* (C207S), *tcpH*, Km^r	(2)
pACYC-tcpPH(C218S)	pACYC117-derived, *tcpP*(C218S),*tcpH,* Km^r	(2)
pWM91-Δvc0034(dsbA)	pWM91-derived, inframe deletion fragment of *dsbA*. Amp^r	(2)
pAH6-toxT-LacZ	*toxT* - *lacZ* transcriptional fusion reporter. Cm^r	(6)
pUT18C-toxRS(C236S)	pUT18C-derived, *toxR(C236S)*,*toxS,*Amp^r	This study
pUT18C-toxRS	pUT18C-derived, *toxRS*, Amp^r	This study
pUT18C-toxRS(C293S)	pUT18C-derived, *toxR(C293S)*,*toxS,* Amp^r	This study
pUT18C-C_R-T_R	pUT18C-derived, *toxR* (1-193 aa),*toxS,*Amp^r	This study
pUT18C-C_R	pUT18C-derived, *toxR* (1-177 aa),toxS,Amp^r	This study
pUT18C-T_R-P_R	pUT18C-derived, *toxR*(178-293 aa), *toxS,*Amp^r	This study

Plasmid	Relevant property	Reference
pKT25-toxRS	pKT25-derived, *toxRS*, Km^r	This study
pSRKtc-toxRS (C293S)	pSRKtc-derived, *toxR(C293S)*,*toxS,* Tc^r	This study
pSRKtc-toxRS (C236S)	pSRKtc-derived, *toxR(C236S)*,*toxS,* Tc^r	This study
pSRKtc-toxRS	pSRKtc-derived, *toxRS*, Tc^r	This study
pUT18C-C_R-T_P-P_P-tcpH	pUT18C-derived, *toxR(*1-177 aa*)-tcpP(*143-223 aa*), tcpH,*Amp^r	This study
pUT18C-C_P-T_R-P_R	pUT18C-derived, *tcpP(*1-142 aa*)-toxR(*178-293 aa*),*Amp^r	This study
pUT18C-C_R-T_R-P_P-tcpH	pUT18C-derived, *toxR(*1-193 aa*)-tcpP(*177-223 aa*), tcpH,*Amp^r	This study
pKNT25-AphB	pKNT25-derived, *aphB,* Km^r	This study
pUT18-AphB	pUT18-derived, *aphB,* Amp^r	This study
pKNT25-AphB(C235S)	pKNT25-derived, *aphB(C235S),*Km^r	This study
pUT18-AphB(C235S)	pUT18-derived, *aphB(C235S),* Amp^r	This study
pKNT25-ToxT	pKNT25-derived, *toxT,* Km^r	This study
pUT18-ToxT	pUT18-derived, toxT*,* Amp^r	This study
pACYC-AphB(C235S)	pACYC117-derived, containing *bad* promoter, and *aphB(C235S)*, Km^r	(7)

Table S3. Oligonucleotide primers used in this study.

Oligonucleotides	Sequence (5' – 3')[a]	Used in plasmid
toxRS-Nde1-R	GCC CATATGTTCGGATTAGGACACAACTC	pSRKTc-toxRS
toxRS-Spe1-F	CCGACTAGTTTAAGAATTACTGAACAGTACGG	

Oligonucleotides	Sequence (5' – 3')[a]	Used in plasmid
toxR ^{C293S}-F	GCAAGATCCTACTC**AGA**CACTTTGATGG	Overlap PCR primer for *toxR*
toxR ^{C293S}-R	CCATCAAAGTG**TCT**GAGTAGGATCTTGC	*(C293S)toxS* fragment
toxR ^{C236S}-F	GAACTG**TCC**GTTAAAAAATAC	Overlap PCR primer for *toxR*
toxR ^{C236S}-R	GTATTTTTTAAC**GGA**CAGTTC	*(C236S)toxS* fragment
toxRS-XbaI-F1	CGC TCTAGA G ATGTTCGGATTAGGACAC	pKT25-toxRS
toxRS-BamHI-R	CCG GGATCC TTAAGAATTACTGAACAGTACGG	
toxRS-KpnI-R	CGG GGTACC TTAAGAATTACTGAACAGTACGG	pUT18C-toxRS
toxRS-XbaI-F1	CGC TCTAGA G ATGTTCGGATTAGGACAC	pUT18C-toxRS(C293S) pUT18C-toxRS(C236S)
toxRcyto-toxS-overlap-F	ATCCTACTCACACACTGGAGCCGATTTATTCGTCAC	pUT18C-C_R
toxRcyto-toxS-overlap-R	AATAAATCGGCTCCAGTGTGTGAGTAGGATCTTGCTATGC	
toxRcytoTm-toxS-overlap-F	ATCCTACTCACACACGGGAAGTAAGACCGCTATCAGAATAAG CAG	pUT18C-C_R-T_R
toxRcytoTM-toxS-overlap-R	GCGGTCTTACTTCCCGTGTGTGAGTAGGATCTTGCTATGC	
toxR Tm--XbaI-F	CGC TCTAGA GGGGAATCGACTGCTTATTC	pUT18C-T_R-P_R
toxR-Tm-peri-- KpnI-F	CGG GGTACC AGTCAGGTTAAGAATTACTG	
toxR cyto-XbaI-F	CGC TCTAGA GTTCGGATTAGGACACAAC	pUT18C-C_R-T_R-P_P-tcpH
toxR cyto Tm tcpP peri-overlap-R	CATAACTATTGACGGC TACGCCATCGACAAC	
toxR cyto Tm tcpP peri-overlap-F	GTTGTCGATGGCGTA GCCGTCAATAGTTATG	
toxR cyto Tm tcpP peri-KpnI-R	CGG GGTACC CTAAAAATCGCTTTGAC	
toxR cyto-XbaI-F	CGC TCTAGA GTTCGGATTAGGACACAAC	pUT18C-C_R-T_P-P_P-tcpH

Oligonucleotides	Sequence (5' – 3')[a]	Used in plasmid
toxR cyto tcpP Tm-peri-overlap-R	GTTTATCTTCCCCCACTCAATCGATTTATTCGTCAC	
toxR cyto tcpP Tm-peri-overlap-F	GTGACGAATAAATCGATTGAGTGGGGGAAGATAAAC	
toxR cyto tcpP Tm-peri-KpnI-R	CGG GGTACC CTAAAAATCGCTTTGAC	
tcpP-cyto- XbaI-F	CGC TCTAGA GATGGGGTATGTCCGCG	pUT18C-C_P-T_R-P_R
tcpP cyto toxR Tm peri-overlap-R	CGATTCCCCAAGTTTGGAGCGTTTATCTTCCCCCAC	
tcpP cyto toxR Tm peri-overlap-F	GTGGGGGAAGATAAAC GCTCCAAACTTGGGGAATCG	
tcpP cyto toxR Tm peri-KpnI-R	CGGGGTACCGCATAGCAAGATCCTACTCACACAC	
toxT-F-PstI	GACTGCAGGATTGGGAAAAAATCTTTTCAAAC	pKNT25-toxT
toxT-R-KpnI	GTGGTACCGCTTCTGCAACTCCTGTCAAC	pUT18-toxT
aphB-F-PstI	GCGCTGCAGGATGCAACATAATGTGTCAG	pKNT25-aphB
aphB-R-EcoRI	GCGGAATTCGATTGCAGGTGGTAGCCAATCAC	pUT18-aphB and their C235S mutants

[a]Restriction sites are underlined.

[b]Bold letters indicate amino acid change.

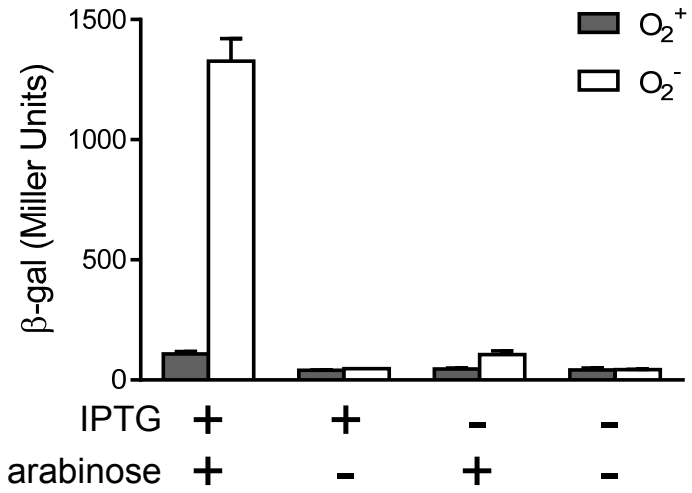

Fig. S1. Both ToxR and TcpP are required for *toxT* expression. *ΔtoxRS ΔtcpPH* mutants containing P_{BAD}-*tcpPH,* P_{lac}-*toxRS* and P_{toxT}-*lacZ* reporter plasmids were grown in AKI aerobically or anaerobically with or without 0.01% arabinose and 1μM IPTG until $OD_{600} \approx 0.3$. β-galactosidase activity was then measured. Data represent the mean ± SD of three independent experiments.

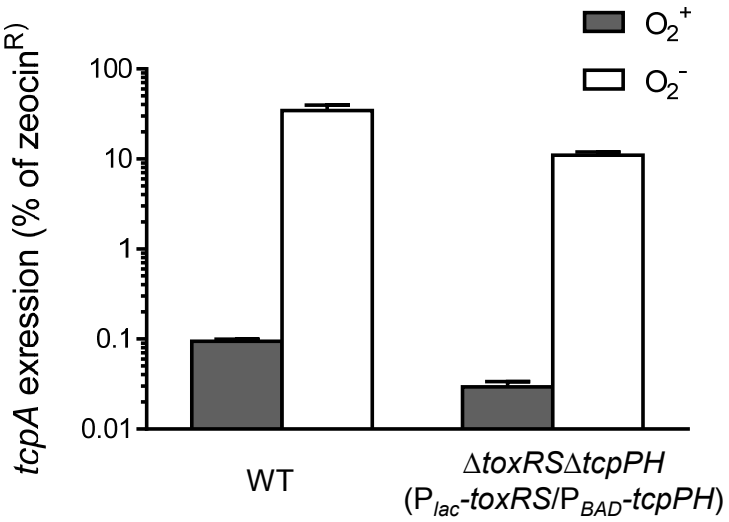

Fig. S2. The effects of ToxR-TcpP interaction on *tcpA* expression. *ΔtoxRS ΔtcpPH* mutants containing P_{BAD}-*tcpPH,* P_{lac}-*toxRS* and P_{toxT}-*lacZ* reporter plasmids were grown in AKI aerobically or anaerobically with or without 0.01% arabinose and 1μM IPTG until $OD_{600} \approx 0.3$. This strain also harbored a P_{tcpA}-*sh ble* reporter (7). The cultures were then treated with 25 μg/ml zeocin in 0.5x LB (pH 8.5) for 30' and the percentage of zeocinR cells were calculated based on the CFU counting.

References

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