

Table S1 Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristic	Source
<i>E. coli</i> strains		
S17-1 λ pir	<i>recA, thi, pro, hsdR-M+</i> [RP4-2-Tc::Mu::Km ^R Tn7] (λ pir); Tp ^R Str ^R	(1)
MC4100	F- <i>araD139</i> Δ (<i>argF-lac</i>)U169 <i>rpsL150 relA1 flbB5301</i> <i>deoC1 ptsF25 rbsR</i> ; Str ^R	(2)
<i>V. cholerae</i> strains		
O395	Classical O1, Ogawa, Str ^R derivative	(3)
RT4368	O395 Δ <i>tcpB1</i> ; Str ^R	(4)
RT4372	O395 Δ <i>tcpF1</i> ; Str ^R	(4)
CL101	O395 CTX-Km Φ ; Str ^R /Km ^R	(4)
TK223	O395 Δ <i>tcpB</i> pTK85, Str ^R /Ap ^R	Laboratory collection
JMM191	O395 Δ <i>tcpB</i> pBAD22, Str ^R /Ap ^R	Laboratory collection
CAH034	O395 Δ <i>tcpB</i> pCHG003, Str ^R /Ap ^R	This study
CAH038	O395 Δ <i>tcpB</i> pCHG005; Str ^R /Ap ^R	This study
CAH067	O395 Δ <i>tcpB</i> pCHG016; Str ^R /Ap ^R	This study
CAH068	O395 Δ <i>tcpB</i> pCHG017; Str ^R /Ap ^R	This study

Strain or plasmid	Relevant characteristic	Source
JMM283	O395 $\Delta tcpB$ pJMA32; Str ^R /Ap ^R	This study
JMM285	O395 $\Delta tcpB$ pJMA33; Str ^R /Ap ^R	This study
JMM329	O395 $\Delta tcpB$ pJMA34; Str ^R /Ap ^R	This study
JMM301	O395 $\Delta tcpB$ pJMA35; Str ^R /Ap ^R	This study
JMM318	O395 $\Delta tcpB$ pJMA36; Str ^R /Ap ^R	This study
JMM319	O395 $\Delta tcpB$ pJMA37; Str ^R /Ap ^R	This study
JMM321	O395 $\Delta tcpB$ pJMA38; Str ^R /Ap ^R	This study
JMM322	O395 $\Delta tcpB$ pJMA39; Str ^R /Ap ^R	This study
JMM323	O395 $\Delta tcpB$ pJMA40; Str ^R /Ap ^R	This study
JMM324	O395 $\Delta tcpB$ pJMA41; Str ^R /Ap ^R	This study
JMM325	O395 $\Delta tcpB$ pJMA42; Str ^R /Ap ^R	This study
JMM327	O395 $\Delta tcpB$ pJMA43; Str ^R /Ap ^R	This study
JMM330	O395 $\Delta tcpB$ pJMA45; Str ^R /Ap ^R	This study
JMM328	O395 $\Delta tcpB$ pJMA46; Str ^R /Ap ^R	This study
JMM371	O395 $\Delta tcpB$ pJMA47; Str ^R /Ap ^R	This study

Strain or plasmid	Relevant characteristic	Source
JMM373	O395 $\Delta tcpB$ pJMA48; Str ^R /Ap ^R	This study
JMM377	O395 $\Delta tcpB$ pJMA49; Str ^R /Ap ^R	This study
JMM369	O395 $\Delta tcpB$ pJMA50; Str ^R /Ap ^R	This study
JMM375	O395 $\Delta tcpB$ pJMA51; Str ^R /Ap ^R	This study
JMM379	O395 $\Delta tcpB$ pJMA52; Str ^R /Ap ^R	This study
JMM382	O395 $\Delta tcpB$ pJMA53; Str ^R /Ap ^R	This study
JMM438	O395 $\Delta tcpB$ pJMA54; Str ^R /Ap ^R	This study
JMM427	O395 $\Delta tcpB$ pJMA55; Str ^R /Ap ^R	This study
JMM428	O395 $\Delta tcpB$ pJMA56; Str ^R /Ap ^R	This study
JMM419	O395 $\Delta tcpB$ pJMA57; Str ^R /Ap ^R	This study
JMM420	O395 $\Delta tcpB$ pJMA58; Str ^R /Ap ^R	This study
JMM421	O395 $\Delta tcpB$ pJMA59; Str ^R /Ap ^R	This study
JMM422	O395 $\Delta tcpB$ pJMA60; Str ^R /Ap ^R	This study
JMM423	O395 $\Delta tcpB$ pJMA61; Str ^R /Ap ^R	This study
JMM424	O395 $\Delta tcpB$ pJMA62; Str ^R /Ap ^R	This study

Strain or plasmid	Relevant characteristic	Source
JMM429	O395 $\Delta tcpB$ pJMA63; Str ^R /Ap ^R	This study
JMM430	O395 $\Delta tcpB$ pJMA64; Str ^R /Ap ^R	This study
JMM431	O395 $\Delta tcpB$ pJMA65; Str ^R /Ap ^R	This study
JMM432	O395 $\Delta tcpB$ pJMA66; Str ^R /Ap ^R	This study
JMM433	O395 $\Delta tcpB$ pJMA67; Str ^R /Ap ^R	This study
JMM434	O395 $\Delta tcpB$ pJMA68; Str ^R /Ap ^R	This study
JMM435	O395 $\Delta tcpB$ pJMA69; Str ^R /Ap ^R	This study
JMM425	O395 $\Delta tcpB$ pJMA70; Str ^R /Ap ^R	This study
JMM426	O395 $\Delta tcpB$ pJMA71; Str ^R /Ap ^R	This study
JMM448	O395 $\Delta tcpB$ pJMA72; Str ^R /Ap ^R	This study
JMM449	O395 $\Delta tcpB$ pJMA73; Str ^R /Ap ^R	This study
JMM450	O395 $\Delta tcpB$ pJMA74; Str ^R /Ap ^R	This study
JMM451	O395 $\Delta tcpB$ pJMA75; Str ^R /Ap ^R	This study
JMM452	O395 $\Delta tcpB$ pJMA76; Str ^R /Ap ^R	This study
JMM458	O395 $\Delta tcpB$ pJMA82; Str ^R /Ap ^R	This study

Strain or plasmid	Relevant characteristic	Source
JMM459	O395 $\Delta tcpB$ pJMA83; Str ^R /Ap ^R	This study
JMM460	O395 $\Delta tcpB$ pJMA84; Str ^R /Ap ^R	This study
JMM461	O395 $\Delta tcpB$ pJMA85; Str ^R /Ap ^R	This study
JMM462	O395 $\Delta tcpB$ pJMA86; Str ^R /Ap ^R	This study
RT4634	O395 <i>tcpB</i> :E5V; Str ^R	This study
RT4635	O395 <i>tcpB</i> :R26E; Str ^R	This study
RT4637	O395 <i>tcpB</i> :E83R; Str ^R	This study
RT4638	O395 <i>tcpB</i> :C85A; Str ^R	This study
RT4639	O395 <i>tcpB</i> :C85S; Str ^R	This study
RT4640	O395 <i>tcpB</i> :C107A; Str ^R	This study
RT4641	O395 <i>tcpB</i> :C107S; Str ^R	This study
RT4642	O395 <i>tcpB</i> :LCWD249-252AAAA; Str ^R	This study
RT4644	O395 <i>tcpB</i> :C250A; Str ^R	This study
RT4645	O395 <i>tcpB</i> :C250S; Str ^R	This study
RT4646	O395 <i>tcpB</i> :W251A; Str ^R	This study

Strain or plasmid	Relevant characteristic	Source
RT4648	O395 <i>tcpB</i> :C261A; Str ^R	This study
RT4649	O395 <i>tcpB</i> :C261S; Str ^R	This study
RT4650	O395 <i>tcpB</i> :K278A; Str ^R	This study
RT4651	O395 <i>tcpB</i> :D281A; Str ^R	This study
RT4652	O395 <i>tcpB</i> :F307A; Str ^R	This study
RT4653	O395 <i>tcpB</i> :K314A; Str ^R	This study
RT4654	O395 <i>tcpB</i> :C321A; Str ^R	This study
RT4655	O395 <i>tcpB</i> :C321S; Str ^R	This study
RT4656	O395 <i>tcpB</i> :KD351-2AA; Str ^R	This study
RT4657	O395 <i>tcpB</i> :S353A; Str ^R	This study
RT4658	O395 <i>tcpB</i> :H366A; Str ^R	This study
RT4659	O395 <i>tcpB</i> :W383A; Str ^R	This study
RT4660	O395 <i>tcpB</i> :Δ392-401, RNPK408-411AKNA; Str ^R	This study
RT4661	O395 <i>tcpB</i> :W420A; Str ^R	This study
RT4662	O395 <i>tcpB</i> :C421S; Str ^R	This study

Strain or plasmid	Relevant characteristic	Source
Plasmids		
pKAS32	pGP704 <i>rpsL</i> ; Ap ^R	(5)
pMIN1	pACYC184 Gm cassette from pUCGM; Gm ^R /Cm ^R	(6)
pTK85	pBAD22 6His- <i>tcpB</i> ; Ap ^R	Laboratory collection
pBAD22	pMB1 <i>Para</i> promoter, <i>araC</i> ; Ap ^R	(7)
pCHG003	pBAD22 6His- <i>tcpB</i> :C421S; Ap ^R	This study
pCHG005	pBAD22 6His- <i>tcpB</i> :W420A; Ap ^R	This study
pCHG016	pBAD22 6His- <i>tcpB</i> :L249A; Ap ^R	This study
pCHG017	pBAD22 6His- <i>tcpB</i> :W251A; Ap ^R	This study
pCHG018	pBAD22 6His- <i>tcpB</i> :D252A; Ap ^R	This study
pJMA32	pBAD22 6His- <i>tcpB</i> :K363A; Ap ^R	This study
pJMA33	pBAD22 6His- <i>tcpB</i> :D384A; Ap ^R	This study
pJMA34	pBAD22 6His- <i>tcpB</i> :KD351-2AA; Ap ^R	This study
pJMA35	pBAD22 6His- <i>tcpB</i> :KD359-60AA; Ap ^R	This study
pJMA36	pBAD22 6His- <i>tcpB</i> :RNPK408-411AAAA; Ap ^R	This study

Strain or plasmid	Relevant characteristic	Source
pJMA37	pBAD22 6His- <i>tcpB</i> :C85A; Ap ^R	This study
pJMA38	pBAD22 6His- <i>tcpB</i> :C250A; Ap ^R	This study
pJMA39	pBAD22 6His- <i>tcpB</i> :C261A; Ap ^R	This study
pJMA40	pBAD22 6His- <i>tcpB</i> :C321A; Ap ^R	This study
pJMA41	pBAD22 6His- <i>tcpB</i> :R328A; Ap ^R	This study
pJMA42	pBAD22 6His- <i>tcpB</i> :E348A; Ap ^R	This study
pJMA43	pBAD22 6His- <i>tcpB</i> :K381A; Ap ^R	This study
pJMA45	pBAD22 6His- <i>tcpB</i> :LCWD249-252AAAA; Ap ^R	This study
pJMA46	pBAD22 6His- <i>tcpB</i> :L262P, KD351-352AA; Ap ^R	This study
pJMA47	pBAD22; 6His- <i>tcpB</i> :D309A; Ap ^R	This study
pJMA48	pBAD22 6His- <i>tcpB</i> :K295A; Ap ^R	This study
pJMA49	pBAD22 6His- <i>tcpB</i> :K278A; Ap ^R	This study
pJMA50	pBAD22 6His- <i>tcpB</i> :K314A; Ap ^R	This study
pJMA51	pBAD22 6His- <i>tcpB</i> :D281A; Ap ^R	This study
pJMA52	pBAD22 6His- <i>tcpB</i> :K272A; Ap ^R	This study

Strain or plasmid	Relevant characteristic	Source
pJMA53	pBAD22 6His- <i>tcpB</i> :K297A; Ap ^R	This study
pJMA54	pBAD22 6His- <i>tcpB</i> :C107A; Ap ^R	This study
pJMA55	pBAD22 6His- <i>tcpB</i> :E392A; Ap ^R	This study
pJMA56	pBAD22 6His- <i>tcpB</i> :D402A; Ap ^R	This study
pJMA57	pBAD22 6His- <i>tcpB</i> :RNPK408-411ANPA; Ap ^R	This study
pJMA58	pBAD22 6His- <i>tcpB</i> :D252A; Ap ^R	This study
pJMA59	pBAD22 6His- <i>tcpB</i> :H366A; Ap ^R	This study
pJMA60	pBAD22 6His- <i>tcpB</i> :K286A; Ap ^R	This study
pJMA61	pBAD22 6His- <i>tcpB</i> :D276A; Ap ^R	This study
pJMA62	pBAD22 6His- <i>tcpB</i> :E274A; Ap ^R	This study
pJMA63	pBAD22 6His- <i>tcpB</i> :W383A; Ap ^R	This study
pJMA64	pBAD22 6His- <i>tcpB</i> :S370A; Ap ^R	This study
pJMA65	pBAD22 6His- <i>tcpB</i> :L369A; Ap ^R	This study
pJMA66	pBAD22 6His- <i>tcpB</i> :S362A; Ap ^R	This study
pJMA67	pBAD22 6His- <i>tcpB</i> :S353A; Ap ^R	This study

Strain or plasmid	Relevant characteristic	Source
pJMA68	pBAD22 6His- <i>tcpB</i> :SS334-335AA; Ap ^R	This study
pJMA69	pBAD22 6His- <i>tcpB</i> :F307A; Ap ^R	This study
pJMA70	pBAD22 6His- <i>tcpB</i> :E392A, D402A; Ap ^R	This study
pJMA71	pBAD22 6His- <i>tcpB</i> :Δ392-401, RNPK408-411AKNA; Ap ^R	This study
pJMA72	pBAD22 6His- <i>tcpB</i> :C85S; Ap ^R	This study
pJMA73	pBAD22 6His- <i>tcpB</i> :C107S; Ap ^R	This study
pJMA74	pBAD22 6His- <i>tcpB</i> :C321S; Ap ^R	This study
pJMA75	pBAD22 6His- <i>tcpB</i> :C250S; Ap ^R	This study
pJMA76	pBAD22 6His- <i>tcpB</i> :C261S; Ap ^R	This study
pJMA82	pBAD22 6His- <i>tcpB</i> :E5V; Ap ^R	This study
pJMA83	pBAD22 6His- <i>tcpB</i> :R26E; Ap ^R	This study
pJMA84	pBAD22 6His- <i>tcpB</i> :E83R; Ap ^R	This study
pJMA85	pBAD22 6His- <i>tcpB</i> :K81A; Ap ^R	This study
pJMA86	pBAD22 6His- <i>tcpB</i> :W79A; Ap ^R	This study
pTRNS101	pKAS32 <i>tcpB</i> ; Ap ^R	This study

Strain or plasmid	Relevant characteristic	Source
pCHG006	pKAS32 <i>tcpB</i> :E5V; Ap ^R	This study
pCHG010	pKAS32 <i>tcpB</i> :R26E; Ap ^R	This study
pCHG007	pKAS32 <i>tcpB</i> :E83R; Ap ^R	This study
pCHG008	pKAS32 <i>tcpB</i> :C85A; Ap ^R	This study
pCHG011	pKAS32 <i>tcpB</i> :C85S; Ap ^R	This study
pCHG014	pKAS32 <i>tcpB</i> :C107A; Ap ^R	This study
pCHG009	pKAS32 <i>tcpB</i> :C107S; Ap ^R	This study
pCHG015	pKAS32 <i>tcpB</i> :C250A; Ap ^R	This study
pTRNS118	pKAS32 <i>tcpB</i> :C250S; Ap ^R	This study
pCHG020	pKAS32 <i>tcpB</i> :W251A; Ap ^R	This study
pCHG012	pKAS32 <i>tcpB</i> :C261A; Ap ^R	This study
pTRNS119	pKAS32 <i>tcpB</i> :C261S; Ap ^R	This study
pTRNS112	pKAS32 <i>tcpB</i> :K278A; Ap ^R	This study
pTRNS109	pKAS32 <i>tcpB</i> :D281A; Ap ^R	This study
pCHG013	pKAS32 <i>tcpB</i> :F307A; Ap ^R	This study

Strain or plasmid	Relevant characteristic	Source
pTRNS110	pKAS32 <i>tcpB</i> :K314A; Ap ^R	This study
pTRNS111	pKAS32 <i>tcpB</i> :C321A; Ap ^R	This study
pTRNS116	pKAS32 <i>tcpB</i> :C321S; Ap ^R	This study
pTRNS113	pKAS32 <i>tcpB</i> :KD351-2AA; Ap ^R	This study
pTRNS132	pKAS32 <i>tcpB</i> :S353A; Ap ^R	This study
pTRNS115	pKAS32 <i>tcpB</i> :H366A; Ap ^R	This study
pTRNS114	pKAS32 <i>tcpB</i> :W383A; Ap ^R	This study
pTRNS120	pKAS32 <i>tcpB</i> :Δ392-401, RNPK408-411AKNA; Ap ^R	This study
pTRNS133	pKAS32 <i>tcpB</i> :C421S; Ap ^R	This study

Abbreviations used: Ap^R - ampicillin resistance; Km^R - kanamycin resistance; Str^R - streptomycin resistance; Tp^R - trimethoprim resistance

|

Literature Cited.

1. **DeLorenzo RA, Vista JP.** 1994. Another hazard of hypertonic dextrose. *Am J Emerg Med* **12**:262-263.
2. **Silhavy TJ, Berman ML, Enquist LW, Cold Spring Harbor Laboratory.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. **Taylor RK, Miller VL, Furlong DB, Mekalanos JJ.** 1987. Use of *phoA* gene fusions to identify a pilus colonization factor coordinately regulated with cholera toxin. *Proc Natl Acad Sci U S A* **84**:2833-2837.
4. **Kirn TJ, Bose N, Taylor RK.** 2003. Secretion of a soluble colonization factor by the TCP type 4 pilus biogenesis pathway in *Vibrio cholerae*. *Mol Microbiol* **49**:81-92.
5. **Skorupski K, Taylor RK.** 1996. Positive selection vectors for allelic exchange. *Gene* **169**:47-52.
6. **Nye MB, Pfau JD, Skorupski K, Taylor RK.** 2000. *Vibrio cholerae* H-NS silences virulence gene expression at multiple steps in the ToxR regulatory cascade. *J Bacteriol* **182**:4295-4303.
7. **Guzman LM, Belin D, Carson MJ, Beckwith J.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J Bacteriol* **177**:4121-4130.