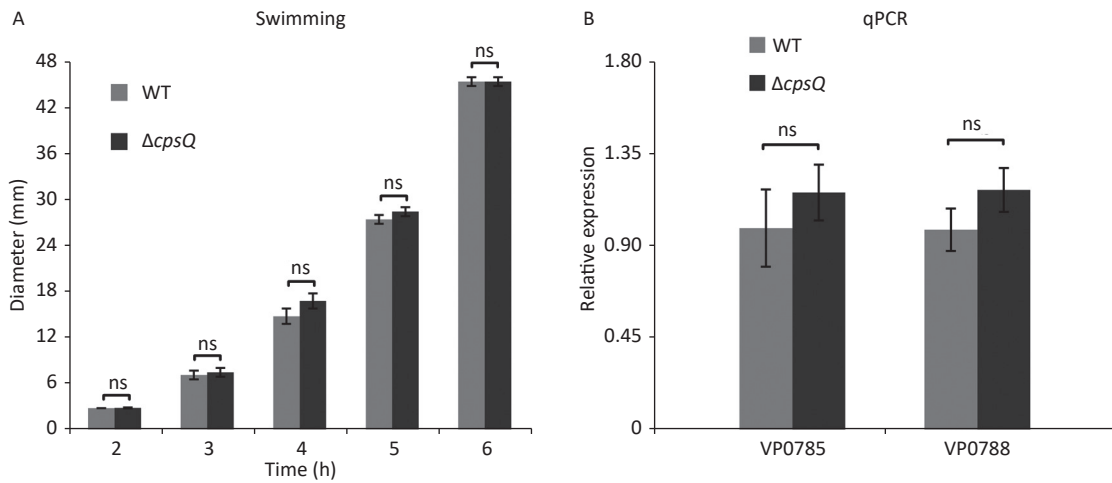
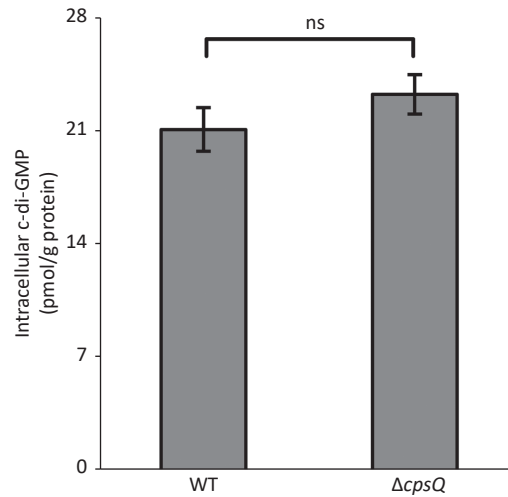


**Supplementary Figure S1.** Growth curves of WT and  $\Delta cpsQ$ . *V. parahaemolyticus* strains were grown in HI broth at 37 °C with shaking at 200 rpm, and the OD<sub>600</sub> values of each culture were monitored at 1 h intervals. Experiments were performed thrice with three different colonies per experiment.



**Supplementary Figure S2.** CpsQ did not affect the swimming motility of *V. parahaemolyticus*. (A) Swimming motility of WT and  $\Delta cpsQ$  was evaluated by measuring the diameter of swimming areas in a semi-solid agar. (B) qPCR. Relative mRNA levels of each target gene were compared between WT and  $\Delta cpsQ$ . Results were analyzed using paired Student's *t*-test. ns,  $P > 0.05$ .



**Supplementary Figure S3.** CpsQ did not affect the c-di-GMP pool in *V. parahaemolyticus*. Data are expressed as the mean  $\pm$  SD of values from at least three independent experiments. Results were analyzed using paired Student's *t*-test. ns,  $P > 0.05$ .

**Supplementary Table S1.** Selected genes from the CpsQ regulon

Locus	Name	FoldChange	Product
Lateral flagella			
VPA0261		2.077	flagellar export chaperone FlgN
Polar flagellum			
VP0774		0.358	protein-glutamate O-methyltransferase
VP0776	<i>flgC</i>	0.300	flagellar basal body rod protein FlgC
VP0777	<i>flgD</i>	0.212	flagellar hook assembly protein FlgD
VP0778	<i>flgE</i>	0.463	flagellar hook protein FlgE
VP0780		0.180	flagellar basal body rod protein FlgF
VP0781	<i>flgG</i>	0.243	flagellar basal-body rod protein FlgG
VP0782	<i>flgH</i>	0.274	flagellar basal body L-ring protein FlgH
VP0783		0.218	flagellar basal body P-ring protein FlgI
VP0784	<i>flgJ</i>	0.253	flagellar assembly peptidoglycan hydrolase FlgJ
VP0785	<i>flgK</i>	0.281	flagellar hook-associated protein FlgK
VP0786	<i>flgL</i>	0.280	flagellar hook-associated protein FlgL
VP0788	<i>flaC</i>	0.254	flagellin
VP2111		0.231	OmpA family protein
VP2225		0.458	chemotaxis protein CheW
VP2227		0.441	ParA family protein
VP2229		0.391	chemotaxis protein CheA
VP2231	<i>cheY</i>	0.486	chemotaxis response regulator CheY
VP2232		0.478	RNA polymerase sigma factor FliA
VP2233		0.415	MinD/ParA family protein
VP2237	<i>fliR</i>	0.483	flagellar type III secretion system protein FliR
VP2241	<i>fliN</i>	0.265	flagellar motor switch protein FliN

Continued

Locus	Name	FoldChange	Product
VP2242	<i>fliM</i>	0.460	flagellar motor switch protein FliM
VP2244		0.465	flagellar hook-length control protein FliK
VP2246	<i>fliI</i>	0.380	flagellar protein export ATPase FliI
VP2249	<i>fliF</i>	0.432	flagellar M-ring protein FliF
VP2251		0.414	sigma-54 dependent transcriptional regulator
VP2252		0.444	PAS domain-containing protein
VP2256	<i>fliD</i>	0.483	flagellar filament capping protein FliD
Scv exopolysaccharide			
VP1464		0.300	O-antigen ligase family protein
VP1469	<i>scvE</i>	3.975	sigma-54 dependent transcriptional regulator
Type IV pilin			
VP2524	<i>pilB</i>	3.295	type IV-A pilus assembly ATPase PilB
c-di-GMP			
VP1255		0.375	GGDEF-only
VP1483		0.481	GGDEF-only
VP1881	<i>tpdA</i>	0.421	EAL-only
VP1979		3.129	EAL-only
VPA0360		0.320	GGDEF-only
VPA0518		2.100	hybrid
T3SS1			
VP1657	<i>vopB</i>	2.380	type III secretion system translocon subunit VopB
T3SS2			
VPA1326		2.725	hypothetical protein
VPA1327	<i>vopT</i>	2.644	T3SS effector ADP-ribosyltransferase toxin VopT
VPA1329		3.741	conjugal transfer protein TraA
VPA1345		2.128	hypothetical protein
VPA1346	<i>vopA</i>	4.834	type III secretion system YopJ family effector VopA
T6SS1			
VP1416		0.462	hypothetical protein
T6SS2			
VPA1024		5.741	hypothetical protein
VPA1025		2.905	PAAR domain-containing protein
VPA1026	<i>vgrG</i>	10.472	type VI secretion system tip protein VgrG
VPA1027		10.854	type VI secretion system tube protein Hcp
VPA1028	<i>tssH</i>	8.675	type VI secretion system ATPase TssH
VPA1029	<i>tssG</i>	14.030	type VI secretion system baseplate subunit TssG
VPA1030	<i>tssF</i>	13.979	type VI secretion system baseplate subunit TssF
VPA1031	<i>tssE</i>	5.256	type VI secretion system baseplate subunit TssE
VPA1032		7.011	protein of avirulence locus
VPA1033	<i>tssC</i>	10.549	type VI secretion system contractile sheath large subunit

Continued

Locus	Name	FoldChange	Product
VPA1034	<i>tssC</i>	3.575	type VI secretion system contractile sheath large subunit
VPA1035	<i>tssB</i>	7.754	type VI secretion system contractile sheath small subunit
VPA1036	<i>tssA</i>	9.924	type VI secretion system protein TssA
VPA1037		6.385	protein phosphatase 2C domain-containing protein
VPA1038	<i>tagF</i>	8.848	type VI secretion system-associated protein TagF
VPA1039	<i>tssM</i>	5.244	type VI secretion system membrane subunit TssM
VPA1040	<i>tssL</i>	4.422	type VI secretion system protein TssL%2C long form
VPA1041	<i>tssK</i>	6.433	type VI secretion system baseplate subunit TssK
VPA1042	<i>tssJ</i>	3.863	type VI secretion system lipoprotein TssJ
VPA1043	<i>tagH</i>	4.076	type VI secretion system-associated FHA domain protein TagH
VPA1044		4.890	serine/threonine protein kinase
VPA1045		3.486	response regulator
VPA1046		4.304	hypothetical protein
Extracellular proteases			
VPA1071		2.654	S8 family serine peptidase
Putative regulatory genes			
VP0080		2.651	sigma-54 dependent transcriptional regulator
VP0358		0.399	DeoR family transcriptional regulator
VP0838	<i>seqA</i>	0.318	replication initiation negative regulator SeqA
VP1032	<i>torR</i>	0.439	two-component system response regulator TorR
VP1382		2.345	LysR family transcriptional regulator
VP1469	<i>scvE</i>	3.975	sigma-54 dependent transcriptional regulator
VP1649		2.449	GntR family transcriptional regulator
VP1906		0.415	MarR family transcriptional regulator
VP1939		2.411	transcriptional regulator
VP2037		0.421	chemotaxis protein CheV, response regulator
VP2229		0.391	chemotaxis protein CheA, histidine kinase
VP2231		0.486	chemotaxis response regulator CheY, response regulator
VP2251		0.414	sigma-54 dependent transcriptional regulator
VP2252		0.444	PAS domain-containing protein
VP2299	<i>glnB</i>	0.430	nitrogen regulatory protein P-II
VP2603		3.631	LysR family transcriptional regulator
VP2710	<i>scrP</i>	2.352	helix-turn-helix transcriptional regulator
VPA0358		0.229	helix-turn-helix transcriptional regulator
VPA0420		0.486	TetR/AcrR family transcriptional regulator
VPA0431		0.455	chemotaxis protein, response regulator
VPA0619		3.870	MerR family DNA-binding transcriptional regulator
VPA0663		2.174	AraC family transcriptional regulator
VPA0717		0.253	LysR family transcriptional regulator
VPA0740		3.494	LysR family transcriptional regulator

Continued

Locus	Name	FoldChange	Product
VPA0746		0.414	chemotaxis protein CheV, response regulator
VPA0826	<i>pgtB</i>	0.388	phosphoglycerate transport regulatory protein, histidine kinase
VPA1045		3.486	response regulator
VPA1162		0.405	response regulator
VPA1423		2.961	AraC family transcriptional regulator
VPA1562		2.684	TetR/AcrR family transcriptional regulator
VPA1729		0.407	helix-turn-helix transcriptional regulator
Antioxidative genes			
VPA1418	<i>katE1</i>	2.095	catalase
VPA1683	<i>ahpC1</i>	2.349	alkyl hydroperoxide reductase subunit C
Outer membrane proteins			
VP1008		0.059	porin
VP1454		0.424	porin family protein
VP1634		2.687	TolC family outer membrane protein
VP2176	<i>aqpZ</i>	2.701	aquaporin Z
VP2362		0.344	outer membrane protein OmpK
VP2385		2.115	aquaporin
VP2467		3.833	porin
VPA0222		2.670	porin

**Supplementary Table S2.** Primers used in this study

Target	Primers (forward/reverse, 5'-3')
Construction of mutant	
<i>cpsQ</i>	GTGACTGCAGGGTTCTCCAAGGCGATATG/CTTGTGGCTTGGCTCCTATGCTTTTCCGTGACTGTTCC GAACAGTACACGGAAAAGCATAGGACGCAAGCCACAAG/GTGAGCATGCCACCAGTTAGACGATCATTG GTGACTGCAGGGTTCTCCAAGGCGATATG/GTGAGCATGCCACCAGTTAGACGATCATTG
qPCR	
VP0785	GCCGTCAGTCAGTGATTC/GTAGAGGACAGGTTGAGTTC
VP0788	AATAAAGCGACCAACGAGCTG/TGCCACATCAAGACCACGAGA
VP1469	GACAGGTCGTGATGCCATTC/GGCGATGATGACCGAAGTG
VPA1027	TAAAGGTGAAGCGACAGCG/AATCATATAGGCGTGTTC
VPA1043	TGACCATAACGAGTTTCCAC/TTTAATCAATTCGCCGTGAG
VPA1044	ATAGCAGCGATAGCGGAG/TTTGAGACAGTTTGTATCC
VPA1327	ATAGCAGCGATAGCGGAG/TTTGAGACAGTTTGTATCC
VP1255	GTAAGCTCGTCATCACACCTG/GCCGTAAGTACCCACCTTCG
VP1483	TCAAAGTGATCGACGGACCA/AATTACCTCGTACCCGTTG
VP1979	TTAAAGCCAGCGATGTAACCC/GGCGAATCTGTTCTAACGCAA
VPA0360	TCGTTCTTTACCTACGCCTTA/TGCCAATAAAGACTCGATAGAGC
VPA0518	GAAACATTAACGCAAGCC/TCACCAATGTAGTTCCCGTTG
VP1906	AAAGCTGAGCAAAGAGTCGGG/GTTGCTTGACTCATATTGGTG
VPA0663	AGGCGATTCAAGTTATGCGAAA/GTGGCTCCAGTTTTGGGTC
VPA0717	CTATATTTAACCAACCAGCC/GATCGAAGACTTCAGCCCCTA
VPA1418	GATTTAGTCGGCAACAACAC/ATCCCAGTTGTTGTCGAGC
VPA1683	AGACCACTACGAAGAGCTAC/GTACTGGATCTTGCCGATTG
VP1071	TCAGAACAATGGCATCTCGAC/ATGGCTTGCCTAAGTTGGTG
VP1008	AGGCAACATCTACGACAACGG/AGCCCAAAGGTCAACGTCTAC
VP2362	ACCTAGCGTCAGACAAAGGC/TGAACTGGACCGAAAGACAGG
LacZ fusion	
VPA1027	GCGCGTCGACTATTACCTTACTTGCCTCTCGG/GCGCGAATTCTGCTTCACGGTCCATTGC
VPA1043	GCGCGTCGACTTTGTTGATAGGTTGGTATTGTG/ATATGAATTCTGAGCGTCCGAAGGTTAC