

1 **Supplemental Material for:**

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3 **The Cpx system regulates virulence gene expression in *Vibrio cholerae***

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11 Running Title: Cpx regulation of CT and TcpA elaboration

12 Key words: Cpx / envelope stress / cholera toxin / toxin-coregulated pilus/ virulence

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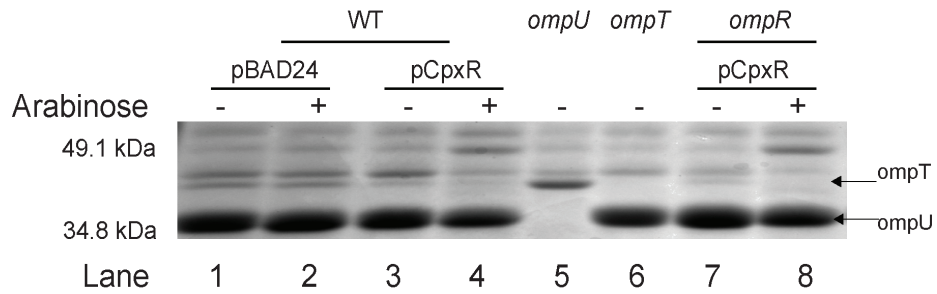
14 **Table S1.** Primers used in this study.

<b>Primer name</b>	<b>Oligonucleotide sequence 5' to 3' (restriction sites underlined)</b>
F1	TTGGATCC CTTTACCTTCTTCACGCAGAT
F2	TTAGATGTTCCGATTAGGAAAAGTGTGTGAGTAGGATC
R1	GATCCTACTCACACACTTTTCCTAATCCGAACATCTAA
R2	CTGCGGCCGCCTTCAGCACTTTGGCTTCT
F3	CTGGATCCCAGGAAAACCGACATTTC
F4	AATTATGTCTAGAAGGATTTCAACCAAAGCCTAAGAGG
R3	CCTCTTAGGCTTTGGTTGAAATCCTTCTAGACATAATT
R4	TTGCGGCCGCAGAAACGCTCCGTAAAC
luxFtoxR	TTGAATTC <sup>CCG</sup> TACCCGATTTAGCAA
luxRtoxR	TTGGATCC <sup>CG</sup> TGCTGCCTAATCGAA
luxctxAF	TTGAATTCACGGCTTACACGACAATCCA
luxctxAR	TTGGATCCTGGCATAAGACCACCTGACT
luxtcpAF	TTGAATTCAGCCGCCTAGATAGTCTGTG
luxtcpAR	TTGGATCCATCAATCGCACGCTGAGCCA
luxtcpPF	TTGAATTCCTTGTGCCTGCTGAGAACT
luxtcpPR	TTGGATCCTGGTGTACCAATCAGCCT
luxtoxTF	TTGAATTC <sup>CT</sup> TGGTGCAATGATCGCAGT
luxtoxTR	TTGGATCCAAGCTTTGCAATTCCACT

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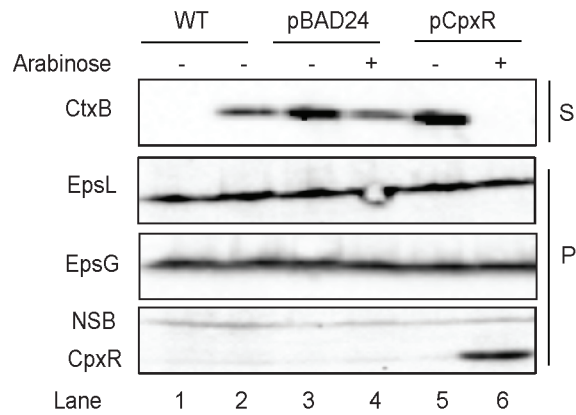
toxSF	CATCGCCATGGGTATTCTTC	15
toxSR	GTCACTCCCCCAATATAACCAG	
toxRF	GATTAGGCAGCAACGAAAGC	
toxRR	AATCACCTCGTTTGGACGTT	
ctxBF	GCGATTGAAAGGATGAAGGA	
ctxBR	ATCGCATGAGGCGTTTTATT	
tcpAF	TTGGTCAGCCTTGGTAAGGT	
tcpAR	CCCCATAGCTGTACCAGTGAA	
tcpPF	TGAAAGTCTAACTCAGGCAATCAA	
tcpPR	TTTCGATCAACGTCTTATGTTCA	
aphAF	AACCGTGCGTGATGAGTTTA	
aphAR	GGTAAGGTTCTGCCGATTGT	
aphBF	GATGCTGCGTGAATTTCTTG	
aphBR	TGAGCTCCAATCCGACAGTA	
crpF	TCAGGTCAAATGGCTCGTC	
crpR	ACGTCTAGGAACGCAAGGTC	

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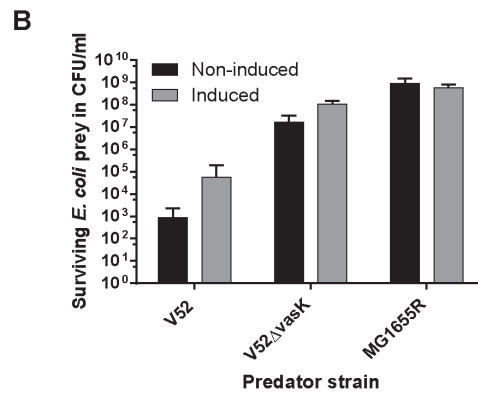
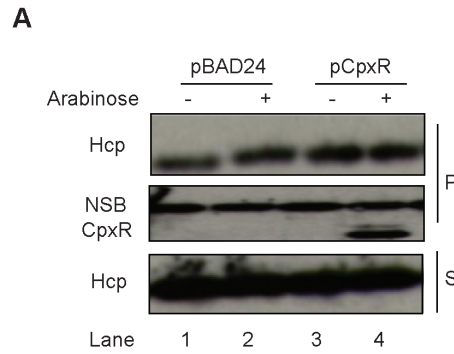
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17 **Figure S1. Cpx-mediated negative regulation of OmpT expression is OmpR-**  
 18 **independent.** Outer membrane (OM) protein profiles of wild-type strain C6706 carrying  
 19 either pBAD24 (lane 1 and 2) or pCpxR (lane 3 and 4), *ompU* (lane 5), *ompT* (lane 6) and  
 20 *ompR* strain carrying pCpxR (lane 7 and 8) grown in AKI conditions at 37°C as described  
 21 in Materials and Methods. OM proteins were resolved by 10% SDS-PAGE followed by  
 22 staining with Coomassie blue. The Cpx pathway was activated by over-expressing CpxR  
 23 from an arabinose inducible promoter in the presence of 0.1% of arabinose. Samples were  
 24 collected from each strain at least three times; one representative SDS-PAGE is shown.



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26 **Figure S2. Activation of the Cpx pathway in *V. cholerae* does not affect the**  
 27 **expression of the T2SS.** Cell pellets (P) and supernatants (S) were collected from *V.*  
 28 *cholerae* C6706 grown in LB (lane 1) or AKI medium (lanes 2 to 6) as described in  
 29 Materials and Methods. Subcultures were grown for 6 h statically at 37°C before the  
 30 addition of 0.1% arabinose to induce CpxR over-expression, followed by an additional 16  
 31 h of incubation at 37°C. Western analysis using antibodies directed against CtxB, EpsL,  
 32 EpsG and CpxR from wild-type C6706 strain (lanes 1 and 2), C6706 carrying the vector  
 33 control (pBAD24) (lanes 3 and 4) or the over-expression plasmid pCpxR (lanes 5 and 6).  
 34 Non-specific band (NSB).



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36 **Figure S3. The Cpx response does not affect Hcp expression in *V. cholerae* V52.** (A)

37 Cell pellets (P) and supernatants (S) were collected from *V. cholerae* V52 carrying the

38 vector control (pBAD24, lanes 1 and 2) or pCpxR (lanes 3 and 4) grown in LB as

39 described in Materials and Methods. Subcultures were grown for 1.5 h at 37°C before the

40 addition of 0.1% arabinose to induce CpxR over-expression. Western blot analysis was

41 performed using antibodies directed against Hcp and CpxR. Non-specific bands (NSB).

42 (B) Bacterial killing assay using *E. coli* MG1655R (pBAD24) as prey strain and *V.*

43 *cholerae* strains V52 (pCpxR) and V52 $\Delta$ vasK (pBAD24) as predator strains. *vasK*

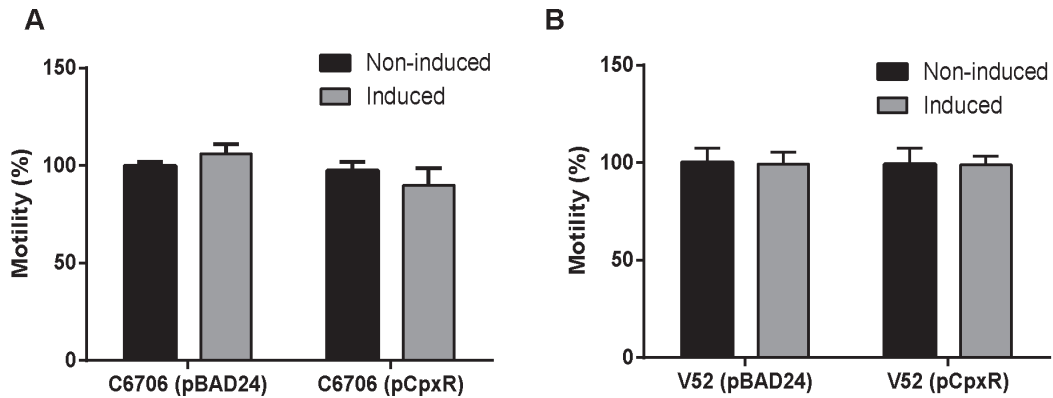
44 encodes a core protein of the T6SS necessary for its function. *E. coli* MG1655R

45 (pBAD24) was also included as predator strain for a negative control of T6SS-mediated

46 killing. Surviving *E. coli* prey was calculated by counting the number of viable cells after

47 4 h of killing in the absence (non-induced) (black bars) or presence of 0.1% arabinose  
48 (induced) (grey bars).

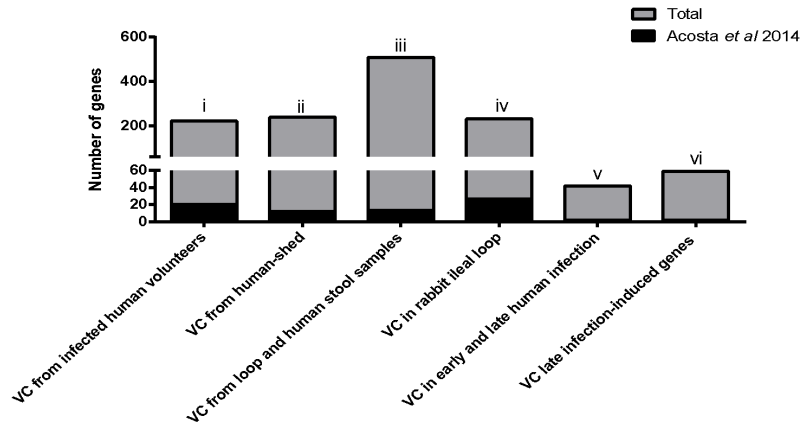
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51 **Figure S4. Motility of *V. cholerae* C6706 and V52 when the Cpx pathway is**  
52 **activated.** Overnight cultures for C6706 (A) and V52 (B) strains carrying either the  
53 vector control (pBAD24) or pCpxR were inoculated onto 0.3% LB agar plates (non-  
54 induced) or 0.3% LB agar plates containing 0.1% arabinose (induced). The growth  
55 diameter was recorded after 16 h. Each strain was inoculated by triplicate and the average  
56 and standard deviation are indicated.

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59 **Figure S5. Comparative analysis between genes that changed expression when the**  
 60 **Cpx pathway was activated and previous genomics research on *V. cholerae*.**

61 Comparative analysis of the 174 genes that we found to be CpxR regulated (55) with  
 62 genes that were either induced or repressed in samples from: i) human healthy volunteers  
 63 infected with *V. cholerae* (97) ii) human cholera stool respect to stationary phase in vitro  
 64 (98), iii) genes shared in intestinal loop model and human cholera stools (17), iv) rabbit  
 65 intestinal loop model respect to exponential phase (99), v) stool or vomitus sample during  
 66 early and late stages of human infection (101); and vi) late infection-induced genes in  
 67 mice (100). VC: *Vibrio cholerae*.

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