SUPPORTING INFORMATION

Article title: Novel cholera toxin variant and ToxT regulon in environmental *Vibrio mimicus* strains: potential resources for the evolution of *Vibrio cholerae* hybrid strains

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KEY WORDS: *Vibrio mimicus*, cholera toxin, CTXΦ, *tcpA*, *toxT*, *Vibrio cholerae* classical and El Tor biotypes

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23 **Table S1: Suckling mice assay showing enterotoxigenic potential of the *ctx*$^{+ve}$ *V. mimicus***

24 **strains**[a]

Set (Strain)	Category	Dose	No. of mice	Avg. FA ratio	±SD	Diarrhea
A (VC O395, pos contr)	Live cell	*~10^{7} CFU*	**5**	0.098	0.010	**5/5**
	CFS	*~200 ng CT*	**3**	0.104	0.008	**2/2**
B (VM ATCC, neg contr)	Live cell	*~10^{7} CFU*	**5**	0.062	0.002	**0/5**
	CFS	*No CT*	**3**	0.063	0.002	**0/2**
C (Vm 2, low CT producer)	Live cell	*~10^{7} CFU*	**5**	0.068	0.002	**0/5**
	CFS	*~0.05 ng CT*	**3**	0.069	0.003	**0/2**
	Live cell	*>5 x 10^{9} CFU*	**3**	0.066	0.001	**0/2**
D (Vm 7, high CT producer)	Live cell	*~10^{7} CFU*	**5**	0.087	0.003	**5/5**
	CFS	*~100 ng CT*	**3**	0.085	0.005	**2/2**
	Live cell	*~10^{6} CFU*	**3**	0.086	0.004	**3/3**
	Live cell	*~10^{5} CFU*	**3**	0.072	0.003	**0/3**

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26 [a]VC, VM, CFS, and FA indicates *V. cholerae*, *V. mimicus*, Cell free supernatant, and fluid

27 accumulation, respectively.

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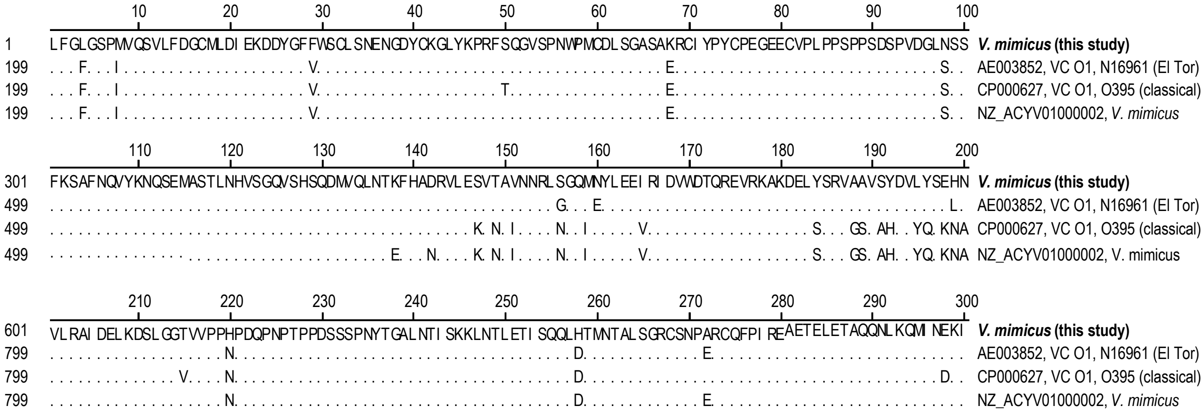

Fig. S1. Comparative variations in deduced amino acids of *orfU* gene sequences in selected *V. mimicus* and *V. cholerae* strains. Sequences were aligned by ClustalW algorithm. Amino acid positions are shown as a heading scale. Strain details are shown on the right border at each row. In comparison to *V. mimicus* strain in this study, only mismatched amino acids of *orfU* genes in other selected strains are shown while their identical amino acids are indicated by dots.

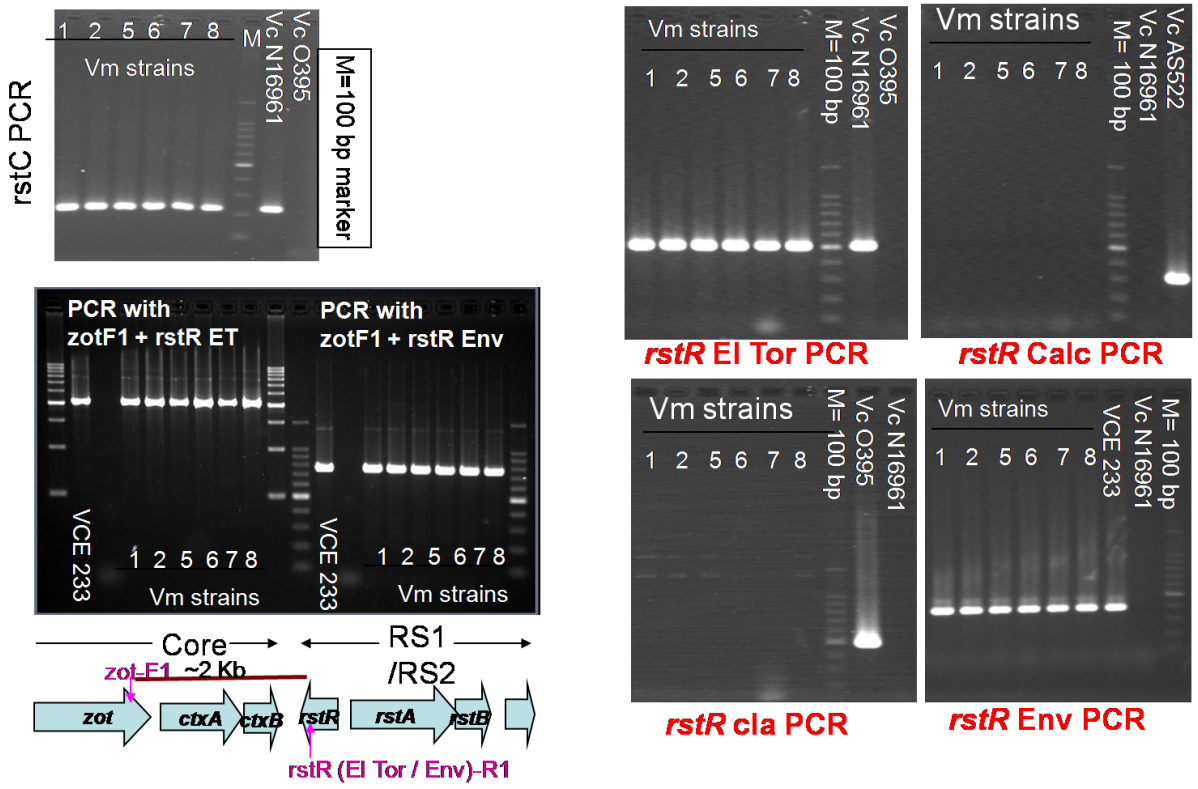

Fig. S2. PCR detection of the *rstC* (RS1), *rstR*$^{\text{El Tor}}$, *rstR*^{Calc}, *rstR*^{Cla} and *rstR*^{Env} genes, and the presence or absence of *ctxAB* in the El Tor type CTXΦ and environmental type CTXΦ in *V. mimicus* (Vm) strains. *V. cholerae* (Vc) strains belonging to O1 El Tor (N16961), O1 classical (O395), and non-O1/non-O139 (AS522 and VCE233) were used as controls. Environmental *V. mimicus* strains were positive for *rstC* (RS1 element), *rstR*$^{\text{El Tor}}$, and *rstR*^{Env} genes but did not contain *rstR*^{Calc} and *rstR*^{Cla} genes. Similar to a *V. cholerae* non-O1/non-O139 strain, VCE233, all of the environmental *V. mimicus* strains contained *ctxAB* in the El Tor type CTXΦ but did not possess any *ctxAB* in the environmental type CTXΦ.

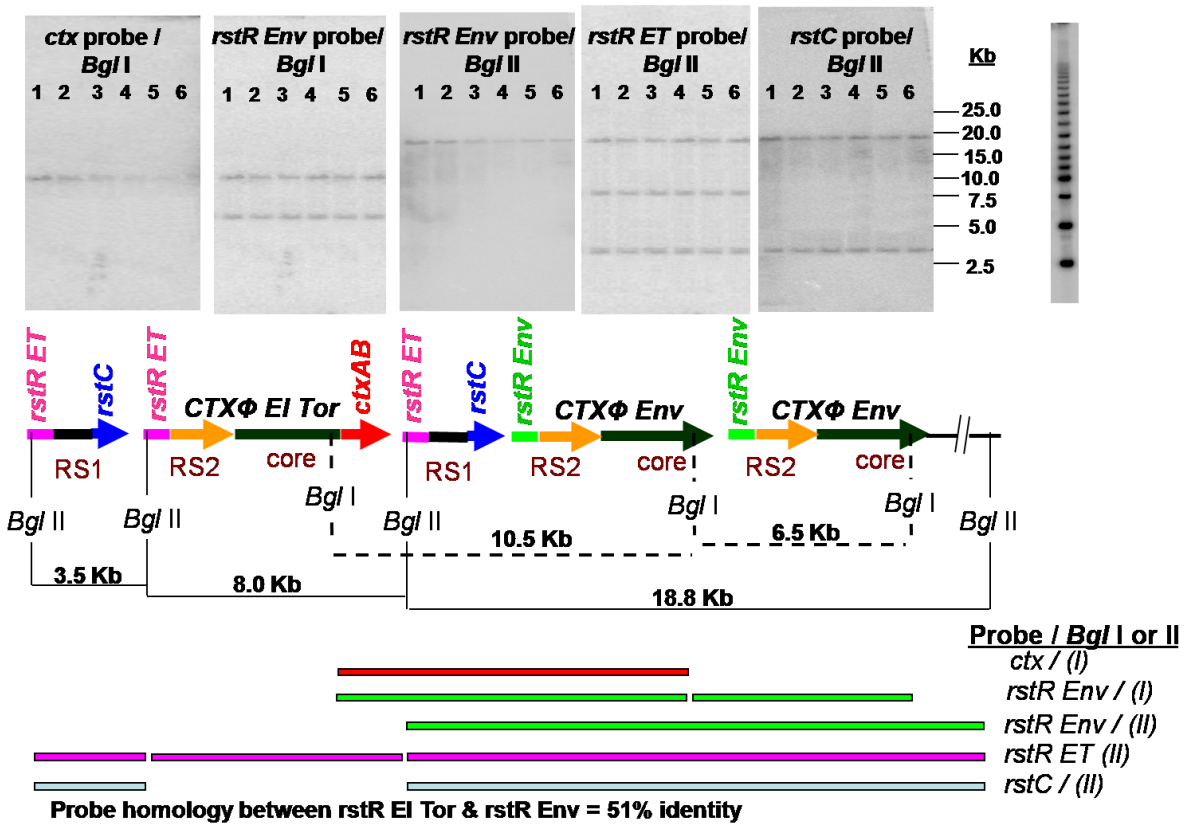

Fig. S3. Probable genetic organization of El Tor and environmental types of CTXΦ, and RS1 element, deduced by comparison of the restriction map of the marker genes, i.e., *ctx*, *rstR*$^{El\ Tor}$, *rstR*^{Env}, and *rstC,* respectively, in *V. mimicus* strains. Top panel: autoradiographed images of gDNA, of *V. mimicus* strains, digested by restriction enzymes (*Bgl*I or *Bgl*II) and detected by ^{32}P-labelled PCR products of the marker genes. Bottom panel: a schematic diagram with location ofRS1, RS2 and Core of the CTX prophages, with lines (filled and dotted) showing the distance between the restriction sites, and bars mimicking the results of Southern hybridization using different probes. Taken together, the results indicated an array of RS1-CTXΦ$^{El\ Tor\ (ET)}$ (with *ctxAB*)- RS1-CTXΦ^{Env} (without *ctxAB*)-CTXΦ^{Env} (without *ctxAB*).

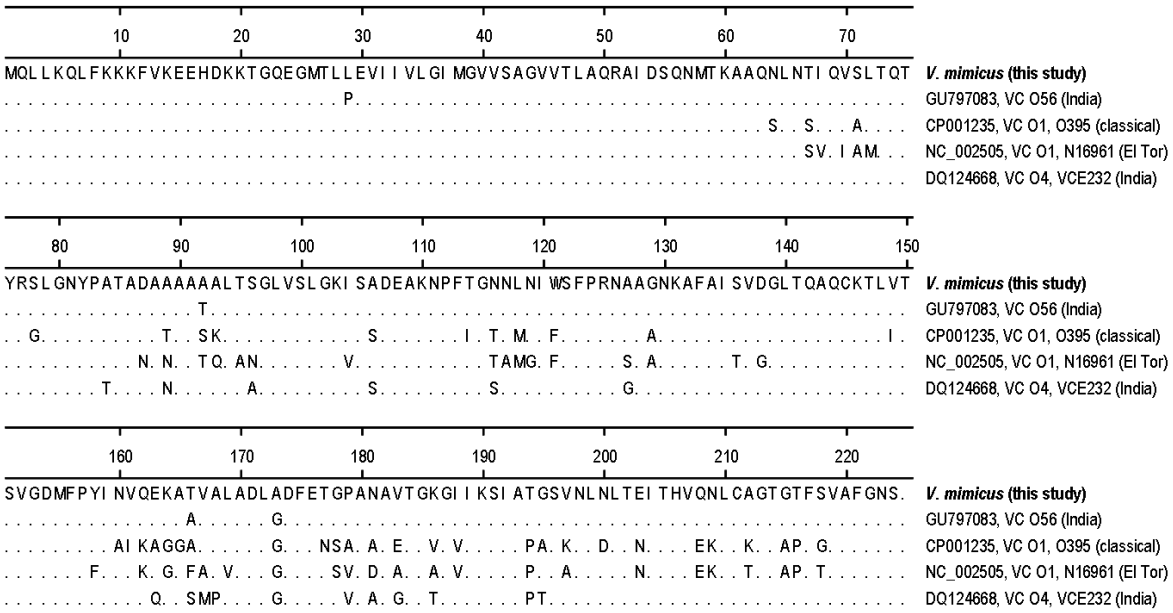

Fig. S4. Genetic diversity of amino acid residues in the novel variant *tcpA* in *V. mimicus* strains of this study in comparison to that of the selected reference strains of *V. cholerae.* Sequences were aligned by ClustalW algorithm. Amino acid positions are shown as a heading scale. Strain details are shown on the right border at each row. In comparison to *V. mimicus* strain in this study, only mismatched amino acids of *tcpA* genes of other selected strains are shown while their identical amino acids are indicated by dots.

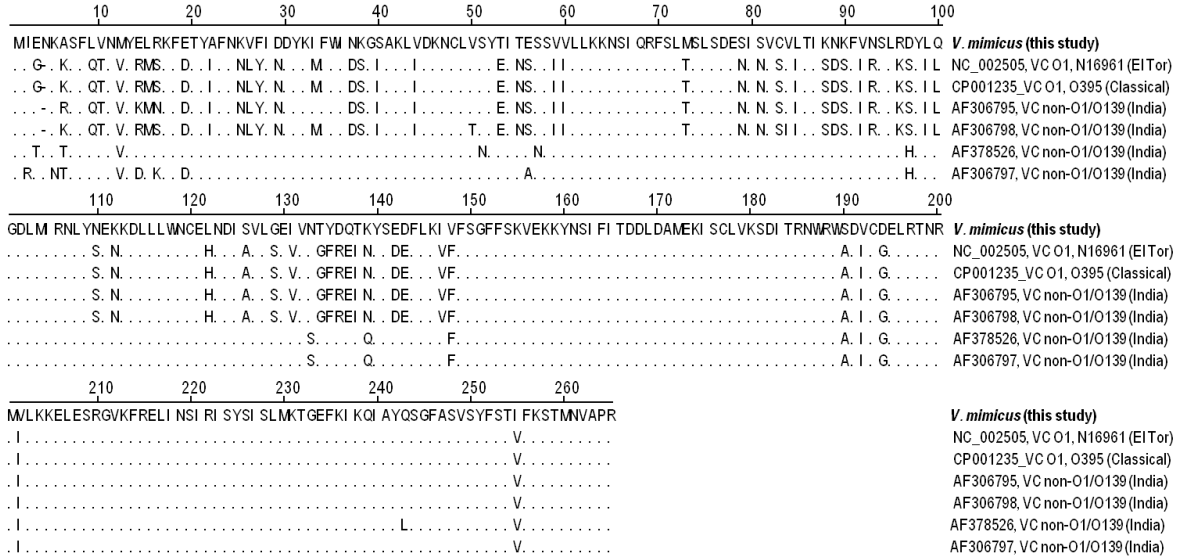

Fig. S5. Variation in amino acid residues in the novel variant *toxT* in *V. mimicus* strains of this study in comparison to other *toxT* genes in selected reference strains of *V. cholerae.* Sequences were aligned by ClustalW algorithm. Amino acid positions are shown as a heading scale. Strain details are shown on the right border at each row. In comparison to *V. mimicus* strain in this study, only mismatched amino acids of *toxT* genes in other selected strains are shown while their identical amino acids are indicated by dots.