Supplementary material for Kamruzzaman *et al*.,

RS1 satellite phage promotes diversity of toxigenic *Vibrio cholerae* by driving CTX prophage loss and elimination of lysogenic immunity

Table S1-S4, Figure S1

Table S1. Description of *Vibrio cholerae* strains and plasmids included in the study

Strain/plasmid	Description	Reference
pRS1-Km	Replicative form of the RS1 satellite phage genome carrying a kanamycin resistance (Km^R) gene cassette in an intergenic region	1
pRstC	A 454 bp *Ban*II fragment of pRS1-Km containing the *rstC* gene cloned into pUC18	This study
O395 (pRS1-Km)	Strain O395 carrying pRS1-Km	1
pCTX-Km	Replicative form of genetically marked CTXΦ genome derived from strain SM44	2
pCTX-Cm	Replicative form of genetically marked CTXΦ genome carrying a cloramphenicol resistance gene	This study
O395 (pCTX-Km)	Strain O395 carrying the RF of CTX-KmΦ derived from strain SM44	2
MJ-1236, MJ-1485, MG-116926, G-7555, MG-116226, G-3985, P-27457, Syria-3, AK-17334	*Vibrio cholerae* O1 El Tor biotype strains	Laboratory collection
AR-02214021, AL-11089,	*Vibrio cholerae* O139 clinical isolates	Laboratory collection
RV508	*V. cholerae* O1 classical biotype strain which constitutively express CT, TCP and other toxR regulated gene products	Laboratory collection
O395, S224	*V. cholerae* O1 classical biotype strains	Laboratory collection
O395-NT	Derivative of classical biotype strain O395 carrying a kanamycin resistance marker in the CTX prophage.	3
CTX-Kmϕ	Km^R-marked CTXϕ	1
RS1-Kmϕ	Km^R-marked RS1ϕ	1

Table S2. *V. cholerae* strains sequenced with Illumina Next-Generation Sequencing to determine assemblages of phage genetic islands and junctions.

Strain ID	Strain	Description	Accession #
G7555	G7555	Wild type	SAMN02630754
SF20011	G7555 ΔCTX⁻	Lost CTX	SAMN02630755
SF20012	G7555 ΔCTX-RS1-TLC)	Lost CTX, RS1, TLC	SAMN02630756
SF20013	G7555 ΔCTX + CTX-Kan	G7555 CTX⁻ reinfected with Kan^R labeled CTX ϕ	SAMN02630757
SF20014	G7555 ΔCTX + 0395 NT DNA	Total DNA of 0395 NT (CTX marked with Kan^R gene disrupting *ctxAB*) used to transform (G7555 CTX-) using chitin induction	SAMN02630758
SF20015	G7555 ΔCTX-RS1- TLC) + CTX-Kan	(G7555 CTX^- $RS1^-$ TLC^-) reinfected with Kan^R labeled CTXϕ	SAMN02630759
P27457	P27457	Wild type	SAMN02630760
SF20016	P27457 ΔCTX		SAMN02630761
SF20017	P27457 ΔCTX + CTX-Kan	(P27457 CTX-) reinfected with Kan^R labeled CTX ϕ	SAMN02630762

Table S3. Susceptibility of RS1-mediated non-toxigenic derivatives of various toxigenic *V. cholerae* strains to CTX-Kmϕ under in vitro laboratory conditions

Parent Strain	CTX negative derivatives	Frequency of Transduction
MG-116926	SF2	5.6×10^{-5}
MG-116226	SF3A	2.1×10^{-4}
MG-116226	SF3B	2.9×10^{-4}
G-7555	SF20011	3.6×10^{-4}
G-3985	SF69-2	1.5×10^{-4}
P-27457	SF20017	2.3×10^{-4}
P-27457	SF20019	1.3×10^{-4}

Table S4. Acquisition of CTX^{class} phage genome by nontoxigenic derivatives of El Tor strains arising from RS1 phage mediated excision of CTX prophage

Parent Strain	Nontoxigenic derivatives	Transformation[a] frequency in the presence of shrimp shell chitin
MG-116926	SF2	1.6×10^{-4}
MG-116226	SF3A	4.1×10^{-4}
AR-02214021	SF2002	6.3×10^{-4}
AI-1836	SF93	2.5×10^{-4}
AK-17334	SF95	7.3×10^{-4}
G-7555	SF20011	8.6×10^{-4}
G-3985	SF69-2	7.5×10^{-4}
P-27457	SF20017	6.5×10^{-4}

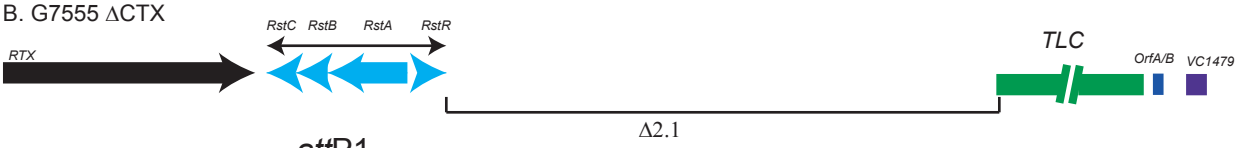
[a]Transformation frequency was based on acquisition of a genetically marked CTX^{class} prophage from total genomic DNA of a classical biotype strain in chitin induced transformation. Presence of different representative genes of the prophage was confirmed using DNA probes and PCR assays.

References.

1. Faruque SM, Asadulghani, Kamruzzaman M, Nandi RK, Ghosh AN, Nair GB, Mekalanos JJ, Sack DA. 2002. RS1 element of *Vibrio cholerae* can propagate horizontally as a filamentous phage exploiting the morphogenesis genes of CTXphi. Infect. Immun.**70**:163-70.
2. Waldor MK, Mekalanos JJ. 1996. Lysogenic conversion by a filamentous bacteriophage encoding cholera toxin. Science. **272**:1910-1914.
3. Mekalanos JJ, Swartz DJ, Pearson GD, Harford N, Groyne F, de Wilde M. 1983. Cholera toxin genes: nucleotide sequence, deletion analysis and vaccine development. Nature. **306**:551-557.

A. G7555

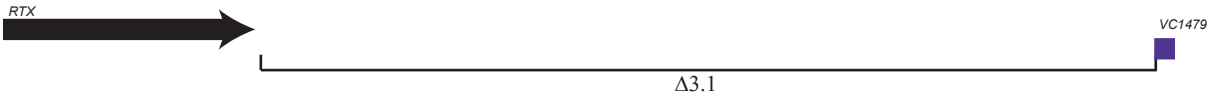
B. G7555 ΔCTX



Δ2.1 *att*P1

CCCTCAACATAATGCCGCGCCACATAAT_GCGCACTAGGAACATTTTGT

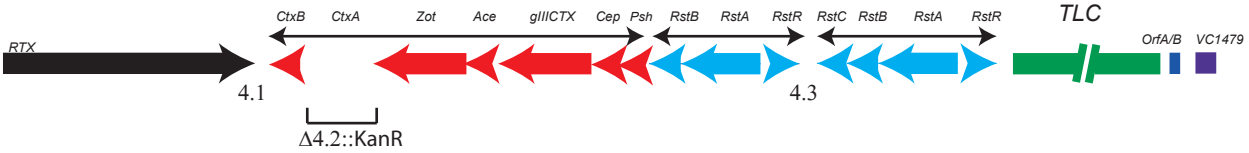
C. G7555 ΔCTX ΔRS1 ΔTLC



Δ3.1 *att*B

TTATGCCTTAATTTAACATA_ACATACATAATA_TGCACTGAGGTATTTTGGTTTTT

D. G7555 ΔCTX (CTXϕ KanR)



4.1 *dif1*

TTAATTTAACATAACAT_ACATAATACGCACTAAGG_ATGTGGAATAAAAACATCATAC

Δ4.3 *att*P1

ACGACTGCCCCTCAACATAATGCCGCGCC_ACATAATACGCACTAAGG_CGGCGGAATCTAGGGTT

E. G7555 ΔCTX (O395 DNA with CTXϕ KanR)

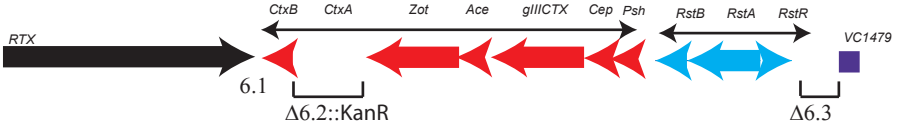
5.1 *dif2*

TTGCGACCTCTTTTTATGCCTTAATTTAACATAA_CG_CACGTAATACGCACTAAGGATGTGGAATA

Δ5.3

CTTTTTGCTCAGGGGTGCGAGCCATACGGT_TCTCCCAACGTCGCCATGT

F. G7555 ΔCTX ΔRS1 ΔTLC (CTXϕ KanR)



6.1 *dif1*

TTAATTTAACATAACAT_ACATAATACGCACTAAGG_ATGTGGAATAAAAACATCATAC

Δ6.3 *att*P1

GACTGCCCCTCAACATAATGCCGCGCC_ACATAAT_ATGCACTGAGGTATTTTGGTT

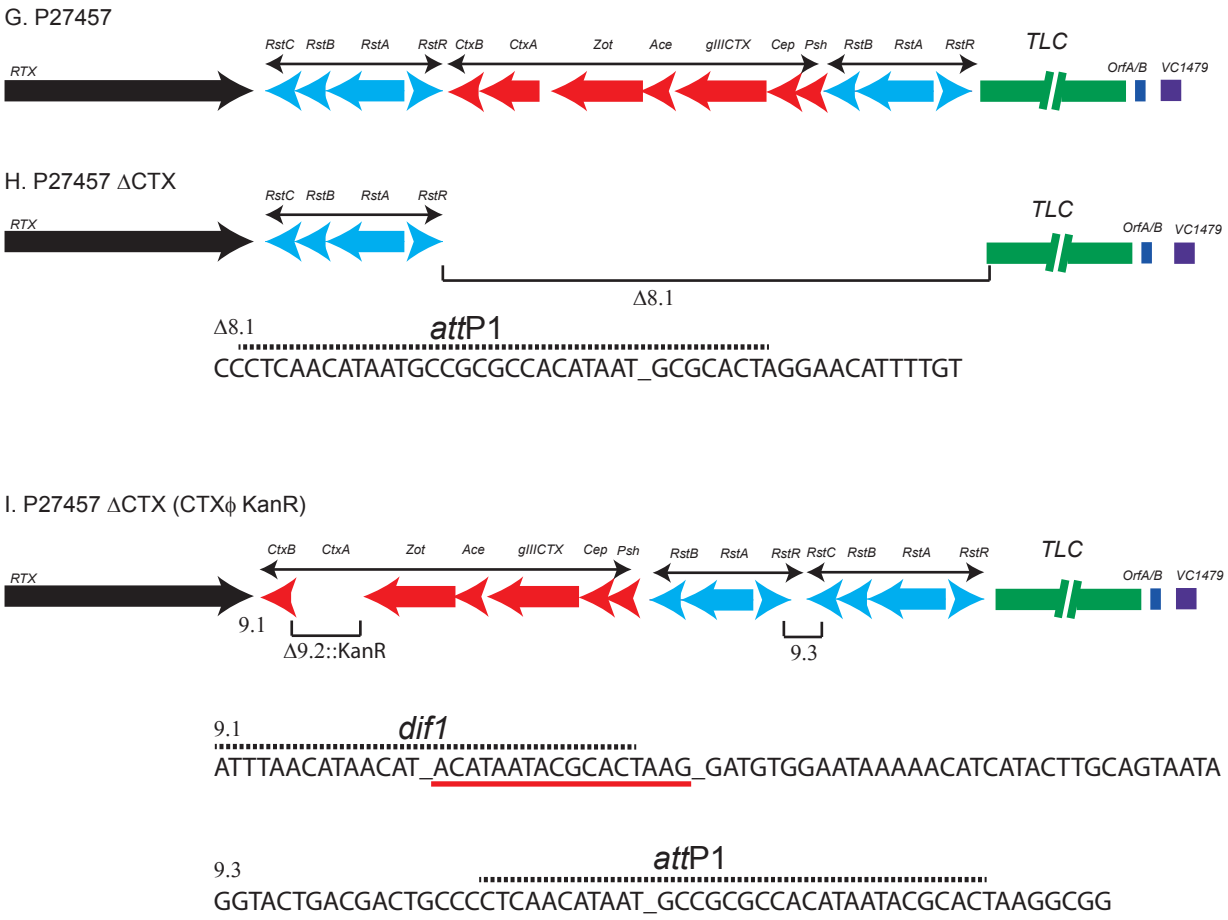


Figure S1. The gene order and sequenced junctions of the CTX/RS1/TLC region of *V.cholerae* El Tor strains G7555 (A,B,C,D,E, & F) and P27457 (G,H, & I) and spontaneous deletions of phage elements. Some strains are derivatives of isolates that have deletions of CTX (B,D,E,H,& I) or CTX/RS1/TLC (C,F). The reacquisition of KanR-marked CTX-ϕ phage (D,F, and I) or via chitin-induced DNA uptake of Classical strain CTX-ϕ DNA (E) and integration into the genome is also diagrammed and the sequences of newly created junctions are provided. Bacterial and phage attachment sites (*att*B/*att*P) and *dif* sites are indicated when present and repetitive sequences found in both newly joined sequences are underlined in red