

# **Supporting Information**

## **Interbacterial competition and anti-predatory behavior of environmental *Vibrio cholerae* strains**

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**Supporting tables and legends**

**Table S1.** *Vibrio cholerae*, *Escherichia coli* and *Klebsiella pneumoniae* strains used in this study.

Strain names	Genotype / description	Internal strain number	Reference
<b><i>V. cholerae</i></b>			
A1552 (WT)	Wild-type, O1 El Tor Inaba; Rif <sup>R</sup>	MB_1	(Yildiz and Schoolnik, 1998)
W10G	Environmental isolate (clade A) collected in Waddell Creek (CA, USA) in October 2004	MB_5537	(Keymer <i>et al.</i> , 2007)
SA3G	Environmental isolate (clade B) collected in Old Salinas River (CA, USA) in March 2004	MB_957	(Keymer <i>et al.</i> , 2007)
SA5Y	Environmental isolate (clade B) collected in Old Salinas River (CA, USA) in May 2004	MB_353	(Keymer <i>et al.</i> , 2007)
SL4G	Environmental isolate (clade B) collected in San Lorenzo River (CA, USA) in April 2004	MB_955	(Keymer <i>et al.</i> , 2007)
SL5Y	Environmental isolate (clade B) collected in San Lorenzo River (CA, USA) in May 2004	MB_954	(Keymer <i>et al.</i> , 2007)
SO5Y	Environmental isolate (clade B) collected in Soquel Creek (CA, USA) in May 2004	MB_960	(Keymer <i>et al.</i> , 2007)
L6G	Environmental isolate (clade C) collected in Lagunitas Creek (CA, USA) in June 2004	MB_956	(Keymer <i>et al.</i> , 2007)
SL6Y	Environmental isolate (clade C) collected in San Lorenzo River (CA, USA) in June 2004	MB_953	(Keymer <i>et al.</i> , 2007)
SP6G	Environmental isolate (clade C) collected in San Pedro Creek (CA, USA) in June 2004	MB_964	(Keymer <i>et al.</i> , 2007)
SP7G	Environmental isolate (clade C) collected in San Pedro Creek (CA, USA) in July 2004	MB_952	(Keymer <i>et al.</i> , 2007)
W6G	Environmental isolate (clade C) collected in Waddell Creek (CA, USA) in June 2004	MB_354	(Keymer <i>et al.</i> , 2007)
W7G	Environmental isolate (clade C) collected in Waddell Creek (CA, USA) in July 2004	MB_962	(Keymer <i>et al.</i> , 2007)
E7G	Environmental isolate (clade D) collected in Moss Landing Harbor (CA, USA) in July 2004	MB_963	(Keymer <i>et al.</i> , 2007)
SA7G	Environmental isolate (clade D) collected in Old Salinas River (CA, USA) in July 2004	MB_959	(Keymer <i>et al.</i> , 2007)
SA10G	Environmental isolate (clade D) collected in Old Salinas River (CA, USA) in October 2004	MB_5539	(Keymer <i>et al.</i> , 2007)
A1552Δ <i>hlyA</i>	A1552 with <i>aph</i> cassette in <i>hlyA</i> (A1552VC_A02993; homolog to VCA0219*); Rif <sup>R</sup> , Kan <sup>R</sup>	MB_3934	This study
W10GΔ <i>hlyA</i>	W10G with <i>aph</i> cassette in <i>hlyA</i> (HPY12_14900); Kan <sup>R</sup>	MB_8548	This study
SA5YΔ <i>hlyA</i>	SA5Y with <i>aph</i> cassette in <i>hlyA</i> (VC-Sa5Y_02920); Kan <sup>R</sup>	MB_4622	This study
SL6YΔ <i>hlyA</i>	SL6Y with <i>cat</i> cassette in <i>hlyA</i> (HPY06_14140); Cm <sup>R</sup>	MB_8549	This study
SA10GΔ <i>hlyA</i>	SA10G with <i>aph</i> cassette in <i>hlyA</i> (HPY11_14585); Kan <sup>R</sup>	MB_8550	This study
A1552Δ <i>vipA</i>	A1552 with <i>aph</i> cassette in <i>vipA</i> (A1552VC_A02892; homolog to VCA0107); Rif <sup>R</sup> , Kan <sup>R</sup>	MB_3012	This study
W10GΔ <i>vipA</i>	W10G with <i>aph</i> cassette in <i>vipA</i> (HPY12_14395); Kan <sup>R</sup>	MB_6850	This study
SA5YΔ <i>vipA</i>	SA5Y with <i>aph</i> cassette in <i>vipA</i> (VC-Sa5Y_02822); Kan <sup>R</sup>	MB_5772	This study
L6GΔ <i>vipA</i>	L6G with <i>aph</i> cassette in <i>vipA</i> (HPY16_13230); Kan <sup>R</sup>	MB_8628	This study
SL6YΔ <i>vipA</i>	SL6Y with <i>aph</i> cassette in <i>vipA</i> (HPY06_13635); Kan <sup>R</sup>	MB_8551	This study
SP7GΔ <i>vipA</i>	SP7G with <i>aph</i> cassette in <i>vipA</i> (HPY08_13415); Kan <sup>R</sup>	MB_8629	This study
SP7GΔ <i>vipA</i> -Rif <sup>R</sup>	SP7G with <i>aph</i> cassette in <i>vipA</i> (HPY08_13415); transformed with PCR of <i>rpoB</i> gene containing mutation (S531F) that confers rifampicin resistance; Kan <sup>R</sup> , Rif <sup>R</sup>	MB_8630	This study
SA10GΔ <i>vipA</i>	SA10G with <i>aph</i> cassette in <i>vipA</i> gene (HPY11_14100); Kan <sup>R</sup>	MB_8552	This study
A1552-Rif <sup>S</sup>	A1552 sensitive to Rifampicin (please check Material and Methods for details on construction)	MB_5161	This study
A1552Δ <i>lacZ</i> ::FRT-Kan-pheS*-FRT	A1552 with construct Δ <i>lacZ</i> ::FRT-Kan-pheS*-FRT; Rif <sup>R</sup> , cPhe <sup>S</sup>	MB_4609	(Van der Henst <i>et al.</i> , 2018)
A1552-Rif <sup>S</sup> Δ <i>vipA</i>	A1552-Rif <sup>S</sup> with <i>aph</i> cassette in <i>vipA</i> ; Rif <sup>R</sup> , Kan <sup>R</sup>	MB_8553	This study

SL6Y-vgrG1ΔACD	SL6Y with truncated <i>vgrG1</i> through insertion of a stop codon and insertion of an <i>aph</i> cassette that replaces the ACD-encoding sequence; Kan <sup>R</sup>	MB_8557	This study
SA10G-vgrG1ΔACD	SA10G with truncated <i>vgrG1</i> through insertion of a stop codon and insertion of an <i>aph</i> cassette that replaces the ACD-encoding sequence; Kan <sup>R</sup>	MB_8558	This study
ATCC25872	<i>V. cholerae</i> non-O1 strain (O37); isolated in 1965, Czechoslovakia; intermediate resistant to Strep	MB_276	Aldová <i>et al.</i> , 1968
ATCC25872Δ <i>vipA</i>	ATCC25872 deleted for <i>vipA</i> (TransFLP); intermediate resistant to Strep	MB_5065	Van der Henst <i>et al.</i> , 2018
ATCC25872 - vgrG1ΔACD	ATCC25872 with truncated <i>vgrG1</i> through insertion of a stop codon and insertion of an <i>aph</i> cassette that replaces the ACD-encoding sequence; Kan <sup>R</sup> , intermediate resistant to Strep	MB_8559	This study
W10G-Rif <sup>R</sup>	W10G selected for rifampicin resistance	MB_8562	This study
SA3G-Rif <sup>R</sup>	SA3G transformed with PCR of <i>rpoB</i> gene containing mutation (S531F) that confers rifampicin resistance	MB_8563	This study
SA5Y-Rif <sup>R</sup>	SA5Y selected for rifampicin resistance	MB_8564	This study
SL4G-Rif <sup>R</sup>	SL4G selected for rifampicin resistance	MB_8565	This study
SL5Y-Rif <sup>R</sup>	SL5Y selected for rifampicin resistance	MB_8566	This study
SO5Y-Rif <sup>R</sup>	SO5Y selected for rifampicin resistance	MB_8567	This study
L6G-Rif <sup>R</sup>	L6G transformed with PCR of <i>rpoB</i> gene containing mutation (S531F) that confers rifampicin resistance	MB_8568	This study
SL6Y-Rif <sup>R</sup>	SL6Y selected for rifampicin resistance	MB_8569	This study
SP6G-Rif <sup>R</sup>	SP6G selected for rifampicin resistance	MB_8570	This study
SP7G-Rif <sup>R</sup>	SP7G transformed with PCR of <i>rpoB</i> gene containing mutation (S531F) that confers rifampicin resistance	MB_8571	This study
W6G-Rif <sup>R</sup>	W6G selected for rifampicin resistance	MB_8572	This study
W7G-Rif <sup>R</sup>	W7G selected for rifampicin resistance	MB_8573	This study
E7G-Rif <sup>R</sup>	E7G selected for rifampicin resistance	MB_8574	This study
SA7G-Rif <sup>R</sup>	SA7G selected for rifampicin resistance	MB_8575	This study
SA10G-Rif <sup>R</sup>	SA10G selected for rifampicin resistance	MB_8576	This study
SP7GΔ <i>Aux6</i>	SP7G with <i>aph</i> cassette in the end of T6SS <i>Aux6</i> (HPY08_16370-16380); Kan <sup>R</sup>	MB_8631	This study
SP7GΔ <i>Aux6</i> -Rif <sup>R</sup>	SP7G with <i>aph</i> cassette in the end of T6SS <i>Aux6</i> (HPY08_16370-16380); Kan <sup>R</sup> ; Rif <sup>R</sup>	MB_8632	This study
<b><i>E. coli</i> and <i>K. pneumoniae</i></b>			
TOP 10	F- <i>mcrA</i> Δ( <i>mrr</i> - <i>hsdRMS</i> - <i>mcrBC</i> ) φ80 <i>lacZ</i> Δ <i>M15</i> Δ <i>lacX74</i> <i>nupG</i> <i>recA1</i> <i>araD139</i> Δ( <i>ara-leu</i> )7697 <i>galE15</i> <i>galK16</i> <i>rpsL</i> ( <i>StrR</i> ) <i>endA1</i> λ-	MB_741	This study
<i>K. pneumoniae</i>	<i>Klebsiella</i> sp. / pANT5 (GFP)	MB_6380	(Benghezal <i>et al.</i> , 2006)

\*reference locus tags belong to reference strain N16961 according to Heidelberg *et al.*, 2000.

**Table S2.** Information on long-read whole genome sequencing data and assemblies of NCBI BioProject PRJNA633476.

Strain name	Strain ID	BioSample	GenBank accession numbers	Number of bases	Number of reads	Mean read length	Total number of contigs	Contig length after circularization	Total genome size	Mean coverage	GC% content
<b>W10G</b>	MB#5537	SAMN14944057	CP053794 (chr1) CP053795 (chr2)	2,050,336,308 bp	119,162	17,206 bp	2 (chr1+chr2)	3,033,188 bp (chr1) 1,028,867 bp (chr2)	4,062,055 bp	292 x	47.7% (chr1) 47.2% (chr2)
<b>SA3G</b>	MB#957	SAMN14944058	CP053744 (chr1) CP053745 (chr2)	1,239,715,817 bp	70,375	17,615 bp	2 (chr1+chr2)	3,020,084 bp (chr1) 1,159,698 bp (chr2)	4,179,782 bp	201 x	47.6% (chr1) 47.0% (chr2)
<b>SA5Y#</b>	MB#353	SAMN08813238	CP028892 (chr1) CP028893 (chr2)	635,540,812 bp	35,390	17,958 bp	2 (chr1+chr2)	2,955,400 bp (chr1) 1,095,478 bp (chr2)	4,050,878 bp	133 x	47.8% (chr1) 46.8% (chr2)
<b>SL4G</b>	MB#955	SAMN14944065	CP053796 (chr1) CP053797 (chr2)	1,083,962,388 bp	55,665	19,472 bp	2 (chr1+chr2)	2,954,462 bp (chr1) 1,173,143 bp (chr2)	4,127,605 bp	186 x	47.8% (chr1) 46.4% (chr2)
<b>SL5Y</b>	MB#954	SAMN14944067	CP053798 (chr1) CP053799 (chr2)	1,623,840,987 bp	84,121	19,303 bp	2 (chr1+chr2)	2,947,299 bp (chr1) 1,086,276 bp (chr2)	4,033,575 bp	336 x	47.8% (chr1) 46.9% (chr2)
<b>SO5Y</b>	MB#960	SAMN14944069	CP053800 (chr1) CP053801 (chr2)	865,600,319 bp	47,657	18,163 bp	2 (chr1+chr2)	2,947,270 bp (chr1) 1,049,609 bp (chr2)	3,996,879 bp	245 x	47.9% (chr1) 47.0% (chr2)
<b>L6G</b>	MB#956	SAMN14944070	CP053802 (chr1) CP053803 (chr2)	1,134,851,816 bp	70,295	16,144 bp	2 (chr1+chr2)	2,829,740 bp (chr1) 1,195,206 bp (chr2)	4,024,946 bp	225 x	48.1% (chr1) 46.3% (chr2)
<b>SL6Y</b>	MB#953	SAMN14944072	CP053804 (chr1) CP053805 (chr2)	826,125,289 bp	48,156	17,155 bp	2 (chr1+chr2)	2,900,064 bp (chr1) 1,143,503 bp (chr2)	4,043,567 bp	175 x	47.8% (chr1) 46.7% (chr2)
<b>SP6G</b>	MB#964	SAMN14944080	CP053806 (chr1) CP053807 (chr2)	874,659,808 bp	47,923	18,251 bp	2 (chr1+chr2)	2,947,818 bp (chr1) 1,229,641 bp (chr2)	4,177,459 bp	154 x	47.8% (chr1) 46.7% (chr2)
<b>SP7G</b>	MB#952	SAMN14944081	CP053808 (chr1) CP053809 (chr2)	1,339,636,117 bp	73,934	18,119 bp	3 (chr1+chr2)	2,868,038 bp (chr1) 1,217,051 bp (chr2)	4,085,089 bp	217 x	48.0% (chr1) 46.5% (chr2)
<b>W6G</b>	MB#354	SAMN14944082	CP053810 (chr1) CP053811 (chr2) CP053812 (plasmid)	1,247,754,152 bp	69,891	17,852 bp	3 (chr1+chr2+plasmid)	2,967,360 bp (chr1) 1,202,132 bp (chr2) 306,484 bp (plas)	4,475,976 bp	192 x	47.8% (chr1) 46.6% (chr2) 42.2% (plas)
<b>W7G</b>	MB#962	SAMN14944083	CP053813 (chr1)	1,490,677,399 bp	86,303	17,272 bp	3 (chr1+chr2+plasmid)	2,967,793 bp (chr1) 1,202,155 bp (chr2) 306,481 bp (plas)	4,476,429 bp	223 x	47.8% (chr1) 46.6% (chr2) 42.2% (plas)

			CP053814 (chr2) CP053815 (plasmid)								
<b>E7G</b>	MB#963	SAMN14944111	CP053822 (chr1) CP053823 (chr2) CP053824 (plasmid)	1,477,482,932 bp	83,856	17,619 bp	3 (chr1+chr2+ plasmid)	3,040,343 bp (chr1) 1,199,805 bp (chr2) 80,726 bp (plas)	4,320,874 bp	194 x	47.8% (chr1) 46.5% (chr2) 46.3% (plas)
<b>SA7G</b>	MB#959	SAMN14944112	CP053816 (chr1) CP053817 (chr2) CP053818 (plasmid 1) CP053819 (plasmid 2)	1,689,606,494 bp	98,160	17,212 bp	4 (chr1+chr2+ plasmid 1 + 2)	3,004,584 bp (chr1) 1,182,929 bp (chr2) 79,397 bp (plas1) 80,721 bp (plas2)	4,347,631 bp	245 x	47.8% (chr1) 46.5% (chr2) 42.2% (plas1) 46.3% (plas2)
<b>SA10G</b>	MB#5539	SAMN14944113	CP053820 (chr1) CP053821 (chr2)	1,643,277,942 bp	91,387	17,981 bp	2 (chr1+chr2)	3,010,605 bp (chr1) 1,166,701 bp (chr2)	4,177,306 bp	269 x	47.8% (chr1) 46.6% (chr2)

#Data based on Matthey *et al.*, 2018

**Table S3.** BlastP-predicted T6SS effector and immunity proteins<sup>1</sup> of pandemic, non-pandemic toxigenic, and environmental *V. cholerae* strains.

Clade	Strain name	Major cluster E/I	Aux cluster 1 <i>vgrG1</i>	Aux cluster 1 E/I	Aux cluster 2 E/I	Aux cluster 3 E/I
<b>Pandemic</b>	<b>A1552</b>	Encoded protein with 1018 aa; evolved VgrG3 with predicted peptidoglycan hydrolase domain; A-type.	Encoded protein with 1164 aa; evolved VgrG1 with an actin crosslinking domain (ACD).	Encoded protein with 642 aa; lipase class 3; A-type.	Encoded protein with 1086 aa; VasX; pore forming activity <sup>2</sup> ; A-type.	Encoded protein with 224 aa; papain-like NlpC/P60 peptidase <sup>3</sup> ; A-type.
<b>D</b>	<b>SA10G*</b>	Encoded protein with 995 aa; evolved VgrG3 with predicted peptidoglycan hydrolase and pesticin-like lysozyme domains; J-type.	Encoded protein with 1164 aa; evolved VgrG1 with an actin crosslinking domain (ACD).	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1194 aa; predicted lysin motif (binds peptidoglycan), but also with pore formation activity <sup>4</sup> ; E-type.	Aux cluster 3 is absent.
	<b>E7G*</b>	Encoded protein with 995 aa; evolved VgrG3 with predicted peptidoglycan hydrolase and pesticin-like lysozyme domains; J-type.	Encoded protein with 1164 aa; evolved VgrG1 with an actin crosslinking domain (ACD).	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1197 aa; predicted lysin motif (binds peptidoglycan), but also with pore formation activity <sup>4</sup> ; E-type.	Aux cluster 3 is absent.
	<b>SA7G*</b>	Encoded protein with 995 aa; evolved VgrG3 with predicted peptidoglycan hydrolase and pesticin-like lysozyme domains; J-type.	Encoded protein with 1164 aa; evolved VgrG1 with an actin crosslinking domain (ACD).	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1197 aa; predicted lysin motif (binds peptidoglycan), but also with pore formation activity <sup>4</sup> ; E-type.	Aux cluster 3 is absent.
<b>C</b>	<b>SL6Y</b>	Encoded protein with 977 aa; evolved VgrG3 with a predicted peptidoglycan hydrolase and phage-like lysozyme domains. E-type.	Encoded protein with 1164 aa; evolved VgrG1 with an actin crosslinking domain (ACD).	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1086 aa; 98.3% identical to A1552's VasX; pore forming activity <sup>2</sup> ; A-type.	Aux cluster 3 is absent.
	<b>SP6G*</b>	Encoded protein with 1052 aa; evolved VgrG3 with a predicted peptidoglycan hydrolase domain; H-type.	Encoded protein with 1164 aa; evolved VgrG1 with an actin crosslinking domain (ACD).	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1087 aa; 94.8% identical to A1552's VasX; pore forming activity <sup>2</sup> ; A-type.	Aux cluster 3 is absent.
	<b>L6G*</b>	Encoded protein with 989 aa; evolved VgrG3 with a predicted peptidoglycan hydrolase and lambda phage-like lysozyme domains; K-type.	Encoded protein with 1167 aa; evolved VgrG1 with an actin crosslinking domain (ACD).	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1194 aa; predicted lysin motif (binds peptidoglycan), but also with pore formation activity <sup>4</sup> ; E-type.	Aux cluster 3 is absent.
	<b>W6G*</b>	Encoded protein with 1052 aa; evolved VgrG3 with a predicted peptidoglycan hydrolase domain; H-type.	Encoded protein with 1164 aa; evolved VgrG1 with an actin crosslinking domain (ACD).	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1088 aa; 94.1% identical to A1552's VasX; pore forming activity <sup>2</sup> ; A-type.	Aux cluster 3 is absent.
	<b>W7G*</b>	Encoded protein with 1052 aa; evolved VgrG3 with a predicted peptidoglycan hydrolase domain; H-type.	Encoded protein with 1164 aa; evolved VgrG1 with an actin crosslinking domain (ACD).	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1088 aa; 94.1% identical to A1552's VasX; pore forming activity <sup>2</sup> ; A-type.	Aux cluster 3 is absent.

	<b>SP7G**</b>	Encoded protein with 439 aa, independent from VgrG3 (structural only); cell adhesion function <sup>4</sup> ; B-type.	Encoded protein with 1164 aa; evolved VgrG1 with an actin crosslinking domain (ACD).	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1194 aa; predicted lysin motif (binds peptidoglycan), but also with pore formation activity <sup>4</sup> ; E-type.	Aux cluster 3 is absent.
<b>A</b>	<b>W10G</b>	Encoded protein with 1018 aa; evolved VgrG3 with predicted peptidoglycan hydrolase domain; A-type.	Encoded protein with 684 aa; structural VgrG1 protein.	PacBio sequencing artifact in the effector gene (HPY12_07370). Region was Sanger sequenced and manually corrected before translation to allow typing. Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	PacBio sequencing artifact in the effector gene (HPY12_14010). Region was Sanger sequenced and manually corrected before translation to allow typing. Encoded protein with 1086 aa; VasX; pore forming activity <sup>2</sup> ; A-type.	Aux cluster 3 is absent.
<b>B</b>	<b>SA3G</b>	Encoded protein with 1018 aa; evolved VgrG3 with predicted peptidoglycan hydrolase domain; A-type.	Encoded protein with 684 aa; structural VgrG1 protein.	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1087 aa; 94.3% identical to A1552's VasX; pore forming activity <sup>2</sup> ; A-type.	Aux cluster 3 is absent.
	<b>SA5Y</b>	Encoded protein with 995 aa; evolved VgrG3 with predicted peptidoglycan hydrolase and pesticin-like lysozyme domains; J-type.	Encoded protein with 684 aa; structural VgrG1 protein.	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1088 aa; 93.7% identical to A1552's VasX; pore forming activity <sup>2</sup> ; A-type.	Aux cluster 3 is absent.
	<b>SL4G*</b>	Encoded protein with 995 aa; evolved VgrG3 with predicted peptidoglycan hydrolase and pesticin-like lysozyme domains; J-type.	Encoded protein with 684 aa; structural VgrG1 protein.	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1088 aa; 93.7% identical to A1552's VasX; pore forming activity <sup>2</sup> ; A-type.	Aux cluster 3 is absent.
	<b>SL5Y*</b>	Encoded protein with 995 aa; evolved VgrG3 with predicted peptidoglycan hydrolase and pesticin-like lysozyme domains; J-type.	Encoded protein with 684 aa; structural VgrG1 protein.	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1088 aa; 93.7% identical to A1552's VasX; pore forming activity <sup>2</sup> ; A-type.	Aux cluster 3 is absent.
	<b>SO5Y*</b>	Encoded protein with 1022 aa; evolved VgrG3 with predicted peptidoglycan hydrolase and muramidase (N-acetylmuramidase) domains; I-type.	Encoded protein with 684 aa; structural VgrG1 protein.	Encoded protein with 710 aa; uncharacterized alpha/beta hydrolase domain (DUF2235); C-type.	Encoded protein with 1220 aa; predicted lysin motif (binds peptidoglycan); D-type.	Aux cluster 3 is absent.
<b>Non-pandemic</b>	<b>ATCC25872</b>	Encoded protein with 1018 aa; evolved VgrG3 with predicted peptidoglycan hydrolase domain; A-type.	Encoded protein with 1164 aa; evolved VgrG1 with an actin crosslinking domain (ACD).	Encoded protein with 642 aa; lipase class 3; A-type.	Encoded protein with 1086 aa; VasX; pore forming activity <sup>2</sup> ; A-type.	Encoded protein with 224 aa; papain-like NlpC/P60 peptidase <sup>3</sup> ; A-type.

<sup>1</sup> Typing according to Unterweger *et al.*, 2014 & Kirchberger *et al.*, 2017.

<sup>2</sup> According to Miyata *et al.*, 2011.

<sup>3</sup> According to Hersch *et al.*, 2020.

<sup>4</sup> According to Unterweger *et al.*, 2014.

\* In PGAP annotation, *vgrG3* gene was annotated without the effector portion. Prokka annotation used instead for this study.

\*\* Strain SP7G (clade C) contains an additional T6SS cluster, called Aux 6, which has the same gene order as auxiliary clusters 1 and 2 (see Fig. 4).

**Table S4.** Matrices of % identity (percentage of residues which are identical) among VgrG1-ACD proteins in T6SS auxiliary cluster 1 from clade C and D environmental *V. cholerae* strains and pandemic strain A1552.

VgrG1-ACD full protein sequence										
	A1552	SA10G	E7G	SA7G	SL6Y	SP6G	L6G	W7G	W6G	SP7G
A1552		98.2	98.2	98.2	98.6	98.8	97.3	98.5	98.5	98.4
SA10G			100	100	98.9	98.9	97.2	99.1	99.1	98.9
E7G				100	98.9	98.9	97.2	99.1	99.1	98.9
SA7G					98.9	98.9	97.2	99.1	99.1	98.9
SL6Y						98.9	97.4	98.9	98.9	98.9
SP6G							97.4	99.1	99.1	99.1
L6G								97.7	97.7	97.4
W7G									100	99.4
W6G										99.4
SP7G										

Actin cross-linking domain (ACD) protein sequence only										
	A1552	SA10G	E7G	SA7G	SL6Y	SP6G	L6G	W7G	W6G	SP7G
A1552		99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.4
SA10G			100	100	100	99.6	100	100	100	99.8
E7G				100	100	99.6	100	100	100	99.8
SA7G					100	99.6	100	100	100	99.8
SL6Y						99.6	100	100	100	99.8
SP6G							99.6	99.6	99.6	99.4
L6G								100	100	99.8
W7G									100	99.8
W6G										99.8
SP7G										

Green: 100% identity  
 Yellow: 90% - 99.99% identity



**Table S5.** Matrices of % identity (percentage of residues which are identical) among effector and immunity proteins harbored in the T6SS large cluster from environmental *V. cholerae* strains and pandemic strain A1552.

Large cluster – Effector																
	A1552	SA10G	E7G	SA7G	SL6Y	SP6G	L6G	W7G	W6G	SP7G	W10G	SA3G	SA5Y	SL4G	SL5Y	SO5Y
A1552		8.9	8.9	8.9	7.5	6.7	5.7	6.7	6.7	5.3	100	99.5	8.9	8.8	8.9	9.8
SA10G			100	100	13.9	8.4	8.2	8.4	8.4	7.4	8.9	8.9	98.9	98.9	98.9	10.4
E7G				100	13.9	8.4	8.2	8.4	8.4	7.4	8.9	8.9	98.9	98.9	98.9	10.4
SA7G					13.9	8.4	8.2	8.4	8.4	7.4	8.9	8.9	98.9	98.9	98.9	10.4
SL6Y						8.6	10.6	8.6	8.6	5.6	7.5	7.5	13.8	13.8	13.8	6.5
SP6G							9.2	100	100	5.7	6.7	6.7	8.3	8.3	8.4	10.8
L6G								9.2	9.2	5.4	5.7	5.7	8.7	8.6	8.4	8.5
W7G									100	5.7	6.7	6.7	8.3	8.3	8.4	10.8
W6G										5.7	6.7	6.7	8.3	8.3	8.4	10.8
SP7G											5.3	5.3	7.6	7.6	7.4	5.3
W10G												99.5	8.9	8.8	8.9	9.8
SA3G													8.9	8.8	8.9	9.8
SA5Y														100	100	10.8
SL4G															100	10.8
SL5Y																10.9
SO5Y																

Large cluster – Immunity																
	A1552	SA10G	E7G	SA7G	SL6Y	SP6G	L6G	W7G	W6G	SP7G	W10G	SA3G	SA5Y	SL4G	SL5Y	SO5Y
A1552		11.7	11.7	11.7	7.7	8.1	12.0	8.1	8.1	6.4	100	100	11.7	11.7	11.7	15.8
SA10G			100	100	10.8	6.8	8.4	6.8	6.8	10.9	11.7	11.7	100	100	100	10.5
E7G				100	10.8	6.8	8.4	6.8	6.8	10.9	11.7	11.7	100	100	100	10.5
SA7G					10.8	6.8	8.4	6.8	6.8	10.9	11.7	11.7	100	100	100	10.5
SL6Y						8.5	15.8	8.5	8.5	8.8	7.7	7.7	10.8	10.8	10.8	10.1
SP6G							7.0	100	100	8.4	8.1	8.1	6.8	6.8	6.8	9.7
L6G								7.0	7.0	8.5	12.0	12.0	8.4	8.4	8.4	11.2
W7G									100	8.4	8.1	8.1	6.8	6.8	6.8	9.7
W6G										8.4	8.1	8.1	6.8	6.8	6.8	9.7
SP7G											6.4	6.4	10.9	10.9	10.9	7.5
W10G												100	11.7	11.7	11.7	15.8
SA3G													11.7	11.7	11.7	15.8
SA5Y														100	100	10.5
SL4G															100	10.5
SL5Y																10.5
SO5Y																

Green: 100% identity (same family and subfamily)  
 Yellow: 90% - 99.99% identity (same family, with some polymorphism)  
 Orange: 30% - 90% identity (same family, with considerable polymorphism)  
 Red: <30% identity (different families)

**Table S6.** Matrices of % identity (percentage of residues which are identical) among effector and immunity proteins harbored in the T6SS auxiliary cluster 1 from environmental *V. cholerae* strains and pandemic strain A1552.

Auxiliary cluster 1 – Effector																
	A1552	SA10G	E7G	SA7G	SL6Y	SP6G	L6G	W7G	W6G	SP7G	W10G	SA3G	SA5Y	SL4G	SL5Y	SO5Y
A1552		15.0	15.0	15.0	15.5	15.0	15.5	15.8	15.8	15.8	14.6	15.9	15.9	15.9	14.9	15.3
SA10G			100	100	90.4	99.7	90.6	94.6	94.6	94.6	91.3	94.5	94.5	94.4	92.5	98.9
E7G				100	90.4	99.7	90.6	94.6	94.6	94.6	91.3	94.5	94.5	94.4	92.5	98.9
SA7G					90.4	99.7	90.6	94.6	94.6	94.6	91.3	94.5	94.5	94.4	92.5	98.9
SL6Y						90.4	99.9	92.9	92.9	92.9	87.0	92.8	92.8	92.7	94.5	91.1
SP6G							90.6	94.4	94.4	94.3	91.3	94.2	94.2	94.1	92.3	98.9
L6G								93.1	93.1	93.1	87.2	92.9	92.9	92.8	94.6	91.3
W7G									100	100	90.3	99.6	99.6	99.4	91.0	94.6
W6G										100	90.3	99.6	99.6	99.4	91.0	94.6
SP7G											90.3	99.6	99.6	99.4	91.0	94.6
W10G												90.1	90.1	89.9	89.9	91.9
SA3G													100	99.9	90.8	94.5
SA5Y														99.9	90.83	94.5
SL4G															90.7	94.4
SL5Y																92.7
SO5Y																

Auxiliary cluster 1 – Immunity																
	A1552	SA10G	E7G	SA7G	SL6Y	SP6G	L6G	W7G	W6G	SP7G	W10G	SA3G	SA5Y	SL4G	SL5Y	SO5Y
A1552		15.5	15.5	15.5	15.2	15.5	15.2	15.9	15.9	15.9	14.0	15.9	15.9	15.9	15.1	15.1
SA10G			100	100	72.1	99.6	71.7	85.7	85.7	85.7	78.3	85.7	85.7	85.7	76.2	97.5
E7G				100	72.1	99.6	71.7	85.7	85.7	85.7	78.3	85.7	85.7	85.7	76.2	97.5
SA7G					72.1	99.6	71.7	85.7	85.7	85.7	78.3	85.7	85.7	85.7	76.2	97.5
SL6Y						72.1	99.6	78.7	78.7	78.7	65.6	78.7	78.7	78.7	91.4	72.5
SP6G							71.7	85.7	85.7	85.7	77.9	85.7	85.7	85.7	75.8	97.1
L6G								78.3	78.3	78.3	65.2	78.3	78.3	78.3	90.9	72.1
W7G									100	100	71.3	100	100	100	73.0	85.2
W6G										100	71.3	100	100	100	73.0	85.2
SP7G											71.3	100	100	100	73.0	85.2
W10G												71.3	71.3	71.3	70.2	79.2
SA3G													100	100	73.0	85.2
SA5Y														100	73.0	85.2
SL4G															73.0	85.2
SL5Y																76.3
SO5Y																

Green: 100% identity (same family and subfamily)  
 Yellow: 90% - 99.99% identity (same family, with some polymorphism)  
 Orange: 30% - 90% identity (same family, with considerable polymorphism)  
 Red: <30% identity (different families)

**Table S7.** Matrices of % identity (percentage of residues which are identical) among effector and immunity proteins harbored in the T6SS auxiliary cluster 2 from environmental *V. cholerae* strains and pandemic strain A1552.

Auxiliary cluster 2 – Effector																
	A1552	SA10G	E7G	SA7G	SL6Y	SP6G	L6G	W7G	W6G	SP7G	W10G	SA3G	SA5Y	SL4G	SL5Y	SO5Y
A1552		14.6	14.4	14.4	98.3	94.8	14.8	94.0	94.0	14.8	96.5	94.3	93.6	93.6	93.6	12.7
SA10G			95.2	95.2	14.5	15.3	97.6	15.2	15.2	97.5	14.9	15.0	15.2	15.2	15.2	20.2
E7G				100	14.3	15.0	95.1	15.0	15.0	95.0	14.6	14.7	15.1	15.1	15.1	19.8
SA7G					14.3	15.0	95.1	15.0	15.0	95.0	14.6	14.7	15.1	15.1	15.1	19.8
SL6Y						94.1	14.7	93.7	93.5	14.7	95.7	93.8	93.7	93.7	93.7	12.8
SP6G							15.4	96.1	96.1	15.4	94.7	98.8	95.0	95.0	95.0	12.7
L6G								15.3	15.3	99.9	15.0	15.2	15.4	15.4	15.4	19.8
W7G									100	15.3	93.8	94.8	99.0	99.0	99.0	13.0
W6G										15.3	93.8	94.8	99.0	99.0	99.0	13.0
SP7G											15.0	15.2	15.4	15.4	15.4	19.8
W10G												94.0	93.4	93.4	93.4	12.8
SA3G													94.0	94.0	94.0	12.4
SA5Y														100	100	13.0
SL4G															100	13.0
SL5Y																13.0
SO5Y																

Auxiliary cluster 2 – Immunity																
	A1552	SA10G	E7G	SA7G	SL6Y	SP6G	L6G	W7G	W6G	SP7G	W10G	SA3G	SA5Y	SL4G	SL5Y	SO5Y
A1552		13.2	12.9	12.9	99.6	72.3	12.9	68.7	68.7	12.9	83.9	71.9	68.3	68.3	68.2	12.4
SA10G			73.1	73.1	13.2	13.6	92.5	12.9	12.9	92.2	12.5	13.6	12.9	12.9	12.9	9.4
E7G				100	12.9	11.2	73.9	11.9	11.9	73.9	12.2	11.5	11.9	11.9	11.9	8.1
SA7G					12.9	11.2	73.9	11.9	11.9	73.9	12.2	11.5	11.9	11.9	11.9	8.1
SL6Y						71.9	12.9	68.3	68.3	12.9	83.5	71.5	67.9	67.9	67.9	12.4
SP6G							12.5	80.1	80.1	12.5	74.0	99.6	80.5	80.5	80.5	12.1
L6G								12.5	12.5	99.7	12.2	12.5	12.5	12.5	12.5	9.9
W7G									100	12.5	67.5	79.7	99.2	99.2	99.2	11.0
W6G										12.5	67.5	79.7	99.2	99.2	99.2	11.0
SP7G											12.2	12.5	12.5	12.5	12.5	9.9
W10G												73.6	67.5	67.5	67.5	11.2
SA3G													80.1	80.1	80.1	12.1
SA5Y														100	100	11.0
SL4G															100	11.0
SL5Y																11.0
SO5Y																

Green: 100% identity (same family and subfamily)  
 Yellow: 90% - 99.99% identity (same family, with some polymorphism)  
 Orange: 30% - 90% identity (same family, with considerable polymorphism)  
 Red: <30% identity (different families)

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