

Appendix

A binary effector module secreted by a type VI secretion system

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Appendix Table S1. A list of bacterial strains used in this study.

Strain name	Genotype	Comments	Source
<i>Vibrio parahaemolyticus</i> RIMD 2210633	Wild type	Used for generating deletion strains, as an attacker in bacterial competition assays, and as a template for PCR amplification.	Obtained from Kim Orth; (Makino <i>et al</i> , 2003)
<i>Vibrio parahaemolyticus</i> RIMD 2210633 $\Delta hcp1$	$\Delta hcp1$	RIMD 2210633 derivative containing an in-frame deletion of <i>vp1393</i> ; used as an attacker in bacterial competition assays and for VP1388 and VP1390 expression.	This study
<i>Vibrio parahaemolyticus</i> RIMD 2210633 $\Delta vp1388$	$\Delta vp1388$	RIMD 2210633 derivative containing an in-frame deletion of <i>vp1388</i> ; used as an attacker and prey in bacterial competition assays.	This study
<i>Vibrio parahaemolyticus</i> RIMD 2210633 $\Delta vp1388^{MIX}$	$\Delta vp1388^{MIX}$	RIMD 2210633 derivative containing a deletion of the <i>vp1388</i> region encoding a MIX domain (corresponding to amino acids 242-423); used as an attacker in bacterial competition assays.	This study
<i>Vibrio parahaemolyticus</i> RIMD 2210633 $\Delta vp1389$	$\Delta vp1389$	RIMD 2210633 derivative containing an in-frame deletion of <i>vp1389</i> ; used as an attacker and prey in bacterial competition assays, and in immunoprecipitation assays.	This study
<i>Vibrio parahaemolyticus</i> RIMD 2210633 $\Delta vp1390$	$\Delta vp1390$	RIMD 2210633 derivative containing an in-frame deletion of <i>vp1390</i> ; used as an attacker and prey in bacterial competition assays.	This study
<i>Vibrio parahaemolyticus</i> RIMD 2210633 Δhns	Δhns	RIMD 2210633 derivative containing an in-frame deletion of <i>vp1133</i> ; used as an attacker and prey in bacterial competition	This study

		assays, and in secretion assays.	
<i>Vibrio parahaemolyticus</i> RIMD 2210633 $\Delta hns/\Delta hcp1$	$\Delta hns/\Delta hcp1$	RIMD 2210633 derivative containing an in-frame deletion of <i>vp1133</i> and of <i>vp1393</i> ; used as an attacker in bacterial competition assays, in secretion assays, and in immunoprecipitation assays.	This study
<i>Vibrio parahaemolyticus</i> RIMD 2210633 $\Delta hns/\Delta vp1388^{MIX}$	$\Delta hns/\Delta vp1388^{MIX}$	RIMD 2210633 derivative containing an in-frame deletion of <i>vp1133</i> and of the <i>vp1388</i> region encoding a MIX domain (corresponding to amino acids 242-423); used as an attacker in bacterial competition assays, and in secretion assays.	This study
<i>Vibrio parahaemolyticus</i> RIMD 2210633 $\Delta hns/\Delta vp1390$	$\Delta hns/\Delta vp1390$	RIMD 2210633 derivative containing an in-frame deletion of <i>vp1133</i> and of <i>vp1390</i> ; used as an attacker in bacterial competition assays, and in secretion assays.	This study
<i>Vibrio parahaemolyticus</i> RIMD 2210633 $\Delta hns/\Delta hcp1/\Delta vp1390$	$\Delta hns/\Delta hcp1/\Delta vp1390$	RIMD 2210633 derivative containing an in-frame deletion of <i>vp1133</i> , <i>vp1393</i> , and <i>vp1390</i> ; used for immunoprecipitation assays.	This study
<i>Escherichia coli</i> DH5 α (λ pir)	K-12 derivative laboratory strain containing λ pir	Used for plasmid maintenance and cloning	Obtained from Eric V. Stabb
<i>Escherichia coli</i> XL1 Blue	Laboratory strain	Used as prey in bacterial competition assays	Purchased from Addgene
<i>Escherichia coli</i> sAJM.1506	MG1655-derivative; "Marionette" strain	Used for protein expression, toxicity assays, and microscopy	Purchased from Addgene; (Meyer <i>et al</i> , 2019)
<i>Escherichia coli</i> BL21 (DE3)	Laboratory strain	Used for protein expression	Obtained from Kim Orth

Appendix Table S2. A list of plasmids used in this study.

Plasmid name	Description	Comments	Source
pDM4	a Cm ^R and oriVR6K-containing suicide vector	Used to generate deletions in <i>Vibrio</i> genomes	(O'Toole <i>et al</i> , 1996)
pDM4:vp1388	pDM4 containing 1 kb upstream and 1 kb downstream of vp1388 in its MCS	Used to delete vp1388 in <i>V. parahaemolyticus</i> RIMD 2210633	(Salomon <i>et al</i> , 2014a)
pDM4:vp1388 ^{MIX}	pDM4 containing 1 kb upstream and 1 kb downstream of the vp1388 region encoding the MIX domain in its MCS	Used to delete the vp1388 region encoding the MIX domain (corresponding to amino acids 242-423) in <i>V. parahaemolyticus</i> RIMD 2210633	This study
pDM4:vp1389	pDM4 containing 1 kb upstream and 1 kb downstream of vp1389 in its MCS	Used to delete vp1389 in <i>V. parahaemolyticus</i> RIMD 2210633	This study
pDM4:vp1390	pDM4 containing 1 kb upstream and 1 kb downstream of vp1390 in its MCS	Used to delete vp1390 in <i>V. parahaemolyticus</i> RIMD 2210633	This study
pDM4:hns	pDM4 containing 1 kb upstream and 1 kb downstream of vp1133 in its MCS	Used to delete hns in <i>V. parahaemolyticus</i> RIMD 2210633	(Salomon <i>et al</i> , 2014b)
pDM4:hcp1	pDM4 containing 1 kb downstream and 1 kb upstream of vp1393 in its MCS	Used to delete vp1393 in <i>V. parahaemolyticus</i> RIMD 2210633	(Salomon <i>et al</i> , 2013)
pBAD ^K /Myc-His	pBR322 ori-containing plasmid harboring a Kan ^R cassette, araC, and an MCS following a <i>Pbad</i> promoter	Used for arabinose-inducible expression	(Salomon <i>et al</i> , 2013)
pPER5	pBAD ^K /Myc-His with a PelB signal peptide inserted at the 5' end of the MCS	Used for arabinose-inducible expression of proteins targeted to the periplasm in <i>E. coli</i>	(Dar <i>et al</i> , 2018)
pBAD33.1	p15A ori-containing plasmid carrying a Cm ^R gene, araC, and an MCS following a <i>Pbad</i> promoter.	Used as template to construct pBAD33.1 ^F	Purchased from Addgene; (Chung & Raetz, 2010)
pBAD33.1 ^F	pBAD33.1 with a FLAG tag inserted at the 3' end of the MCS	Used for arabinose-inducible expression	(Fridman <i>et al</i> , 2020)
pVP1388 ^{peri}	pPER5 plasmid containing the CDS of vp1388 in frame with the N-terminal PelB signal peptide and the	Used for arabinose-inducible expression of periplasm-targeted VP1388 in <i>E. coli</i>	This study

	C-terminal Myc-His tag		
pVP1388 ^{Myc}	pBAD ^K /Myc-His plasmid containing the CDS of <i>vp1388</i> in frame with a C-terminal Myc-His tag	Used for arabinose-inducible expression of VP1388 in <i>E. coli</i>	This study
pVP1388	pBAD33.1 ^F plasmid containing the CDS of <i>vp1388</i> in frame with the C-terminal FLAG tag	Used for arabinose-inducible expression of VP1388 in <i>Vibrio</i>	This study
pVP1389	pBAD/Myc-His containing the CDS of <i>vp1389</i> in frame with the C-terminal Myc-His tag	Used for arabinose-inducible expression of VP1389 in <i>Vibrio</i>	This study
pVP1389 ^{ΔSS}	pBAD/Myc-His containing the CDS of <i>vp1389</i> lacking the first 17 amino acids in frame with the C-terminal Myc-His tag	Used for arabinose-inducible expression of VP1389 ^{ΔSS} in <i>Vibrio</i>	This study
pVP1390 ^{peri}	pPER5 plasmid containing the CDS of <i>vp1390</i> in frame with the N-terminal PelB signal peptide and the C-terminal Myc-His tag	Used for arabinose-inducible expression of periplasm-targeted VP1390 in <i>E. coli</i>	This study
pVP1390	pBAD33.1 plasmid containing the CDS of <i>vp1390</i> in frame with the C-terminal FLAG tag	Used for arabinose-inducible expression of VP1390 in <i>Vibrio</i> and in <i>E. coli</i>	This study
pVP1390 ^{cyto}	pBAD/Myc-His containing the CDS of <i>vp1390</i> in frame with the C-terminal Myc-His tag	Used for arabinose-inducible expression of cytoplasmic VP1390 in <i>E. coli</i>	This study
pPoNi	pBAD33.1 plasmid containing the CDS of <i>b5c30_rs14460</i> from <i>V. parahaemolyticus</i> 12-297/B in frame with the C-terminal FLAG tag	Used for arabinose-inducible expression of PoNi in <i>E. coli</i>	(Jana <i>et al</i> , 2019)
pPoNe ^{D335A}	pBAD/Myc-His containing the CDS of <i>b5c30_rs14465</i> from <i>V. parahaemolyticus</i> 12-297/B carrying a mutation in the active site D335A, in frame	Used for arabinose-inducible expression of PoNe ^{D335A} in <i>E. coli</i>	(Jana <i>et al</i> , 2019)

	with the C-terminal Myc-His tag		
psfGFP ^{peri}	pPER5 plasmid containing the CDS of sfGFP in frame with the N-terminal PelB signal peptide and the C-terminal Myc-His tag	Used for arabinose-inducible expression of sfGFP in <i>E. coli</i>	This study
psfGFP	pBAD33.1 ^F plasmid containing the CDS of sfGFP in frame with the C-terminal FLAG tag	Used for arabinose-inducible expression of sfGFP in <i>Vibrio</i>	This study
pBC3020	pBAD33.1 ^F plasmid containing the CDS of BC3020 in frame with the C-terminal FLAG tag	Used for arabinose-inducible expression of BC3020 in <i>Vibrio</i>	(Jana <i>et al</i> , 2019)
pGFP	a Spec ^R -containing, high copy number plasmid for constitutive expression of GFP in vibrios	Used for constitutive expression of GFP in <i>V. parahaemolyticus</i> prey visualized under the microscope	(Ritchie <i>et al</i> , 2012)

Appendix Table S3. A list of primers used in this study.

Primer name	Sequence (5' to 3')	Description
VP1388_MIX_SacI_U P_F	CACCGAGCTCCCTCAACCTTCGGCTATCAGT	Used to amplify 1 kb upstream of the <i>vp1388</i> MIX domain-encoding region to construct pDM4: <i>vp1388-MIX</i>
VP1388_MIX_UP_R	CCGGGATCACTTTCCAACACGTTATCCCACTCATCC	
VP1388_MIX_DN_F	GGATAACGTGTTGGAAAGTGATCCCGGAACCAAG	Used to amplify 1 kb downstream of the <i>vp1388</i> MIX domain-encoding region to construct pDM4: <i>vp1388-MIX</i>
VP1388_MIX_Sall_D N_R	CCACGTCGACCCTAGTTCAATATACTCACCCG	
VP1389_SacI_UP_F	CACCGAGCTCAATTACGGAAAATGGTTAC	Used to amplify 1 kb upstream of <i>vp1389</i> to construct pDM4: <i>vp1389</i>
VP1389_BamHI_UP_ R	CAACGGATCCCTTATATTCTCTTCGTTATC	
VP1389_BamHI_DN_ F	CAACGGATCCGCCTGTAGAGAACTGCAATG	Used to amplify 1 kb downstream of <i>vp1389</i> to construct pDM4: <i>vp1389</i>
VP1389_Sall_DN_R	CAACGTCGACGCAAATTTCCCTAACGGAG	
VP1390_SphI_UP_F	CACCGCATGCAATGCTCAACAGTGGGAAG	Used to amplify 1 kb upstream of <i>vp1390</i> to construct pDM4: <i>vp1390</i>
VP1390_BamHI_UP_ R	CAACGGATCCAAATCCCCCTCTTTAACAAAATC	
VP1390_BamHI_DN_ F	CACCGGATCCATGCAAAGAAAAACCGCC	Used to amplify 1 kb downstream of <i>vp1390</i> to construct pDM4: <i>vp1390</i>
VP1390_SpeI_DN_R	CAACACTAGTTGGTGAAACGGGCGCTGG	
pVP1388 ^{peri} _F	CCCAGCCGGCGATGGCCATGTGGAGAATTCAAATGCATTT AG	Used to amplify

pVP1388 ^{peri} _R	TTTTGTTTCGGGCCCAAGCTTACTTGGCTGATCTTTTTTCTT ATAAG	<i>vp1388</i> to construct pVP1388 ^{peri}
pVP1388 ^{Myc} _F	GCTAACAGGAGGAATTAACCTTGTGGAGAATTCAAATGC	Used to amplify <i>vp1388</i> to construct pVP1388 ^{Myc}
pVP1388 ^{Myc} _R	TTTTGTTTCGGGCCCAAGCTTACTTGGCTGATCTTTTTTCTT ATAAGCC	
pVP1388_F	CTTTAAGAAGGAGATATACATTTGTGGAGAATTCAAATGC	Used to amplify <i>vp1388</i> to construct pVP1388
pVP1388_R	CGTCGTCATCCTTGTAAATCACTTGGCTGATCTTTTTTCTTA TAAGC	
pVP1389_F	GCTAACAGGAGGAATTAACCATGAATCTACGTCATACATT TGC	Used to amplify <i>vp1389</i> to construct pVP1389
pVP1389_R	TTTTGTTTCGGGCCCAAGCTTTAAATCCCCCTCTTTAAC	
pVP1389_ΔSS_F	GCTAACAGGAGGAATTAACCATGTGTAATCAAGAATCGGC CAAGG	Used with pVP1389_R to amplify <i>vp1389</i> to construct pVP1389 ^{ΔSS}
pVP1390 ^{peri} _F	CCCAGCCGGCGATGGCCATGAGCCTTGTAGAGAACTGCA ATG	Used to amplify <i>vp1390</i> to construct pVP1390 ^{peri}
pVP1390 ^{peri} _R	TTTTGTTTCGGGCCCAAGCTTGGAAGGGTTATCAAGTGACT TTTC	
pVP1390 ^{cyto} _F	TTTTGGGCTAACAGGAGGAATTAACCATGAGCCTTGTAGA GAACTGC	Used with pVP1390 ^{peri} _R to amplify <i>vp1390</i> to construct pVP1390 ^{cyto}
pVP1390_F	CTTTAAGAAGGAGATATACATATGAGCCTTGTAGAGAACT GC	Used to amplify <i>vp1390</i> to construct pVP1390
pVP1390_R	CGTCGTCATCCTTGTAAATCGGAAGGGTTATCAAGTGACTT TTC	
psfGFP ^{peri} _F	CCCAGCCGGCGATGGCCATGGTGAGCAAGGGCGAGGAG	Used to amplify <i>sfgfp</i> to construct psfGFP ^{peri}
psfGFP ^{peri} _R	TTTTGTTTCGGGCCCAAGCTTCTTGTACAGCTCGTCCATGC C	
psfGFP_F	CTTTAAGAAGGAGATATACATATGGTGAGCAAGGGCGAGG AGC	Used to amplify <i>sfgfp</i> to construct psfGFP
psfGFP_R	CGTCGTCATCCTTGTAAATCCTTGTACAGCTCGTCCATGCC G	

Appendix References

- Chung HS & Raetz CRH (2010) Interchangeable domains in the Kdo transferases of *Escherichia coli* and *Haemophilus influenzae*. *Biochemistry* 49: 4126–4137
- Dar Y, Salomon D & Bosis E (2018) The antibacterial and anti-eukaryotic Type VI secretion system MIX-effector repertoire in *Vibrionaceae*. *Mar Drugs* 16: 433
- Fridman CM, Keppel K, Gerlic M, Bosis E & Salomon D (2020) A comparative genomics methodology reveals a widespread family of membrane-disrupting T6SS effectors. *Nat Commun* 11: 1085
- Jana B, Fridman CM, Bosis E & Salomon D (2019) A modular effector with a DNase domain and a marker for T6SS substrates. *Nat Commun* 10: 3595
- Makino K, Oshima K, Kurokawa K, Yokoyama K, Uda T, Tagomori K, Iijima Y, Najima M, Nakano M, Yamashita A, *et al* (2003) Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of *V. cholerae*. *Lancet* 361: 743–749
- Meyer AJ, Segall-Shapiro TH, Glassey E, Zhang J & Voigt CA (2019) *Escherichia coli* “Marionette” strains with 12 highly optimized small-molecule sensors. *Nat Chem Biol* 15: 196–204
- O’Toole R, Milton DL & Wolf-Watz H (1996) Chemotactic motility is required for invasion of the host by the fish pathogen *Vibrio anguillarum*. *Mol Microbiol* 19: 625–637
- Ritchie JM, Rui H, Zhou X, Iida T, Kodama T, Ito S, Davis BM, Bronson RT & Waldor MK (2012) Inflammation and Disintegration of Intestinal Villi in an Experimental Model for *Vibrio parahaemolyticus*-Induced Diarrhea. *PLoS Pathog* 8: e1002593
- Salomon D, Gonzalez H, Updegraff BL & Orth K (2013) *Vibrio parahaemolyticus* Type VI secretion system 1 is activated in marine conditions to target bacteria, and is differentially regulated from system 2. *PLoS One* 8: e61086
- Salomon D, Kinch LN, Trudgian DC, Guo X, Klimko JA, Grishin N V., Mirzaei H & Orth K (2014a) Marker for type VI secretion system effectors. *Proc Natl Acad Sci* 111: 9271–9276
- Salomon D, Klimko JA & Orth K (2014b) H-NS regulates the *Vibrio parahaemolyticus* type VI secretion system 1. *Microbiol (United Kingdom)* 160: 1867–1873