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Supporting Information for

Cooperation and cheating orchestrate Vibrio assemblages and polymicrobial synergy in oysters infected with OsHV-1 virus

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Table S1. Prevalence of the Harveyi clade in oyster flesh during Mediterranean POMS episodes.

Oysters were collected during and outside POMS episodes in the Thau lagoon. At each sampling time, a total of 76 to 96 bacterial isolates obtained from oyster flesh were screened for the Harveyi clade (rctB-F/rctB-R primers) and the *V. harveyi* species (Vh-mreB-F/Vh-mreB-R primers). The oyster flesh was simultaneously tested for OsHV-1 µvar detection (OsHVDPFor/OsHVDPRev primers).

Date	Field mortalities	OsHV-1 µvar virus	% Harveyi clade	% *V. harveyi species*
1-Oct-2015	Yes	positive	94.0	75.9
9-June-2016	No	negative	0.0	0.0
23-June-2016	Yes	positive	49.0	32.0
13-March-2017	No	negative	1.0	1.0

Table S2. Samplings in the Thau lagoon.

Sampling date	GPS coordinates	Temp	Juvenile Mortality	Biological material	Time of immersion	No of isolates		No of *Vibrio* isolates*		No of confirmed Harveyi isolates**	
1-Oct-2015	43°25'07.5"N 3°37'21.4"E	18°C	yes	Oysters (n=30)	4 weeks	96		55		54	
1-Oct-2015	43°25'07.5"N 3°37'21.4"E	18°C	yes	Water column > 60µm	n/a	96		59		5	
1-Oct-2015	43°25'07.5"N 3°37'21.4"E	18°C	yes	Water column 5-60 µm	n/a	96	472	61	437	21	53
1-Oct-2015	43°25'07.5"N 3°37'21.4"E	18°C	yes	Water column 1-5 µm	n/a	96		67		23	
1-Oct-2015	43°25'07.5"N 3°37'21.4"E	18°C	yes	Water column 0,2-1 µm	n/a	88		62		4	
9-June-2016	43°22'44.8"N 3°34'17.5"E	24°C	no	Oysters (n=30)	1 week	76		76		0	
23-June-2016	43°22'45.1"N 3°34'16.1"E	22°C	yes	Oysters (n=29)	10.5 weeks	96		96		47	
13-March-2017	43°26.058'N 003°39.878'E	12.6°C	no	Oysters (n=12)	2 weeks	96		96		1	

* based on *hsp60* sequencing

** based on MLST (*hsp60, rctB, topA*, *mreB*)

Table S3. List of primers

Primer name	Primer sequence	Description and references
rctB-Fw-I	5'-TCGTCGGCAGCGTCAGATGTGTATAAGA-GACAGYRTGAATAGGCTCAAATTCGCCGTC -3'	Forward primer for *rctB* gene amplification (barcoding), this study
rctB-Rv-I	5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGA-CAGYRCCWTCSTTCTWYGAAGAAYYGCT -3'	Reverse primer for *rctB* gene amplification (barcoding) , this study
Insert-pLP12-R	5'- CGGCTGACATGGGAATTGC -3'	Forward primer annealing upstream of the multiple cloning site of pLP12, this study
del-pvuA1-A2-OG05-F	5'- GTCATGCTCACGACCAAAC -3'	Forward primer annealing upstream of *pvuA1.* Used to verify integration of pAM010 in *V. rotiferianus* Th15_O_G05 and to verify the presence of *pvuA1* and *pvuA2* genes, this study
del-pvuA1-A2-OG05-R	5'- GGCTTGCTCACTTGGTTG -3'	Reverse primer annealing downstream of *pvuA2.* Used to verify the presence of p*vuA1* and *pvuA2* genes in *V. rotiferianus* Th_15_O_G05, this study
del-exsA-primer1	5'- GTATCGATAAGCTTGATATCGA-ATTCAACTTAGGTCATCGGGTCAG-3'	Deletion of ExsA in *V. harveyi* Th15_O_G11, this study
del-exsA-primer2	5'- CATCTCCAGAACGAACCTTACT-GCGCGAGTCGACCCTTTCTG-3'	Deletion of ExsA in *V. harveyi* Th15_O_G11, this study
del-exsA-primer3	5'- CAGAAAGGGTCGACTCGCGCA-GTAAGGTTCGTTCTGGAGATG-3'	Deletion of ExsA in *V. harveyi* Th15_O_G11, this study
del-exsA-primer4	5'- CCCCCGGGCTGCAGGAATTCAG-GCGCATAGCAAATCGATC-3'	Deletion of ExsA in *V. harveyi* Th15_O_G11, this study
exsA-020123-13	TATCTCCAGACCTAGGTCTC	External primers flanking ExsA, this study
exsA-020123-14	ACACCGTCTGATCCGTCTGC	External primers flanking ExsA, this study
hsp60-F	5'- GAATTCGAIIIIGCIGGIGAYGGIACIACIAC -3'	Forward primer for hsp60 gene amplification (MLST) (1)
hsp60-R	5'- CGCGGGATCCYKIYKITCICCRAAICCIGGIGCYTT -3'	Reverse primer for hsp60 gene amplification (MLST), (1)
VtopA400F	5'- GAGATCATCGGTGGTGATG -3'	Forward primer for topA gene amplification (MLST) (2)
VtopA1200R	5'- GAAGGACGAATCGCTTCGTG -3'	Reverse primer for topA gene amplification (MLST) (2)
rctB-F	5'- GCTGATGAAAAAATTCTGATTAAAGC -3'	Forward primer for Harveyi clade rctB gene amplification (MLST), this study
rctB-R	5'- TGAATAGGCTCAAATTCGCCGTC -3'	Reverse primer for Harveyi clade rctB gene amplification (MLST), this study
VmreB12F	5'- ACTTCGTGGCATGTTTTC -3'	Forward primer for mreB gene amplification (MLST) (2)
VmreB999R	5'- CCGTGCATATCGATCATTTC -3'	Reverse primer for mreB gene amplification (MLST) (2)
OsHVDPFor	5'- ATTGATGATGTGGATAATCTGTG -3'	Forward primer for OsHV-1 DNA pol gene amplification (quantification) (3)
OsHVDPRev	5'- GGTAAATACCATTGGTCTTGTTCC -3'	Reverse primer for OsHV-1 DNA pol gene amplification (quantification) (3)
Vh-mreB-F	5'- GCTGATGAAAAAATTCTGATTAAAGC -3'	Forward primer for *V. harveyi* mreB gene amplification (detection), this study

Vh-mreB-R	5'- TGAATAGGCTCAAATTCGCCGTC -3'	Reverse primer for *V. harveyi* mreB gene amplification (detection), this study
567F	5'- GGCGTAAAGCGCATGCAGGT -3'	Forward primer for Vibrio 16s rDNA amplification (quantification) (4)
680R	5'- GAAATTCTACCCCCCTCTACAG -3'	Reverse primer for Vibrio 16s rDNA amplification (quantification) (4)
GFP_F522	5'- TGGAAGCGTTCAACTAGCAG -3'	Forward primer for *gfp* amplification (quantification), this study
GFP_R625	5'- AAAGGGCAGATTGTGTGGAC -3'	Reverse primer for *gfp* amplification (quantification), this study
mCherry_F494	5'- AGATCAAGCAGAGGCTGAAGCTGA -3'	Forward primer for *mCherry* amplification (quantification) (5)
mCherry-R655	5'- ACTGTTCCACGATGGTGTAGTCCT -3'	Forward primer for *mCherry* amplification (quantification) (5)
hr-F1	5'- AAGAGGTTCAAGCCCAGACG -3'	Forward primer for *V. harveyi-V. rotiferianus* chemotaxis protein amplification (quantification), this study
hr-R1	5'- AGTCCTGCGCTTGTTTCTCA -3'	Forward primer for *V. harveyi-V. rotiferianus* chemotaxis protein amplification (quantification), this study
oj-F1	5'- CCAGAAGAGCAAACCGTGAT -3'	Forward primer for *V. owensii-V. jasicida ompA* amplification (quantification), this study
oj-R1	5'- CGACTGGCTAACTGCATCAA -3'	Reverse primer for *V. owensii-V. jasicida ompA* amplification (quantification), this study

Table S4. List of genetically modified strains

Strains	Description	Reference
E. coli		
DH5αλpir	*sup* E44, Δ*lacU*169 (Φ*lacZ*ΔM15), *recA1*, *endA1*, *hsdR17*, *thi-1*, *gyrA96*, *relA1*, λpir phage lysogen	LEMAR collection
β3914	F-, RP4-2-Tc::Mu, Δ*dapA*::(*erm*-pir) *gyrA462* zei-298::Tn10, Km^R, Em^R, Tc^R, DAP-	(6)
GEB883 + pEVS104	Strain GEB883 containing pEVS104. Used as a helper strain during bacterial conjugation	(7)
DH5α λpir + pAM010	*E. coli* DH5α λpir containing the pAM010	This study
β3914 + pAM010	*E. coli* β3914 containing the pAM010	This study
DH5α λpir + pFD086	*E. coli* DH5α λpir containing the pFD086 (GFP, $Trim^R$)	(7)
DH5α λpir + pFD096	*E. coli* DH5α λpir containing the pFD096 (mCherry, $Trim^R$)	This study
π3813	B462 Δ*thyA::(erm-pir116)* [Erm^R]	(6)
V. rotiferianus		
Th15_O_G05	*V. rotiferianus* strain, isolated in 2015 in oysters sampled in Thau lagoon	This study
Th15_O_G05 ΔpvuA1-2	*V. rotiferianus* O_G05 strain in which the *pvuA1* and the *pvuA2* genes have been deleted	This study
Th15_O_G05 mCherry	*V. rotiferianus* O_G05 strain in which the pFD096 plasmid was conjugated (mCherry, $Trim^R$)	This study
V. harveyi		
Th15_0_G11	*V. harveyi* strain, isolated in 2015 in oysters sampled in Thau lagoon	This study
Th15_O_G11 GFP	*V. harveyi* O_G11 strain in which the pFD086 plasmid was electroporated (GFP, $Trim^R$)	This study
Th15_O_G11 ΔexsA	*V. harveyi* O_G11 strain in which the *exsA* gene has been deleted	This study

Table S5. List of plasmids used for mutagenesis and labelling

Plasmid name	Description	Reference
pMK-RQ_updn-pvuA1-2	Cloning vector in which the upstream region of *pvuA1* and the downstream region of *pvuA2* of *V. rotiferianus* Th15_O_G05 have been cloned. Km^R	GeneArt
pLP12	Suicide plasmid used for targeted deletion of chromosomal genes of *Vibrio* species. oriT-RP4, oriV-R6K, P_{BAD}-*vmi480*. Cm^R	(8)
pAM010	Derivative of pLP12 in which the upstream region of *pvuA1* and the downstream region of *pvuA2* of *V. rotiferianus* Th15_O_G05 have been inserted. Cm^R	This study
pFD086	Replicative plasmid for *Vibrio* species containing the *gfp* gene under the control of the constitutive P_{lac} promoter. $Trim^R$	(7)
pFD096	Derivative plasmid of pFD086 in which the *gfp* gene has been replaced by the *mcherry* gene of pME9407 (Rochat *et al.*, 2010), using the XbaI and BamHI sites. $Trim^R$	This study
pMRB	High copy number replicative plasmid. P_{lac}-GFP. Cm^R	(9)
pSW7848T	Suicide vector for allele exchange. This pSW23T derivative vector encodes the *ccdB* toxin gene under the control of an arabinose-inducible and glucose-repressible promoter, p_{BAD}	(14)

Table S6. Strains sequenced by high-throughput sequencing.

In this study, 17 Harveyi-related genomes from our collection (4 *V. harveyi*, 5 *V. owensii*, 4 V. *jasicida* and 4 *V. rotiferianus* isolates) were sequenced. The genome sequences have been deposited in the European Nucleotide Archive (ENA) under project accession number PRJEB49488.

Species	Isolates	Origin	Contig number	Genome size (Mb)	CDSs	Accession number
V. harveyi	Th15_O_G11	This study	54	5.88	5,485	ERS9919775
	Th15_F5_F11	This study	106	5.96	5,615	ERS9919777
	Th15_O_D03	This study	64	5.50	5,497	ERS9919776
	Th15_O_A01	This study	99	5.89	5,507	ERS9780105
V. rotiferianus	Th15_O_G05	This study	85	5.29	4,968	ERS9919778
	Th15_O_G10	This study	81	5.16	4,862	ERS9919779
	Th15_O_B06	This study	79	5.17	4,863	ERS9919780
	Th15_O_E12	This study	45	5.33	5,015	ERS9919781
V. jasicida	Th15_F5_H11	This study	52	6.1	5,724	ERS9919787
	Th15_F1_A12	This study	159	7.12	7,262	ERS9919788
	Th15_F1_C08	This study	158	7.12	7,251	ERS9919789
	Th15_F5_G08	This study	61	5.89	5,431	ERS9919790
V. owensii	Th15_Z_G08	This study	98	6.02	5,578	ERS9919782
	Th15_O_D04	This study	46	5.95	5,473	ERS9919783
	Th15_O_A03	This study	95	6.01	5,568	ERS9919784
	Th15_Z_B04	This study	43	5.97	5,474	ERS9919785
	Th15_F1_D04	This study	75	6.25	5,870	ERS9919786

Abbreviation; CDS, coding DNA sequence.

Table S7. Virulence and candidate virulence genes found in Vibrio strains used in mesocosm experiments.

X indicates the presence of T3SS, T6SS systems siderophore gene clusters and siderophore receptors in isolates. Among the T3SS1 associated effectors characterized in Harveyi isolates, we identified VopQ, VopR, VopS and VPA0450 as well as an associated cargo chaperone orthologous to VPA0451, which is needed for translocation of VPA0450 into the host cell membrane (10).

Species	Isolates	% mortality at day 1	T3SS	T3SS effectors	T6SS1	T6SS2	T6SS3	T6SS4	Amphi entero bactin	Entero bactin	Amphi entero bactin R	Enterob actin R
V. harveyi	Th15_O_G11	50	X	VopQ, VopR, VPA0450, VPA0451	X	X	X		X	X	X	X
	Th15_F5_F11	41	X	VopQ, VopR, VPA0450, VPA0451	X	X	X	X	X	X	X	X
	Th15_O_D03	28	X	VopQ, VopR, VPA0450, VPA0451	X	X	X		X	X	X	X
	Th15_O_A01	46	X	VopQ, VopR, VPA0450, VPA0451	X	X	X		X	X	X	X
V. rotiferianus	Th15_O_G05	30			X		X	X				X
	Th15_O_G10	10			X		X	X				X
	Th15_O_B06	0			X		X	X				X
	Th15_O_E12	15			X		X	X				X
V. jasicida	Th15_F5_H11	44	X	VopQ, VopS	X	X	X		X	X	X	X
	Th15_F1_A12	26	X	VopQ, VopS	X	X	X		X	X	X	X
	Th15_F1_C08	28	X	VopQ, VopS	X	X	X		X	X	X	X
	Th15_F5_G08	42	X	VopQ, VopS	X	X	X		X	X	X	X
V. owensii	Th15_Z_G08	0			X	X	X		X	X	X	X
	Th15_O_D04	0			X	X	X		X	X	X	X
	Th15_O_A03	0			X	X	X		X	X	X	X
	Th15_Z_B04	0			X	X	X		X	X	X	X
	Th15_F1_D04	0			X	X	X		X	X	X	X

Table S8. List of *V. harveyi* and *V. rotiferianus* specific genes.

As example, genes corresponding to the isolate *V. harveyi* Th15_O_A1 are indicated in this list. Orthologues of all these genes were identified only in *V. harveyi* and *V. rotiferianus* isolates. A gene cluster encoding components of vibrioferrin transport is indicated in light grey.

Vibrio harveyi Th15_O_A1	Product	Function
VHARVA1S8_v1_100126	protein of unknown function	
VHARVA1S8_v1_190050	Chemotaxis protein	Chemotaxis
VHARVA1S8_v1_260075	protein of unknown function	
VHARVA1S8_v1_260092	conserved membrane protein of unknown function	
VHARVA1S8_v1_360021	conserved protein of unknown function	
VHARVA1S8_v1_460139	conserved exported protein of unknown function	
VHARVA1S8_v1_480079	conserved exported protein of unknown function	
VHARVA1S8_v1_480080	Peptidase M66 family protein	
VHARVA1S8_v1_480410	conserved protein of unknown function	
VHARVA1S8_v1_530104	PvuA1, TonB-dependent siderophore receptor family protein	Vibrioferrin transport
VHARVA1S8_v1_530105	PvuA2, TonB-dependent siderophore receptor family protein	
VHARVA1S8_v1_530106	PvuB, iron-dicitrate transporter subunit ; periplasmic-binding component	
VHARVA1S8_v1_530285	conserved membrane protein of unknown function	
VHARVA1S8_v1_80159	conserved protein of unknown function	
VHARVA1S8_v1_80182	glycosyltransferase family 25 protein	

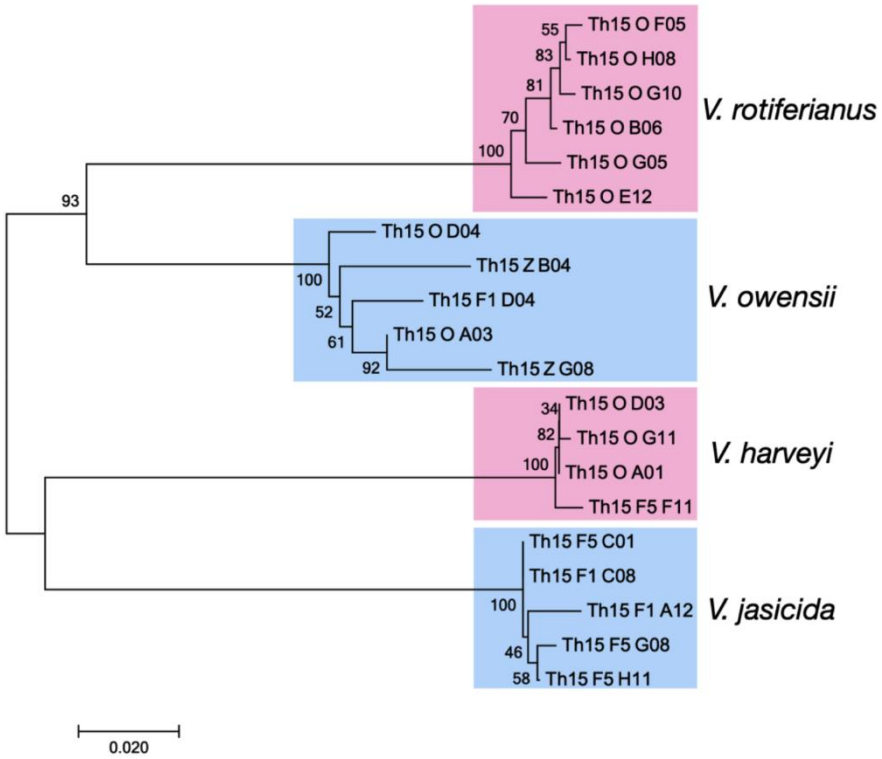
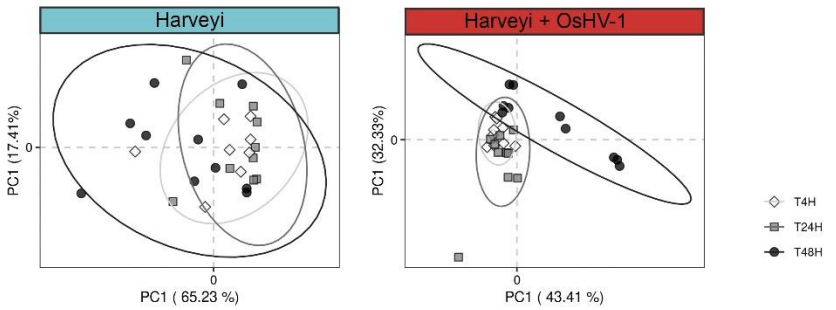


Fig S1. Discrimination of Harveyi species by *rctB* sequencing.
Phylogenetic tree constructed using the Neighbor-Joining method, the optimal tree with the sum of branch length = 0.49310882 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 494 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

A



B

Source of variation	DF	Sum Sq	Mean Sq	F	Pr (>F)
Harveyi.Time-steps	2	0.22743	0.15190	2.2389	0.059
Residual	25	1.26975	0.84810		
Total	27	1.49718	1.00000		
Harveyi+OsHV-1.Time-steps	2	0.75804	0.29006	5.3113	0.001***
Residual	26	1.85537	0.70994		
Total	28	2.61341	1.00000		

Fig S2. Monitoring of the dysbiosis by rctB-metabarcoding
The colonization of oysters by the *Vibrio* Harveyi community in a mesocosm experiment (see Fig. 2) was monitored trough an *rctB* gene-based barcoding. **(A) Principal Component Analysis (PCoA) ordination plots of Bray-Curtis dissimilarities for the *Vibrio* Harveyi community.** PCoA results are presented for each experimental condition. Left panel corresponds to animals exposed to 4 species of the Harveyi clade (V; *V. harveyi, V. rotiferianus, V. owensii, V. jasicida*. Right panels corresponds to oysters exposed simultaneously to the same 4 species of the Harveyi clade and OsHV-1. Each dot corresponds to the Harveyi community of one oyster. Different symbols are used for different time-steps (i.e. 4, 24 and 48 h). Ellipses represent the 95% confidence intervals for each group. Permutational multivariate analysis of variance (PERMANOVA) between the two experimental conditions and the three time-steps indicates significant differences Pr (>F) = 0.001. Data highlight the destabilization of the Harveyi community at 48h in the presence of both OsHV-1 µVar and *Vibrio* (right panel). No significant shift was observed at any time point when oysters were exposed to *Vibrio* only (left panel). **(B) Statistical analyses supporting Harveyi community destabilization at 48h in presence of OsHV-1.** Results of permutational multivariate analysis of variance (PERMANOVA) with 1,000 permutations using the *adonis2* function implemented in the vegan R package.

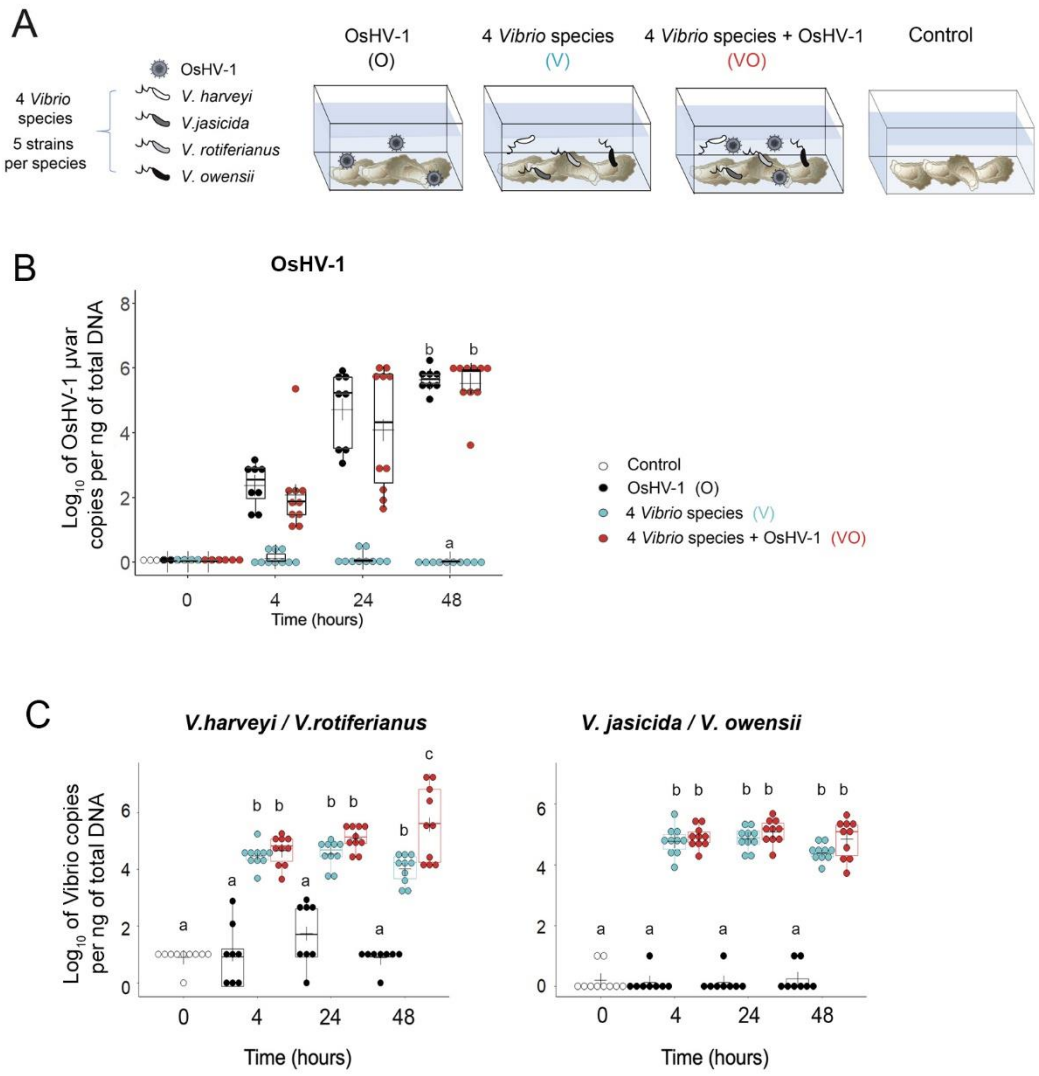


Fig S3. OsHV-1 load in oysters exposed to the mesocosm experiment (Fig. 2)
(A) Design of a mesocosm experiment to recapitulate the complexity of the disease. Oysters were immersed at 20°C in seawater containing OsHV-1 (O), 4 species of the Harveyi clade (V; *V. harveyi, V. rotiferianus, V. owensii, V. jasicida*), or both (VO). Oyster mortalities were monitored daily over 6 days. 10 individuals were sampled at each time point (0, 4, 24 and 48 h) for monitoring pathogens. **(B) Total OsHV-1 µVar quantified on individual oysters by qPCR.** Number of copies of OsHV-1 per ng of total DNA were $\log_{10}(x+1)$ transformed. Final time points were compared by a Kruskal-Wallis test. Significant differences between conditions are indicated with different letters ($p < 0.001$). **(C) qPCR monitoring of species of the Harveyi clade** on individual oysters using primer pairs discriminating *V. harveyi/V. rotiferianus* from *V. jasicida/V. owensii* (hr-F1/R1 and oj-F1/R1, targeting a specific chemotaxis protein and *ompA* gene, respectively). Data show that OsHV-1 significantly enhanced colonization by *V. harveyi*/*V. rotiferianus* (VO) at 48h (mutiple t test, $p < 0.01$). No significant effect of OsHV-1 was observed on *V. jasicida/V. owensii* colonization. Each dot represents an individual.

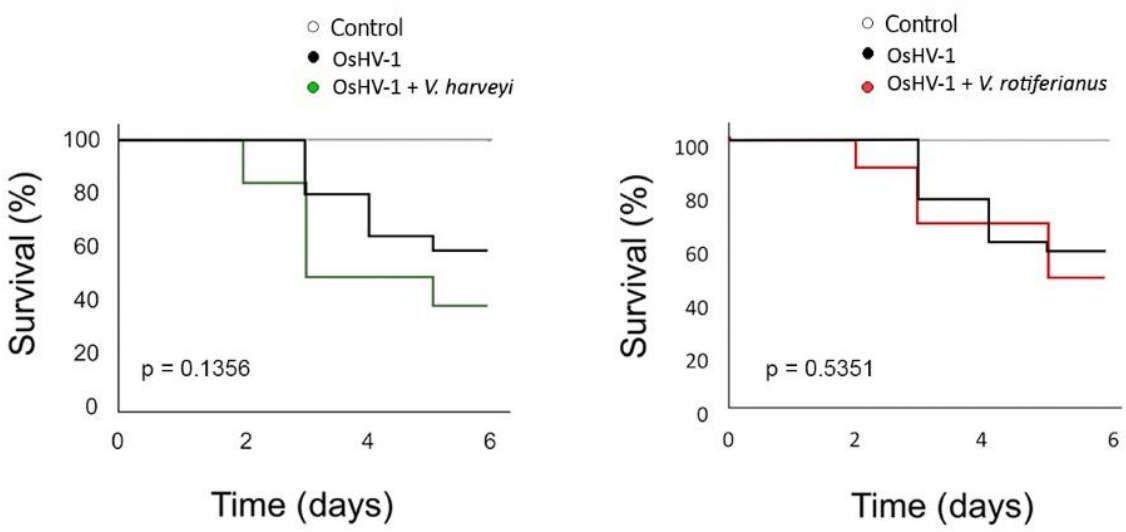


Fig S4. Mortalities of oysters co-infected with OsHV-1 and single Vibrio species
Kaplan-Meier survival curves were generated for oysters exposed to both *V. harveyi* Th15_O_G11 and OsHV-1 (green) or both *V. rotiferianus* Th15_O_G05 and OsHV-1 (red) compared to OsHV-1 only (black). No significant difference was observed between conditions (log-rank test, p = 0.1356 and p = 0.5351 for co-infections with *V. harveyi* and *V. rotiferianus*, respectively).

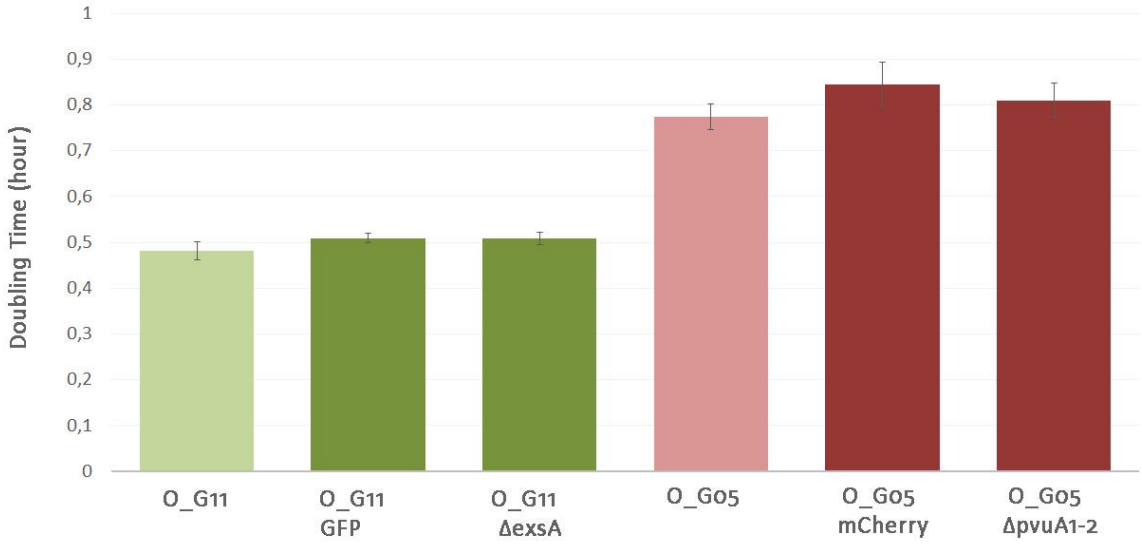


Fig S5. Doubling time of Vibrio strains and mutants
Doubling time of *V. harvey*i O_G11, O_G11 GFP, O_G11 Δ*exsA*, and *V. rotiferianus* O_G05, O_G05 mCherry, O_G05 Δ*pvuA1-2*. Bacteria were grown in LBS with shaking, and OD600nm was measured regularly to calculate the doubling time during exponential phase. A one-way ANOVA (with Tukey test) was performed for every condition. No significant differences was observed between wild-type and mutants of a same strain.

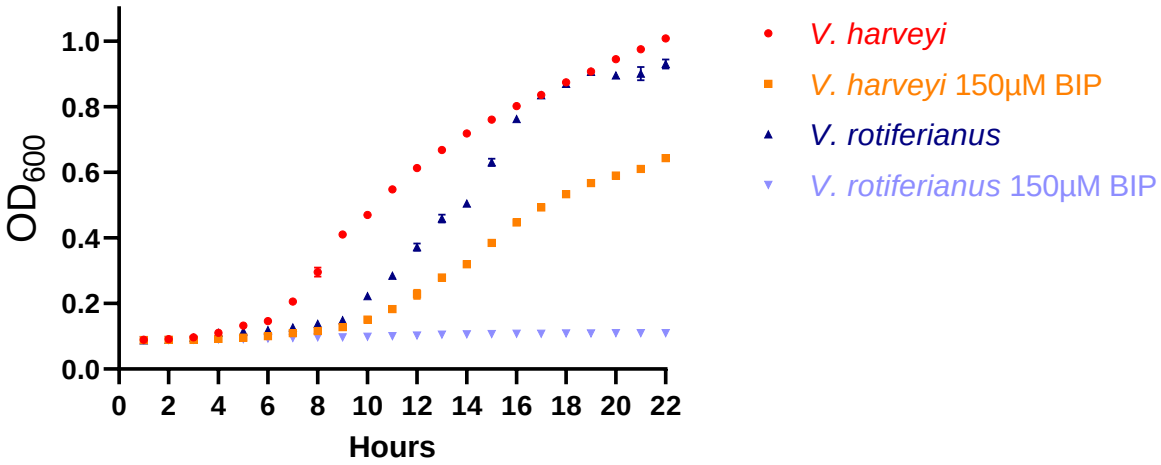
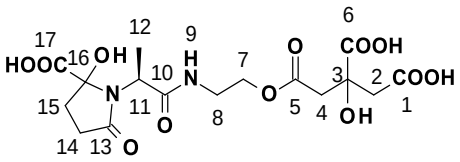
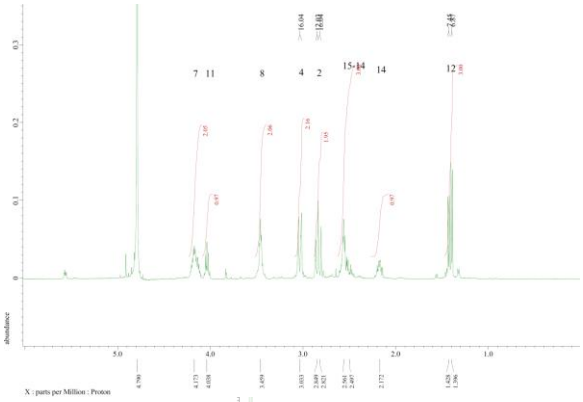


Fig S6. Growth of V. harveyi and V. rotiferianus in iron-depleted conditions.
Strains *V. harveyi* Th15_O_G11 and *V. rotiferianus* Th15_O_G05 were grown in Lewis water 1X supplemented with casaminoacids and Vitamin B. Iron-depletion was obtained by adding 150 µM 2,2'-bipyridine (BIP) to the minimal culture medium. Only *V. harveyi* could grow in such culture conditions.

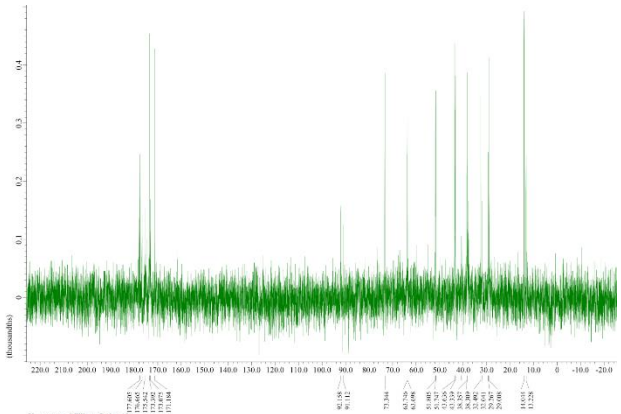


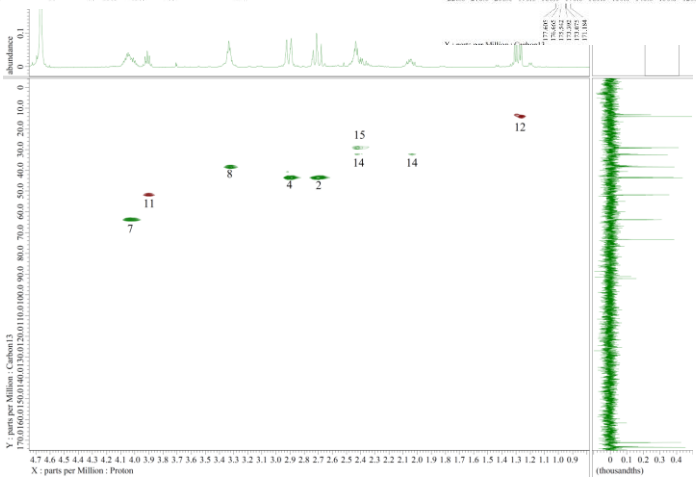
^{1}H NMR (D_2O): [1.39 (d, J=6.9 Hz), 1.43 (d, J=7.5 Hz), 3H], 2.17 (m, 1H), 2.49-2.56 (m, 3H), [2.82 (d, J=16 Hz), 2.85 (d, J=12.0 Hz), 2H], 3.03 (d, J=16.0 Hz), 3.46 (m, 2H), 4.03 (m, 1H), 4.17 (m, 2H)
^{13}C NMR (D_2O): 13.2,14.0 (C12), 29.1, 29.3 (C15), 32.0, 32.4 (C14), 38.3, 38.4 (C8), 43.3, 43.6 (C2-C4), 51.7, 51.8 (C11), 63.7, 63.8 (C7), 73.3 (C3), 91.1, 92.2 (C16), 171.2, 173.0, 173.4, 175.5, 176.6, 177.6 (C1, C5, C6, C10, C13, C17)
HRMS: Calculated for $C_{16}H_{22}N_2O_{12}$ $[M\text{-}H_2O]^+$: 417.1145**, [**M+H]$^+$: 435.1251, $[2M+H]^+$: 869.2424 found for $[M\text{-}H_2O]^+$: 417.1126 **[**M+H]$^+$: 435.1229 $[2M+H]^+$: 869.2392

^{1}H NMR spectra of vibrioferrin **8**



^{13}C NMR spectra of vibrioferrin **8**





HRMS spectra of vibrioferrin **8**

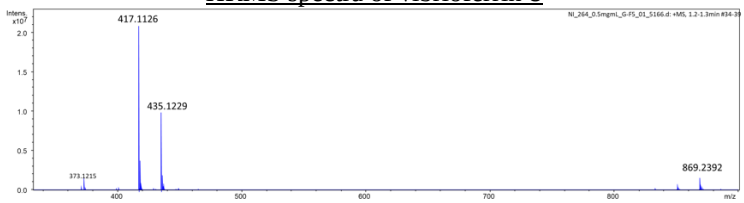


Fig S7. Characterization of racemic vibrioferrin 8.
NMR spectra were recorded on a JEOL ECZ-500R spectrometer, and the HRMS spectra on a Brucker Q-Tof maXis. The NMR or mass spectroscopy data are in good agreement with data from Takeushi et al (11). The chemical shifts for the ^{1}H and ^{13}C NMR spectra are identified using the numbering provided with the vibrioferrin formula. The presented data are sufficient to ascertain that the synthesis of vibrioferrin was successfully completed.

Supplementary Materials and Methods

Oysters

Oysters were maintained under controlled biosecured conditions in Bouin nursery (Vendée, France) with filtered and UV-treated seawater enriched in phytoplankton (*Skeletonema costatum, Isochrysis galbana,* and *Tetraselmis suecica*). The 'specific pathogen-free' (SPF) status of the animals was confirmed by (i) the absence of OsHV-1 DNA detection by qPCR and (ii) a low *Vibrio* presence (~10 CFU. mg of oyster tissue^{-1}) determined by isolation on selective culture medium (thiosulfate-citrate-bile salts-sucrose agar, TCBS). Oysters were observed to remain free of any abnormal mortality until the use.

Bacterial growth conditions

Bacteria were grown for 18 h under shaking at 20°C in Zobell liquid medium (4 g.L^{-1} bactopeptone, 1 g.L^{-1} yeast extract in sterile seawater, pH 7.4) or LB broth adjusted to 0.5M NaCl unless otherwise stated. When necessary, antibiotics were added (Trimethoprim Trim 10 µg. mL^{-1} or Chloramphenicol Cm 10 µg. mL^{-1}). Doubling time of the different Vibrio strains were determined from the OD_{600nm} measured in exponential growth phase of a bacterial culture realised in a 200 µl microplate for 15 h using a TECAN microplate reader.

In vitro cytotoxicity assays

Hemocytes were plated in 96 well-plates (2 x 10^5 cells/well) as previously published (12). After 1 h, plasma was removed and 5 µg/µL Sytox Green (Molecular Probes) diluted in 200 µL sterile seawater was added to each well. Washed *Vibrios* that had been opsonized in plasma for 1 h were then added to the wells at an MOI of 50:1. Sytox Green fluorescence was monitored (λex 480 nm/λem 550 nm) for 15 h using a TECAN microplate reader. Maximum cytolysis was determined by adding 0.1% Triton X-100 to hemocytes.

Experimental infection in mesocosm

For experimental infections, juvenile oysters (10 months) were infected with OsHV-1 virus, *Vibrio,* or both. Microorganisms were prepared as follows.

Viral inoculation - For **Design 1** (Fig. 2), seawater containing OsHV-1 virions was produced to infect pathogen-free juvenile oysters. Briefly, 90 donor oysters were anesthetized, and their adductor muscles were injected with 100 µl of 0.2 µm filtrated viral suspension (10^8 genomic units mL^{-1}). These donor oysters were then placed in a 40 L tank for 24 h. Virion release into the seawater was quantified by qPCR. This OsHV-1-contaminated seawater was used to fill tanks for the different experimental conditions. At day 0, 10 recipient oysters were placed in tanks containing 2 L of OsHV-1-contaminated seawater and were sampled at 4, 24, and 48 h; 15 additional recipient oysters were placed in tanks with 3 L of contaminated seawater to track mortalities daily. An identical design was used for control tanks, in which clean seawater was used instead of OsHV-1-contaminated water. For **Design 2** (Fig. 3), 250 donor oysters were injected

with a filtrated viral suspension as described above. After 24 h, 250 recipient oysters were placed in contact with donor oysters in a 40 L-tank. After another 18 h (day 0), recipient oysters were transferred into aerated clean seawater for mortality recording (10 animals in 0.5 L) or for sampling (30 animals in 1.5 L).

Bacterial inoculation – Seawater tanks were inoculated with bacteria on day 0 (final concentration of 10^7 CFU. mL^{-1}). Briefly, bacterial cultures (Zobell broth, 20°C, 18h) were centrifuged at 1500 x g for 10 min. Bacterial pellets were rinsed and resuspended in sterile seawater and the concentration was adjusted to OD_{600} = 1 (10^9 CFU. mL^{-1}). The bacterial concentration was confirmed by conventional dilution plating and CFU counting on Zobell agar.

At each sampling time point, oysters were sampled together with 100 mL of seawater. The oyster flesh was removed from the shell, snap-frozen in liquid nitrogen and stored at -80°C. 30 mL of seawater were filtered (0.2 µm pore size) and filters were stored at -80 °C. For tissue grinding, individual frozen oysters were shaken for 30 s inside a stainless steel cylinder containing a stainless-steel ball cooled in liquid nitrogen in a Retsch MM400 mixer mill. The pulverized tissue was transferred to a 2 mL screw-capped tube and stored at -80°C until further processing.

Nucleic acid extraction

Total DNA was extracted from either 20 mg of frozen oyster tissue-powder, 25 mg frozen oyster tissue, a pellet from 1 mL of stationary phase bacterial cultures, or a 0.2 µm filter using the Nucleospin tissue DNA extraction kit (Macherey Nagel, ref: 740952.250) with a modified protocol. Briefly, samples were added to a 2 mL screw-capped tube containing Zirconium beads, lysis buffer, and proteinase K and shaken for 12 min at a frequency of 35 cycles/s in a Retsch MM400 mixer mill at room temperature and then incubated for 1h 30 min at 56 °C. The samples were then treated with RNase for 5 min at 20 °C and then 10 min at 70 °C. The following purification steps were carried out according to manufacturer's recommendations. Nucleic acid concentration and purity was assayed using a Nanodrop ND-10000 spectrophotometer (Thermo Scientific).

Mutagenesis and mutant validation

E. coli strains used for mutagenesis (Table S4) were routinely grown at 37°C in Luria-Bertani (LB) medium and *V. rotiferianus* Th15_O_G05 was cultivated at 20°C in LB supplemented with 20 g/l (final concentration) NaCl (LBS). If necessary antibiotics and components were added to the media: kanamycin (Km, 100 µg/ml), chloramphenicol (Cm, 5 µg/ml), D-glucose (D-Glc, 0.3 g/l), L-arabinose (L-ara, 0.2%), diaminopimelic acid (DAP, 0.3 mM) and agar (15 g/l).

The DNA fragment containing *pvuA1* and *pvuA2* was deleted in *V. rotiferianus* Th15_O_G05 by double homologous recombination between a suicide plasmid (Table S5) and the chromosome. Briefly, two fragments of around 800 bp located upstream (up) of the *pvuA1* gene (THOG05_v1_100041) and downstream (down) of the *pvuA2* gene (THOG05_v1_100042) were synthetized and assembled by GeneArt (Invitrogen) and supplied into a pMKRQ plasmid. The synthetic up-down fragment was released from pMKRQ using *Xho*I

and *Eco*RI and ligated into the pLP12 plasmid (Luo *et al.*, 2015) (digested with same enzymes) using T4 DNA ligase (Promega) according to manufacturer recommendations. The ligation mixture was transformed by heat shock into competent cells of *E. coli* DH5α λpir. After PCR-confirmation, a clone containing the pLP12 in which the up-down fragment was inserted (named pAM010) was stored in 20% glycerol at -80°C. The purified pAM010 was transferred by heat shock into competent cells of *E. coli* β3914 (6) carrying a RP4 origin of transfer. Finally, pAM010 was transferred to *V. rotiferianus* Th15_O_G05 by a triparental mating procedure, adapting a recently published method (7). Briefly, after overnight growth, 200 µl of the *E.coli* β3914 donor strain carrying the pAM010 were centrifuged, 200 µl of the *E. coli* GEB883 helper strain carrying the pEVS104 plasmid (13) were added to the pellet, centrifuged, and 800 µl of the *V. rotiferianus* Th15_O_G05 were added to the pellet and centrifuged. The cell pellet was resuspended with 20 µl of LBS+DAP and spotted on a 0.2 µm acetate filter (Sartorius Stedim biotech) placed on the LBS+DAP plate. This plate was then incubated at 20°C for 24 h. The filter was rinsed with 1 ml filter-sterilized seawater (FSSW) and the suspension cell was centrifuged. The pellet was resuspended with 50 µl of LBS+DAP, plated on LBS+D-Glc+Cm plate and then incubated at 20°C for 24 h to 48 h. The obtained colonies were restreaked on LBS+D-Glc+Cm and the integration of pAM010 in the chromosome of *V. rotiferianus* Th15_O_G05 was determined by PCR, using Insert-pLP12-R and del-pvuA1-A2-OG05-F primers. Several colonies with an integration of the suicide plasmid in the "upstream" or in the "downstream" region of the to-be-deleted DNA fragment were grown overnight in LBS at 20°C. Cultures were serially diluted and 100 µl were plated on LBS+L-ara to the excision of the pAM010. After a 24 h-incubation at 20°C, colonies were restreaked on LBS+Cm and LBS+L-ara to control the Cm-sensitivity. The Cm-sensitive clones were tested for deletion of *pvuA1* and *pvuA2* genes by using primers del-pvuA1-A2-OG05-F and del-pvuA1-A2-OG05-R (Table S5). A *V. rotiferianus* Th15_O_G05 mutant strain deleted for the *pvuA1-2* genes was stored in glycerol at -80°C (strain *V. rotiferianus* Th15_O_G05 Δ*pvuA1-2*).

Standard curves for absolute qPCR quantification

Standard curves of known concentration of *Vibrio* genomes were generated using the relation between the concentration of DNA and the theoretical copy number of genomes, calculated on the basis of the DNA mass divided by molecular weight of the genome. They were also validated by the limit dilution method, assuming that the dilution at which 1 replicate in 10 was positive corresponds to 1 copy.

References:

1. Goh SH*, et al.* (1996) HSP60 gene sequences as universal targets for microbial species identification: Studies with coagulase-negative *staphylococci*. *Journal of Clinical Microbiology* 34(4):818-823.
2. Sawabe T*, et al.* (2013) Updating the *Vibrio* clades defined by multilocus sequence phylogeny: Proposal of eight new clades, and the description of *Vibrio tritonius* sp. nov. *Frontiers in Microbiology* 4(DEC).
3. Webb SC, Fidler A, & Renault T (2007) Primers for PCR-based detection of ostreid herpes virus-1 (OsHV-1): Application in a survey of New Zealand molluscs. *Aquaculture* 272(1-4):126-139.
4. Thompson JR*, et al.* (2004) Diversity and dynamics of a north atlantic coastal *Vibrio c*ommunity. *Appl Environ Microbiol* 70(7):4103-4110.
5. Mueller KE, Wolf K, & Fields KA (2016) Gene deletion by fluorescence-reported allelic exchange mutagenesis in *Chlamydia trachomatis*. *mBio* 7(1):e01817-01815.
6. Le Roux F, Binesse J, Saulnier D, & Mazel D (2007) Construction of a *Vibrio splendidus* mutant lacking the metalloprotease gene vsm by use of a novel counterselectable suicide vector. *Appl Environ Microbiol* 73(3):777-784.
7. Morot A*, et al.* (2021) Virulence of *Vibrio harveyi* ORM4 towards the European abalone *Haliotis tuberculata* involves both quorum sensing and a type III secretion system. *Environmental Microbiology* 23(9):5273-5288.
8. Luo P, He X, Liu Q, & Hu C (2015) Developing universal genetic tools for rapid and efficient deletion mutation in vibrio species based on suicide t-vectors carrying a novel counterselectable marker, vmi480. *PLoS ONE* 10(12):1-17.
9. Le Roux F, Davis BM, & Waldor MK (2011) Conserved small RNAs govern replication and incompatibility of a diverse new plasmid family from marine bacteria. *Nucleic Acids Res* 39(3):1004-1013.
10. Waddell B, Southward CM, McKenna N, & Devinney R (2014) Identification of VPA0451 as the specific chaperone for the Vibrio parahaemolyticus chromosome 1 type III-secreted effector VPA0450. *FEMS Microbiol Lett* 353(2):141-150.
11. Takeuchi Y*, et al.* (1999) Synthesis and siderophore activity of vibrioferrin and one of its diastereomeric isomers. *Chemical and Pharmaceutical Bulletin* 47(9):1284-1287.
12. Vanhove AS*, et al.* (2015) Outer membrane vesicles are vehicles for the delivery of *Vibrio tasmaniensi*s virulence factors to oyster immune cells. *Environ Microbiol*.
13. Dunn AK, Millikan DS, Adin DM, Bose JL, & Stabb EV (2006) New rfp- and pES213-derived tools for analyzing symbiotic *Vibrio fischeri* reveal patterns of infection and lux expression *in situ*. *Applied and Environmental Microbiology* 72(1):802-810.
14. Val ME, Skovgaard O, Ducos-Galand M, Bland MJ, Mazel D. (2012). Genome engineering in *Vibrio cholerae*: a feasible approach to address biological issues. *PLoS Genet* **8**: e1002472.