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1 **A core of functionally complementary bacteria colonizes oysters in Pacific Oyster Mortality**
2 **Syndrome.**

3

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24

25 **ABSTRACT**

26 The Pacific oyster *Crassostrea gigas* is one of the main cultivated invertebrate species around the
27 world. Since 2008, oyster juveniles have been confronted with a lethal syndrome, Pacific Oyster
28 Mortality Syndrome (POMS). The etiology of POMS is complex. Recently, we demonstrated that
29 POMS is a polymicrobial disease. It is initiated by a primary infection with the *herpesvirus* OsHV-1 μ
30 Var, and evolves towards a secondary fatal bacteremia that is enabled by the oyster's
31 immunocompromised state. In the present article, we describe the implementation of an unprecedented
32 combination of metabarcoding and metatranscriptomic approaches to show that the sequence of events
33 in POMS pathogenesis is conserved across infectious environments and susceptible oyster genetic
34 backgrounds. We also identify a core colonizing bacterial consortium which, together with OsHV-1 μ
35 Var, forms the POMS pathobiota. This bacterial core is characterized by highly active global
36 metabolism and key adaptive responses to the within-host environment (e.g. stress responses and
37 redox homeostasis). Several marine gamma proteobacteria in the core express different and
38 complementary functions to exploit the host's resources. Such cross-benefits are observed in
39 colonization-related functions, and reveal specific strategies used by these bacteria to adapt and
40 colonize oysters (e.g. adhesion, cell defense, cell motility, metal homeostasis, natural competence,
41 quorum sensing, transport, and virulence). Interdependence and cooperation within the microbial
42 community for metabolic requirements is best exemplified by sulfur metabolism, which is a property
43 of the pathobiota as a whole and not of a single genus. We argue that this interdependence may dictate
44 the conservation of the POMS pathobiota across distinct environments and oyster genetic
45 backgrounds.

46

47 **INTRODUCTION**

48 Introduced from Asia to a broad range of countries, *Crassostrea gigas* has become one of the world's
49 main cultivated species. Since 2008, juvenile stages of *C. gigas* have suffered massive mortality
50 events, especially in France [1]. In subsequent years, this so-called Pacific Oyster Mortality Syndrome
51 (POMS) [2] has become panzootic. POMS has been observed in all coastal regions of France [3-5] and
52 numerous other countries worldwide [6-11].

53 This situation has promoted a research effort that has revealed a series of factors contributing to the
54 disease, including infectious agents that interact with seawater temperature and oyster genetics [12-
55 17]. Dramatic POMS mortality events have coincided with the recurrent detection of *Ostreid*
56 *herpesvirus* variants in moribund oysters [3-5]. The involvement of other etiological agents has also
57 been suspected. In particular, bacterial strains of the genus *Vibrio* have been shown to be associated
58 with the disease [18, 19]. Recently, integrative molecular approaches performed on susceptible and
59 resistant families of oysters have revealed the complex etiology of POMS [20, 21]. These studies
60 demonstrated that infection by *Ostreid herpesvirus* type 1 μ Var (OsHV-1) is a critical step in POMS
61 pathogenesis. OsHV-1 leads to an immunocompromised state by altering hemocyte physiology. This
62 results in dysbiosis and bacteremia, which ultimately results in oyster death [20]. Several bacterial
63 genera are involved in this secondary infection [20]; one of them (*Vibrio*) was found to include
64 opportunistic pathogens that cause hemocyte lysis [21]. This observation occurred in a specific
65 infectious environment in French Brittany [20]. Whether the observed mechanisms of POMS
66 pathogenesis are conserved in other infectious environments remains unknown. Also of importance,
67 *Vibrio* species are not the only bacteria that colonize oyster tissues during the secondary bacterial
68 infection. Several bacterial genera, including *Arcobacter*, *Marinobacterium*, *Marinomonas*, and
69 *Psychrobium* are also found to massively colonize oysters [20]. The mechanisms underlying the
70 colonizing capacity of these species remain to be elucidated.

71 In the present study, we investigated whether POMS pathogenesis is conserved across environments,
72 and which biological functions are expressed by the bacterial consortia that causes oyster death. We
73 compared pathogenesis in oyster biparental families that display contrasting phenotypes (resistant or
74 susceptible to POMS) in two infectious environments (the Atlantic Bay of Brest environment, and the
75 Mediterranean Thau Lagoon environment). We found that the sequence of events is conserved in both
76 infectious environments: intense viral replication in susceptible oysters, followed by a secondary
77 bacteremia caused by a conserved bacterial consortium that results in oyster death. Using
78 metabarcoding and metatranscriptomics, we identified members of the core colonizing bacterial
79 consortium, and characterized their highly active metabolism and key adaptive responses to the
80 within-host environment. We also identified a series of genus-specific functions required for

81 colonization, which reveal specific strategies that the core consortium uses to adapt and colonize
82 oysters. Finally, we identified cooperative processes used by the core consortium to meet their
83 metabolic requirements; these processes may dictate the conservation of the core consortium across
84 environments.

85

86 **METHODS**

87 **Production of biparental oyster families**

88 *C. gigas* families were produced as described in [20, 22]. Briefly, oysters were produced at the Ifremer
89 hatchery in Argenton in March 2015. Three susceptible families (F₁₁, F₁₄, and F₁₅) and three resistant
90 families (F₂₁, F₂₃, and F₄₈) were used as recipients, and 15 families were used as donors. All families
91 were maintained under controlled bio-secured conditions to ensure their specific pathogen-free status.
92 Status was verified by i) the absence of OsHV-1 DNA using qPCR, (see below) and ii) a low *Vibrio*
93 load (~10 cfu/g tissue) on selective culture medium (thiosulfate-citrate-bile salts-sucrose agar) [23].
94 Oysters remained free of any abnormal mortality throughout larval development, at which time
95 experimental infections were started.

96 **Mesocosm experimental infections**

97 The experimental infection protocol consisted of a cohabitation assay between donors (exposed to
98 pathogens naturally present in the environment) and recipient specific pathogen-free oysters [17, 18].
99 Details of the experimental infection protocol (e.g. biomass, oyster weight, experimental duration, and
100 tank volume) were as described in [20, 22]. Briefly, donor oysters were deployed at Logonna Daoulas
101 (lat 48.335263, long 4.317922) in French Brittany (Atlantic environment) and at Thau Lagoon (lat
102 43.418736, long 3.622620) (Mediterranean environment). Oysters were deployed in farming areas
103 during the infectious period, and remained in place until the onset of mortality (< 1%). Donors were
104 then brought back to the laboratory (in Argenton) and placed in tanks, each containing recipient
105 oysters from the three resistant and the three susceptible families. Experimental infections took place
106 in July 2015 and September 2015 for the Atlantic and Mediterranean exposures, respectively. For each

107 experimental infection, mortality rate was monitored, and 10 oysters were sampled in triplicate from
108 each oyster family shucking at 7 time points (0, 6, 12, 24, 48, 60, and 72 hours post-infection). The
109 shell was removed and the whole oyster was flash frozen in liquid nitrogen. Oyster pools were ground
110 in liquid nitrogen in 50 ml stainless steel bowls with 20mm diameter grinding balls (Retsch MM400
111 mill). The powders obtained were stored at -80°C prior to RNA and DNA extraction.

112 **DNA extraction and quantification of OsHV-1 and total bacteria.**

113 DNA extraction was performed as described in [20] using the Nucleospin tissue kit (Macherey-Nagel).
114 DNA concentration and purity were checked with a NanoDrop One (Thermo Scientific).
115 Quantification of OsHV-1 and total bacteria were performed using quantitative PCR (qPCR) with a
116 Roche LightCycler 480 Real-Time thermocycler (qPHD-Montpellier GenomiX platform, Montpellier
117 University, France). Absolute quantity of OsHV-1 was calculated by comparing the observed C_q
118 values to a standard curve of the OsHV-1 DNA polymerase catalytic subunit (AY509253)
119 amplification product cloned into the pCR4-TOPO vector (Invitrogen). Relative quantification of total
120 bacteria 16S rDNA gene was calculated by the $2^{-\Delta\Delta C_q}$ [24] method with the mean of the measured
121 threshold cycle values of two reference genes (*Cg-BPI*, GenBank: AY165040 and *Cg-actin*, GenBank:
122 AF026063) (de Lorgeril, Lucasson et al. 2018).

123

124 **Analyses of bacterial microbiota**

125 Bacterial metabarcoding was performed using 16S rRNA gene amplicon sequencing. Libraries were
126 generated using the Illumina two-step PCR protocol targeting the V3-V4 region [25]. A total of 252
127 libraries (six families × seven sampling time points × three replicates × two infectious environments)
128 were paired-end sequenced with a 2 × 250 bp read length at the Genome Quebec platform on a MiSeq
129 system (Illumina) according to the manufacturer's protocol. A total of 41,012,155 pairs of sequences
130 were obtained. Metabarcoding data was processed using the FROGS pipeline [26]. Briefly, paired
131 reads were merged using FLASH [27]. After cleaning steps and singleton filtering, 26,442,455
132 sequences were retained for further analyses. After denoising and primer/adaptor removal with
133 cutadapt, clustering was performed using SWARM, which uses a two-step clustering algorithm with a

134 threshold corresponding to the maximum number of differences between two Operational Taxonomic
135 Units (OTU) (denoising step $d = 1$; aggregation distance = 3) [28]. Chimeras were removed using
136 VSEARCH [29]. Resulting OTUs were annotated using Blast+ against the Silva database (release
137 128).

138 **Bacterial metatranscriptomic analysis**

139 Powder obtained from the frozen oysters was resuspended in Trizol, and total RNA was extracted
140 using a Direct-zolTM RNA Miniprep kit. Polyadenylated mRNAs (*i.e.* oyster mRNAs) were removed
141 using a MICROBEnrichTM Kit (Ambion). cDNA oriented sequencing libraries were prepared as
142 described in [21] using the Ovation Universal RNA-Seq system (Nugen). Library preparation included
143 steps to remove oyster nuclear, mitochondrial, and ribosomal RNAs, as well as bacterial rRNAs [21].
144 A total of 36 libraries (three families \times two sampling timepoints \times three replicates \times two infectious
145 environments) were sequenced by the Fasteris company (Switzerland, <https://www.fasteris.com>) in
146 paired-end mode (2×150 bp) on an Illumina HiSeq 3000/4000 to obtain 200-300 million clusters per
147 sample (**Supplementary Table 1**).

148 Figure S1 presents a schematic of data processing. Raw Illumina sequencing reads from 72 fastq files
149 (R1 and R2) were trimmed using Trimmomatic v0.38, and rRNA reads (both eukaryotic and bacterial)
150 were removed using Sortme RNA v2.1b with the rRNA Silva database (release 128). At this stage,
151 about 9% of reads were removed, underscoring the efficiency of experimental rRNA removal during
152 library preparation (**Supplementary Figure 1**).

153 To further enrich bacterial sequences, reads were successively mapped by Bowtie2 [30] on a
154 multifasta file containing *Crassostrea gigas* genome sequence v9, complemented by *C. gigas* EST
155 (available from NCBI), and a multifasta file containing the sequences of OsHV-1 (present in diseased
156 oysters) and other viral sequences previously associated with bivalves [31]. Unmapped reads, which
157 represented 4-10% of the starting reads (depending on conditions) were retained for further analysis
158 (**Supplementary Table 1**). After a new filtering step using Trimmomatic, all remaining reads
159 corresponding to the 36 samples were pooled (516,786,580 reads, 36-150 nt) and assembled using
160 Trinity v2.3.2 in paired-end mode to build a reference metatranscriptome (1,091,409 contigs, 201 –

161 15,917 nt). The resulting metatranscriptome was further annotated using Diamond BlastX against the
162 NCBI nr protein database [32]. 48.4 % of the contigs aligned with a protein in the database, and were
163 further assigned to a taxon using Megan 6-LR Community Edition [33]. Sequences were annotated at
164 different taxonomic levels from species to phylum. Out of the 1,091,409 contigs, 352,473 contigs
165 aligned with bacterial proteins by BlastX with an E-value $\leq 01^e-06$.

166 Each protein was assigned to 31 functional categories (**Supplementary Table 2**). Out of the 352,473
167 best hits, 74,015 were annotated as “hypothetical”, “unknown”, or “unnamed”, and were assigned to
168 the category “Unknown function”. The remaining hits corresponded to 15,504 different protein names
169 that were manually assigned to one of the 30 remaining functional categories.

170

171 **Quantification of gene expression and data normalization**

172 For each of the 36 samples used for the assembly of the metatranscriptome, reads were mapped back
173 onto the metatranscriptome by Bowtie2 in paired-end mode. Raw counts per features (*i.e.* per contig)
174 were computed using HTseq-Count [34]. For each contig, and for each sample, raw counts were
175 normalized to TPM (Transcripts per Kilobase / Million = Mapped reads / Length of contig (kb) /
176 Scaling factor with Scaling factor = total reads in a sample / 1,000,000), which corrects for contig
177 length and differences in read number in the different samples. **Supplementary Table 3** presents a
178 complete list of contigs, their length, the encoded feature annotation, and the TPM value of the feature
179 in each sample. In many cases, one protein could be encoded by several contigs, either because gene
180 assembly into a contig was incomplete, or because of the existence of contig isoforms. Altogether, the
181 352,473 contigs encoded 225,965 unique proteins.

182 When analyzing the functional enrichment in the seven genera that predominated in the microbiota of
183 diseased oysters (see **Results** section), contigs that encoded the same protein were merged into a
184 single feature/protein, and their expression levels were summed. The expression level of a category
185 was calculated by summing up the expression level of all the proteins assigned to the category. In
186 addition, expression values were normalized from TPM to a value corrected for the relative proportion
187 of the genus activity in the sample such that the ratio of the average normalized expression level of the
188 three replicates for a given functional category in two conditions (*i.e.* T60 or 72 vs T0) reflected a

189 specific enrichment (Enrichment Factor or EF) of the function, rather than a general increase of the
190 genus in the population. First, a pseudo count of one read was added to each gene in each
191 condition/replicate, and the resulting counts were normalized by dividing by the total number of
192 counts of the genus in a given condition/replicate, then multiplying by 10,000. For a given
193 function/protein or functional category, an enrichment factor (EF) between the two conditions (i.e.
194 T60 or T72 vs T0) was further defined as the ratio of the average normalized expression level of the
195 three replicates at T60 or 72 over the average expression level at T0.

196

197 **Statistical analyses**

198 Survival curves were used to determine differential mortality kinetics between oyster families with the
199 non-parametric Kaplan-Meier test (Mantel–Cox log-rank test, $p < 0.05$, GraphPad_Prism 6.01). For
200 OsHV-1 and total bacteria quantifications, significant differences between resistant and susceptible
201 oyster families were determined using the non-parametric Mann Whitney test ($p < 0.05$,
202 GraphPad_Prism 6.01). For bacterial metabarcoding, statistical analyses were performed using R
203 v3.3.1 (<http://www.R-project.org>, [35]). Principal coordinate analysis (PCoA, “phyloseq”) on a Bray-
204 Curtis distance matrix (ordinate, “phyloseq”) was performed to determine dissimilarities between
205 samples. Multivariate homogeneity of group dispersions was tested between bacterial assemblages of
206 the six oyster families using 999 permutations (permutest, betadisper, “vegan”). DESeq2 (“DESeq”,
207 [36]) from the OTUs to the higher taxonomic ranks was used to identify candidate taxa whose
208 abundance changed between the initial and final time points of the experiment. The abundance of a
209 bacterial taxon was considered significantly modified when the adjusted p-value for multiple testing
210 with the Benjamini-Hochberg procedure, which controls the false discovery rate (FDR), was < 0.05 .
211 Heatmaps of significant genera were computed using relative abundances and the heatmap.2 function
212 “ggplots” [37]. For bacterial metatranscriptomics, significant differences in expression between two
213 conditions (i.e. T60 or T72 vs T0) were assessed by a paired Student’s t-test ($p < 0.05$).

214

215 **Data availability**

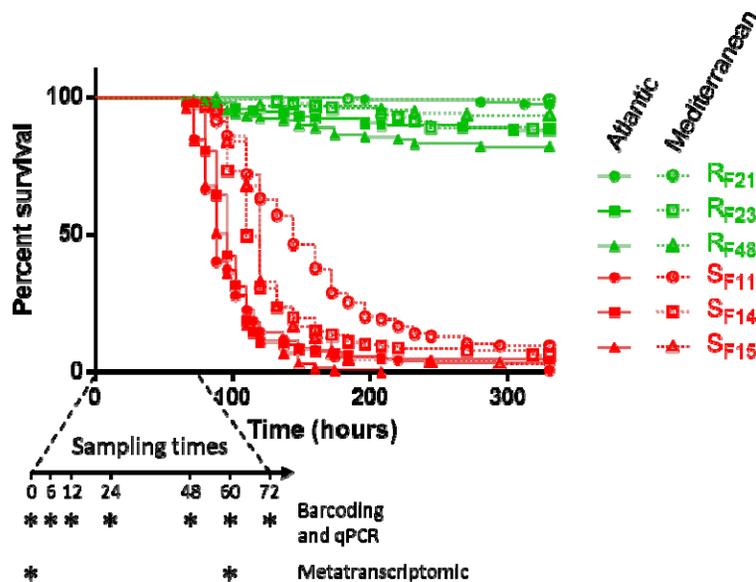
216 Metabarcoding and RNAseq sequence data will be made available through the SRA database
217 (BioProject accession number PRJNA423079 with SRA accession SUB7738644 for bacterial
218 metabarcoding and SUB8110348 for Bacterial metatranscriptomic). Other data generated from this
219 study are included in the published version of this article and its supplementary files.

220

221 RESULTS

222 Primary OsHV-1 infection and secondary bacteremia are conserved in POMS, independent of 223 the infectious environment

224 Six *C. gigas* families were subjected to two experimental infections that mimicked disease
225 transmission in the wild. We previously reported high variability in the dynamics of mortality and
226 percentage survival of oyster families confronted with an Atlantic infectious environment.
227 Specifically, the F11, F14, and F15 families were highly susceptible (survival rate < 4% after 330h) to
228 POMS, whereas the F21, F23, and F48 families were highly resistant (survival rate > 82% after 330h)
229 [20]. Similar results were obtained in the present study when the same oyster families were confronted
230 with a Mediterranean infectious environment: families F11, F14, and F15 were susceptible (survival
231 rates < 9%), whereas families F21, F23, and F48 were resistant (survival rates > 88%) (**Figure 1**).
232 Thus, these oyster families displayed similar phenotypes when confronted with two different
233 infectious environments (Mantel-Cox log-rank test, $p < 0.0001$ for each comparison of resistant vs.
234 susceptible oyster families). Susceptible and resistant oyster families are hereafter referred to as S
235 (S_{F11} , S_{F14} , and S_{F15}) and R (R_{F21} , R_{F23} , and R_{F48}), respectively.



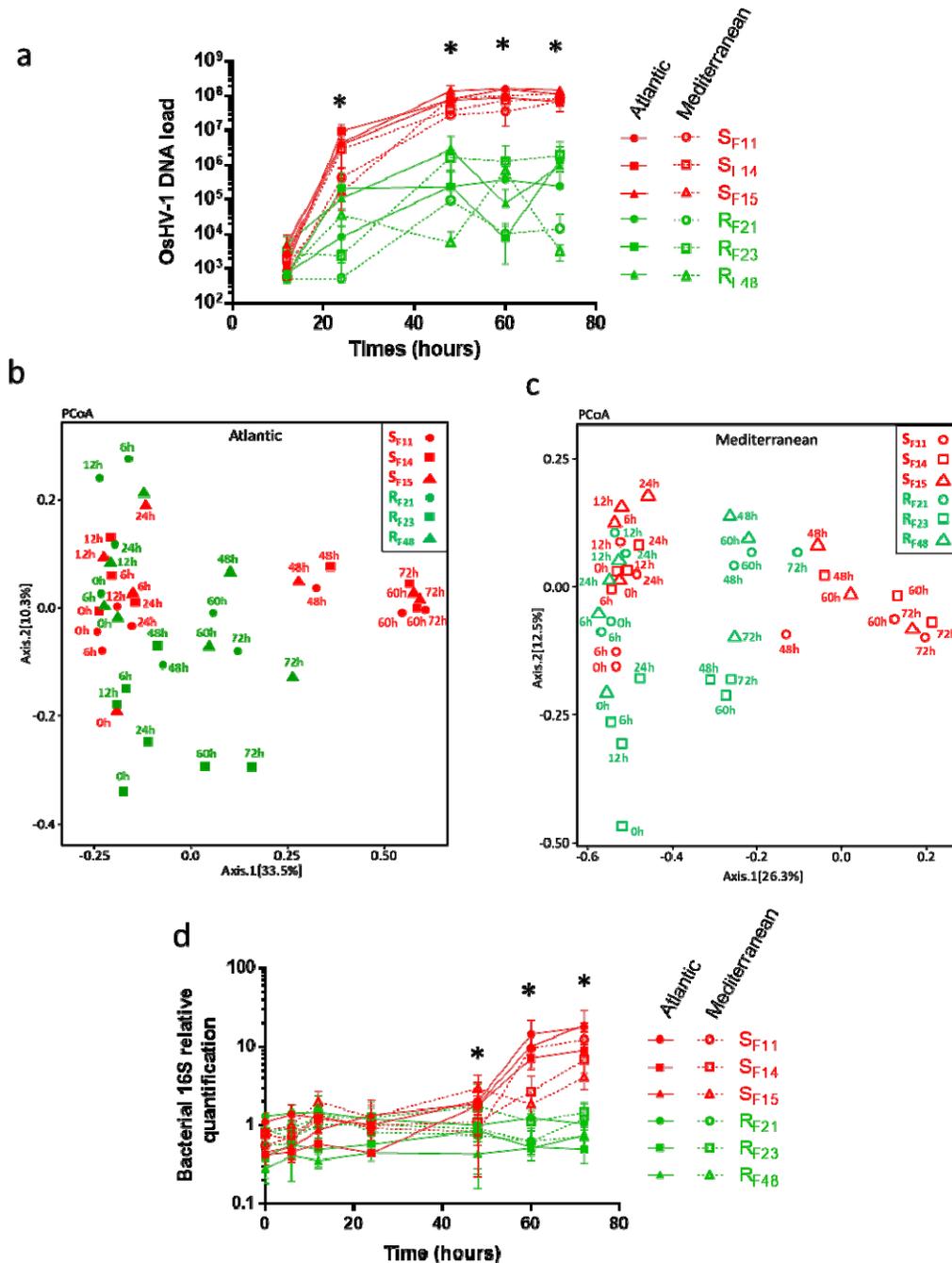
236

237 **Figure 1: Kaplan-Meier survival curves of oyster biparental families confronted with two**
238 **different infectious environments.** Resistant oyster families (R_{F21} , R_{F23} , and R_{F48}) are presented in

239 green, and susceptible oyster families (S_{F11} , S_{F14} , and S_{F15}) are presented in red. At each time point
240 (indicated by asterisks on the arrow), 10 oysters were sampled 3 triplicates from each family in
241 each tank for barcoding, qPCR, and metatranscriptomic analysis. Data for the Atlantic infectious
242 environment are extracted from [20] and shown for comparison.

243

244 We next compared pathogenesis between the two infectious environments by monitoring OsHV-1
245 load, microbiota dynamics, and bacterial abundance in the three resistant and three susceptible oyster
246 families (**Figure 2**). OsHV-1 DNA was detected in all families, regardless of whether they were
247 confronted with the Atlantic or Mediterranean infectious environment (**Figure 2a**). However, very
248 intense viral replication occurred only in the susceptible oyster families: viral DNA loads were 2 to 3
249 logs higher than in resistant oysters at 24 h (**Figure 2a**).



250

251 **Figure 2: Primary OsHV-1 infection, bacterial dysbiosis, and secondary bacteremia are**
 252 **conserved in different infectious environments. (a)** Early and intense replication of OsHV-1 μ Var
 253 occurs in susceptible oysters (red), but not resistant oysters (green), confronted with either the Atlantic
 254 or the Mediterranean infectious environment. OsHV-1 load was quantified by qPCR and expressed as
 255 Viral Genomic Units per ng of oyster DNA (log scale) during experimental infections. Asterisks
 256 indicate significant differences between susceptible and resistant oyster families (Mann Whitney test,
 257 $p < 0.05$). **(b-c)** Principal coordinate analysis (PCoA) plot of the microbiota for susceptible (red) and
 258 resistant (green) oyster families confronted with each infectious environment. Dispersion of oyster
 259 families according to the Bray-Curtis dissimilarity matrix (beta diversity) in **(b)** Atlantic and **(c)**

260 Mediterranean infectious environments. **(d)** Temporal dynamics of total bacteria in susceptible (red)
261 and resistant (green) oyster families confronted with two different infectious environments. Total
262 bacterial quantification based on qPCR amplification of the V3-V4 region of the 16S rRNA gene
263 during experimental infections. Asterisks indicate significant differences between susceptible and
264 resistant oyster families (Mann Whitney test, $p < 0.05$). Data from the Atlantic infectious environment
265 in panels (a) and (d) are extracted from [20] for comparison.

266

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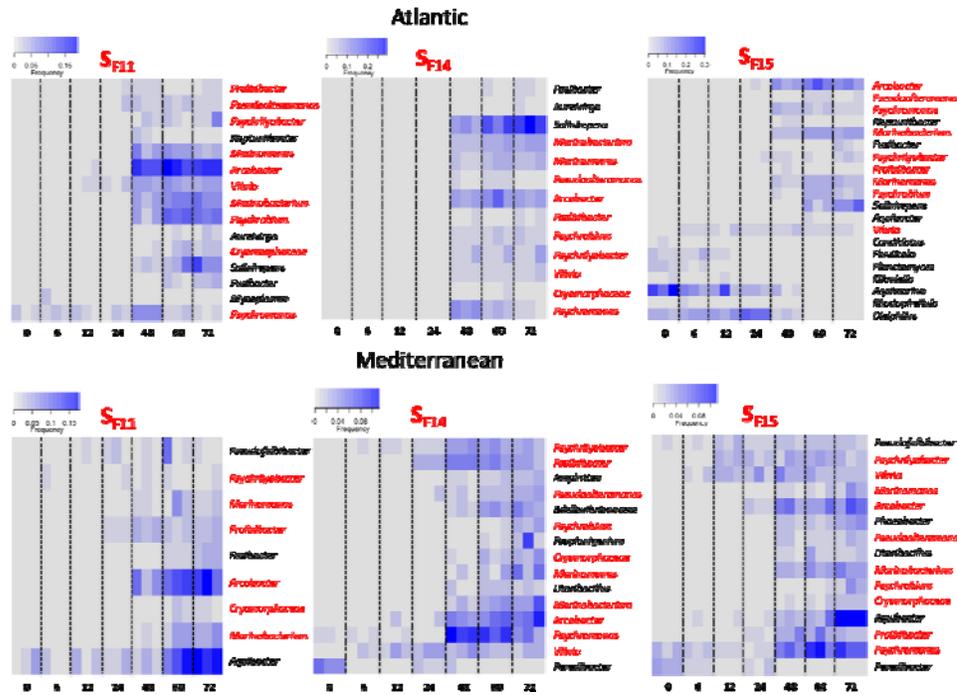
268

269 The dynamics of the oyster microbiota were studied in the six oyster families by monitoring bacterial
270 community composition using 16S rRNA gene metabarcoding over the first 3 days of both
271 experimental infections. A total of 45,686 bacterial OTUs were obtained from the 252 samples and
272 affiliated at different taxonomic ranks (**Supplementary Table 4**). Sufficient sequencing depth was
273 confirmed by species richness rarefaction curves (**Supplementary Figure 2**). Changes in microbiota
274 composition were greater in susceptible oysters than in resistant oysters at all taxonomic ranks
275 (**Supplementary Figure 3**). Indeed, for the Atlantic infectious environment, 52, 43, and 54 OTUs
276 significantly differed (in terms of relative proportion between the start and end of the experiment) in
277 susceptible oysters S_{F11} , S_{F14} and S_{F15} , respectively; only 1, 11, and 9 OTUs significantly differed in
278 resistant oysters R_{F21} , R_{F23} and R_{F48} , respectively (**Supplementary Table 5**). The same trend was
279 observed in the Mediterranean infectious environment. 11, 47, and 43 OTUs significantly differed in
280 S_{F11} , S_{F14} and S_{F15} , respectively, as opposed to 2, 8, and 6 OTUs in R_{F21} , R_{F23} and R_{F48} , respectively.

281 PCoA on a Bray-Curtis dissimilarity matrix (beta diversity) revealed higher microbiota dispersion in
282 susceptible oyster families than in resistant families in both infectious environments (multivariate
283 homogeneity of groups dispersion, d.f. = 1; $p = 0.016$ and $p = 0.020$ for Atlantic and Mediterranean
284 environments, respectively) (**Figure 2b and 2c**). This disruption of the bacterial community structure
285 occurred in susceptible oysters between 24 h and 48 h, concomitantly with the active replication of
286 OsHV-1. In addition, susceptible oyster families displayed a significantly greater bacterial load than
287 resistant oysters when confronted with either the Atlantic or the Mediterranean infectious environment
288 (Mann Whitney test, $p < 0.05$; **Figure 2d**). This increase started at 60 h and continued to until the end
289 of the experiment (72 h). Total bacterial abundance in susceptible oysters was more than 5-fold higher
290 at 72 h than at T0, which indicated bacterial proliferation. In contrast, total bacterial load remained
291 stable in resistant oysters.

292 **A core of bacterial genera colonizes oysters during secondary bacterial infection in POMS**

293 All bacterial genera that changed significantly in abundance during the two experimental infections
294 (Atlantic and Mediterranean) in susceptible oyster families are reported in **Supplementary Table 5**
295 (DESeq2, adjusted $p < 0.05$). We focused on genera representing $> 2\%$ of the bacteria in at least one
296 sample for each susceptible oyster family confronted with each infectious environment (**Figure 3**). In
297 the Atlantic infectious environment, the corresponding OTUs represented a total of 4%, 0.8%, and
298 46% of total bacteria at the beginning of the experiment (T0), as opposed to 73%, 75%, and 72% at 72
299 h for S_{F11} , S_{F14} , and S_{F15} , respectively (**Supplementary Table 5**). In the Mediterranean infectious
300 environment, these OTUs increased from 2%, 6%, and 7% at T0 to 47%, 56%, and 56% at 72 h for
301 S_{F11} , S_{F14} , and S_{F15} , respectively. Nine to twenty genera increased significantly in abundance between
302 T0 and 72 h. Ten genera (*Arcobacter*, *Cryomorphaceae*, *Marinobacterium*, *Marinomonas*,
303 *Proxilibacter*, *Pseudoalteromonas*, *Psychrilyobacter*, *Psychrobium*, *Psychromonas*, and *Vibrio*) were
304 common to almost all (5 of 6) susceptible oyster families and both infectious environments (**Figure 3**).
305 Most of the remaining genera (*Aquibacter*, *Aureivirga*, *Fusibacter*, *Neptunibacter*, *Peredibacter*,
306 *Pseudofulbibacter*) were shared by at least two families in one infectious environment. One genera
307 (*Salinirepens*) increased significantly in all susceptible oysters in the Atlantic infectious environment
308 only. These results show that a core of bacterial genera colonizes oysters during the POMS secondary
309 bacterial infection, independent of the infectious environment. In resistant oyster families, several taxa
310 also vary significantly in abundance over time. Most of these taxa are the same taxa identified in
311 susceptible oyster families (**Supplementary Figure 4**), but they are present in lesser quantities. These
312 taxa represent between 4% to 23% of the reads sequenced at 72h in resistant oysters, whereas they
313 represent 47% to 75% of the reads sequenced in susceptible oysters (**Supplementary Table 5**).



314

315 **Figure 3: Heatmaps of bacterial genera that changed significantly in abundance over the**
 316 **course of infection in susceptible oysters (S_{F11}, S_{F14}, and S_{F15}) in the Atlantic and**
 317 **Mediterranean infectious environments.** Analyses were performed at the genus level. Only
 318 genera that changed significantly in abundance (DESeq2, adjusted $p < 0.05$) and had a relative
 319 proportion greater than 2% in at least one sample are shown. Increased intensity of color (blue)
 320 represents increased relative abundance. Genera that are consistently modified in 5 out of the 6
 321 conditions (3 families and 2 infectious environments) are in red.

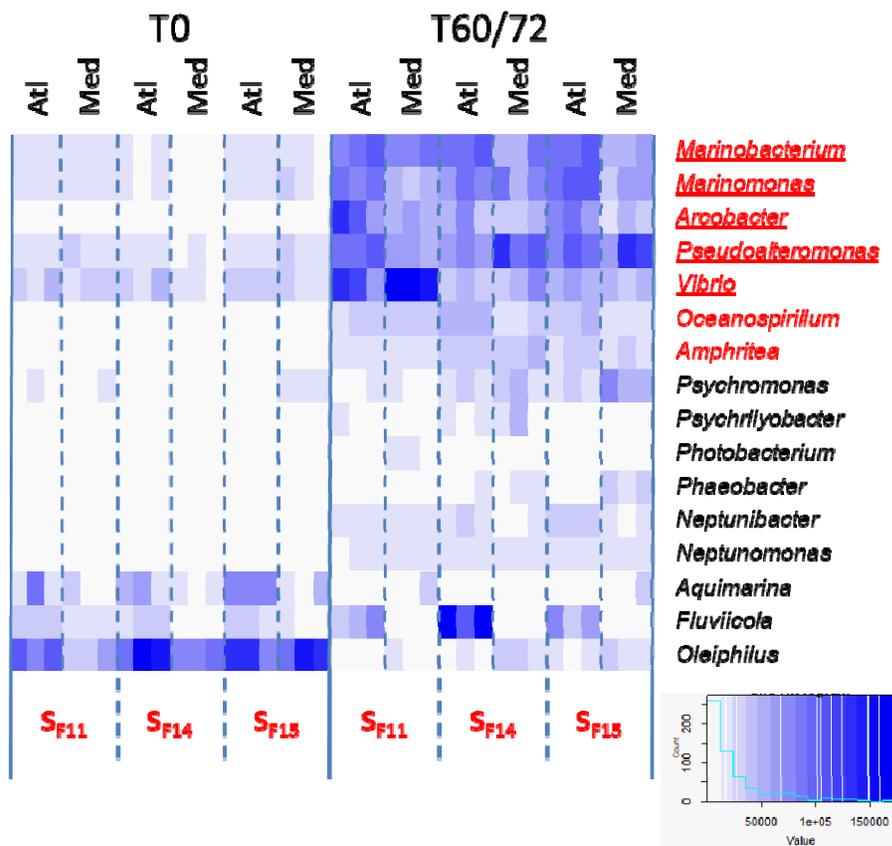
322

323 Seven genera are responsible for most bacterial gene expression in diseased oysters

324 To understand the colonization success of certain genera, we analyzed the gene expression of the
 325 colonizing microbiota. As described in the **Methods** section, we used metatranscriptomics to
 326 determine the functional mechanisms underlying colonization for different bacterial genera in the three
 327 different oyster genetic backgrounds and two different infectious environments. RNAseq was
 328 performed after removal of rRNA, oyster mRNA, and mitochondrial RNA using custom probes. We
 329 sequenced the remaining RNA of the susceptible families S_{F11}, S_{F14}, and S_{F15}, in both the Atlantic and
 330 Mediterranean infectious environments, at T0 and just before oyster mortality occurred (*i.e.* at 60 h
 331 and 72 h for the Atlantic and the Mediterranean infectious environments, respectively). Three
 332 biological replicates were analyzed for each condition, corresponding to a total of 36 biological
 333 samples. After removal of the remaining non-bacterial and ribosomal reads, we assembled and

334 annotated a reference metatranscriptome. We aligned the translated contigs with the NCBI nr protein
335 database, and used MEGAN for taxonomic assignments. We obtained 352,473 contigs that aligned
336 with bacterial proteins with an e-value $\leq 10^{-6}$. In many cases, a single protein could be encoded by
337 several contigs, either because the gene was not fully assembled or because of contig isoforms. In
338 total, our assembled metatranscriptome encoded 225,965 unique proteins. These proteins were
339 functionally and taxonomically annotated, and their expression level was determined for each
340 condition. Further analyses were restricted to proteins assigned at the genus or species levels.

341 To analyze the contribution of each genus to the overall bacterial gene expression, we added the level
342 of expression of all proteins assigned to a given genus (measured in Transcripts Per Million, or TPM).
343 We focused on genera that contributed to more than 2% of total gene expression in at least one oyster
344 sample (Figure 4).



345

346 **Figure 4: Heatmap of transcriptional activity of bacterial genera in susceptible oyster families**
347 **(SF11, SF14, and SF15) in the two infectious environments before infection and in diseased oysters.**
348 For each condition, results of the three replicates are shown. Increased color intensity (blue) indicates

349 increased relative activity of the genus. Genera shown contributed at least 2% of the total
350 transcriptional activity in at least one sample of diseased oysters. Bacterial genera that were
351 overrepresented according to metatranscriptomics alone for all conditions in the diseased oysters are in
352 red, while genera that were overrepresented according to both metabarcoding and metatranscriptomics
353 are underscored. (Atl: Atlantic, Med: Mediterranean, T0: before infection, T60/72: diseased)

354

355 Seven genera were consistently found to contribute the majority of transcriptomic activity in diseased
356 oysters, displaying a strong increase in the number of transcripts compared to healthy oysters.

357 *Amphitrea*, *Arcobacter*, *Marinobacterium*, *Marinomonas*, *Oceanospirillum*, *Pseudoalteromonas*, and

358 *Vibrio* were together responsible for up to 40% of the total bacterial transcriptomic activity detected

359 just before the onset of oyster mortality. All are Gram-negative bacteria, and 6 out of 7 are

360 Gammaproteobacteria. Of the six Gammaproteobacteria, *Amphitrea*, *Marinobacterium*, *Marinomonas*

361 and *Oceanospirillum* belong to the same family (Oceanospirillaceae) and order (Oceanospirillales).

362 *Arcobacter* belongs to the class Epsilonproteobacteria. The genus *Fluviicola*, which belongs to the

363 phylum *Bacteroidetes*, presented an increase of transcriptomic activity in the Atlantic infectious

364 environment only. Finally, the genus *Oleiphilus* (order Oceanospirillales), was represented at a

365 maximum level of 1.9 % in diseased oysters.

366 These results indicate that a limited number of genera participate in the secondary bacteremia that

367 occurs in POMS. These genera are remarkably conserved between the different susceptible oyster

368 families and infectious environments. We therefore focused on these seven genera for additional

369 study.

370 **Secondary bacterial colonizers display genus-specific strategies**

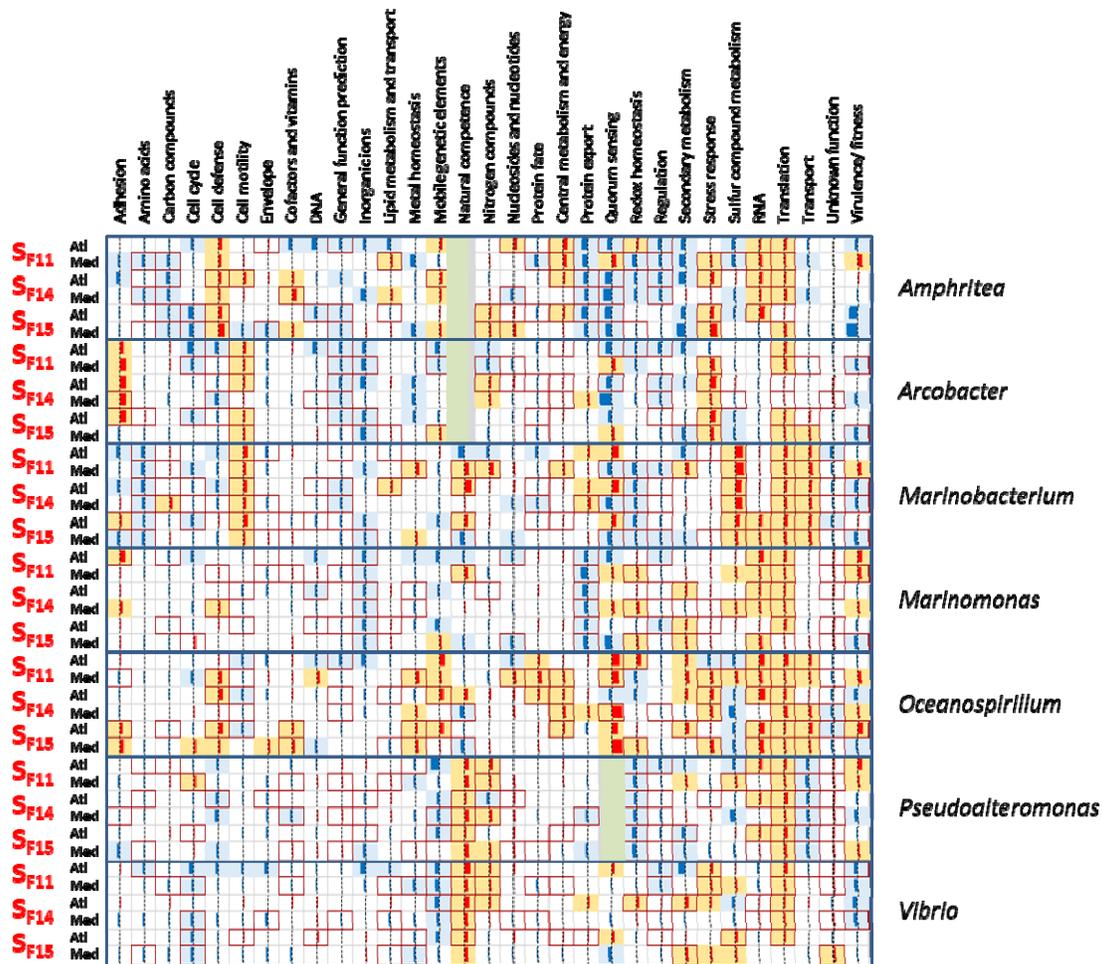
371 We focused on the late phase of the infection process in our examination of the functions underpinning

372 successful colonization by the seven genera discussed above. 31 functional categories were defined,

373 and each transcript-encoded protein was assigned to one of them (**Supplementary Table 2**). For each

374 genus and each category, an enrichment factor (EF) was calculated relative to the general increase of

375 the genus in the bacterial population (See Materials and Methods for details, **Figure 5**).



376

377 **Figure 5. Functional enrichment between T0 and the onset of oyster mortality.** The
 378 enrichment factor (EF) was calculated for each functional category for the seven most active
 379 genera present in all families and both environments. Each overrepresented (red) or
 380 underrepresented (blue) functional category is indicated by a bar with a width proportional to
 381 $\log_2(\text{EF})$. Red boxes around bars indicate $p \leq 0.05$ (Student's t-test). Green-filled cells indicate
 382 functional categories with no gene expression (genes absent from the genome in that genus or
 383 not expressed in any conditions). Light blue-filled cells indicate values lower than the 20th
 384 percentile, while yellow-filled cells indicate values higher than the 20th percentile.

385

386 For all genera except *Arcobacter*, translation and transcription appeared to be globally overrepresented
 387 in diseased oysters. Aside from these functions, we found that each colonizing genus displayed a
 388 specific pattern of functional enrichment, which was generally conserved across oyster families and
 389 environments. For instance, adhesion, cell defense, motility, natural competence, quorum sensing,
 390 stress response, and virulence gene expression were either over- or under-represented according to the

391 genus considered. These results suggest that each genus adopts a specific strategy to ensure
392 colonization success.

393

394 **Enriched functions are potentially important for successful colonization**

395 In a global metatranscriptomic analysis, important overrepresented transcripts/subcategories could be
396 hidden by the under-expression of genes classified as being in the same functional categories.
397 Therefore, we next focused our analysis on overrepresented transcripts (listed in **Supplementary**
398 **Table 6**), and identified several overexpressed genes that are likely to contribute to efficient
399 colonization. **Table 1** summarizes the distribution of these overexpressed genes/subcategories within
400 the seven genera.

Table 1. Specific enrichment of selected functions in the seven genera. Overexpression of function/gene in a given genus is indicated by a filled out green cell. E.C. numbers are indicated whenever applicable. Functions that are enriched in all seven genera are in boldface. The relevant information was extracted from supplementary table S6.

Category	Role/pathways	Enriched functions/genes	E.C. Number	Amphitrea	Acobacter	Marinobacterium	Marinomonas	Oceanospirillum	Pseudomonas	Vibrio
Cell defense										
	Arsenic detoxification	Arsenate reductase	1.6.5.2							
	Cyanide detoxification	Rhodanese like protein	2.8.1.1							
	Formaldehyde detoxification	Formaldehyde dehydrogenase, glutathione-independent	1.2.98.1							
	Drug resistance	Hydroxycyglutathione hydrolase	3.1.2.26							
		S-formylglutathione hydrolase	3.1.2.12							
		Aminoglycoside phosphotransferase	2.7.1.-							
		RND family efflux transporter								
	Hg resistance	TolC family outer membrane protein								
		MerR family DNA-binding transcriptional regulator								
Inorganic ion										
	Calcium transport	Ca ²⁺ transporter								
	Potassium transport	K ⁺ transporter								
	Sodium transport	Na ⁺ /H ⁺ antiporter NhaC								
	Phosphonate utilisation	Alkyl phosphonate utilization PhnA								
	Pi homeostasis	Phosphonate transporter								
		Alkaline phosphatase	3.1.3.1							
		inorganic diphosphatase								
		PhoH (Pi starvation)								
	Selenate transport	Pi transporter								
		Polyphosphate kinase 2	2.7.4.1							
		Selenate transporter								
Metal homeostasis										
	Iron homeostasis	Fe(3+) ABC transporter substrate-binding protein								
		Ferric iron uptake transcriptional regulator								
		Imelysin								
		Bacterioferritin								
		Ferritin								
		Siderophore production								
	Copper homeostasis	TonB-dependent siderophore receptor								
		Copper-translocating P-type ATPase								
	Metal homeostasis	Heavy metal (Co ²⁺ , Zn ²⁺ , W...) transporter								
		Metal transporter								
Nitrogen compound metabolism										
	Nitrosative stress	Hydroxylamine reductase	1.7.99.1							
Precursor metabolite and energy production and conversion										
	ATP synthase	ATP synthase	7.1.2.2							
	Electron transfer	cytochrome C								
		cytochrome C oxidase	7.1.1.9							
		Na(+)-translocating NADH-quinone reductase	7.2.1.1							
		cytochrome B								
		cytochrome o ubiquinol oxidase	7.1.1.3							
		electron transfer flavo protein								
		electron transfer flavo protein-ubiquinone oxidoreductase	1.5.5.1							
		hybrid-cluster NAD(P)-dependent oxidoreductase								
		NADH-quinone oxidoreductase	7.1.1.2							
		pyruvate flavodoxin oxidoreductase	1.2.7.1							
	Acetyl-CoA biosynthesis	ubiquinol oxidase subunit II	7.1.1.7							
		ubiquinol-cytochrome c reductase	1.10.2.2							
		acetate-CoA ligase	6.2.1.1							
	Anaerobic respiration	pyruvate dehydrogenase	1.9.6.1							
		fumarate reductase	1.3.5.4							
		hydrogenase small subunit	1.12.5.1							
		fumarate/nitrate reduction transcriptional regulator Fnr								

Redox homeostasis and oxidative stress			
Oxidative stress	Peroxi redoxin		
	Alkyl hydroperoxide reductase	1.11.1.26	
	Catalase	1.11.1.6	
	Disulfide reductase		
	Glutamate-cysteine ligase	6.3.2.2	
	Peroxidase	1.11.1.7	
Redox homeostasis	Superoxide dismutase	1.15.1.1	
	Gluta redoxin		
Redox homeostasis and oxidative stress	NAD(P) reductase		
	Thioredoxin		
Stress response			
Cold stress	Cold-shock protein		
Starvation/stationary phase	Ribosome-associated translation inhibitor RaiA		
Acid stress	BoIA family transcriptional regulator		
Extracytoplasmic stress	Phage shock protein PspA		
	RIP metalloprotease RseP		
Fe-S cluster repair	RNA polymerase sigma factor RpoE		
	SufS family cysteine desulfurase		
General stress response	Universal stress protein		
Heat-shock	Heat-shock protein		
	RNA polymerase sigma factor RpoH		
Nitrosative stress	Nitric-oxide reductase	1.7.2.5	
	NO-inducible flavohemoprotein		
Osmotic stress: osmoprotectant	Choline ABC transporter substrate-binding protein		
	Choline dehydrogenase	1.1.99.1	
	Dimethylglycine demethylation protein DgcA		
	Glycine betaine/L-proline ABC transporter ATP-binding protein ProV		
	ProQ/FinO family protein		
Signal transduction	Transcriptional regulator BetI		
SOS response	Symmetrical bis(5-nucleosyl)-tetraphosphate		
Starvation/ stationary phase	Transcriptional repressor LexA		
	Stringent starvation protein A		
Stringent response	RNA polymerase sigma factor RpoS		
	Bifunctional (p)ppGpp synthetase/guanosine-3,5-bis		
	GTP diphosphokinase RelA		
Sugar phosphate stress	RNA polymerase-binding protein DksA		
Toxin/antitoxin	Methylglyoxal synthase		
	Toxin/antitoxin system		
Sulfur compound metabolism			
Cysteine degradation	D-cysteine desulfhydrase	4.4.1.15	
	Sulfate adenyltransferase subunit CysD	2.7.7.4	
Sulfate reduction	HTH-type transcriptional regulator CysB		
Sulfite oxidation	Sulfite dehydrogenase	1.8.2.1	
Sulfite transport	Sulfite exporter TauE/SafE family protein		
Sulfolactate utilisation	(S)-sulfolactate dehydrogenase	1.1.1.310	
	Sulfoacetalddehyde acetyltransferase	2.3.3.15	
Sulfur oxidation	Flavocytochrome c sulfide dehydrogenase	1.8.2.3	
Thiosulfate oxidation	Sulfur oxidation c-type cytochrome SoxA	2.8.5.2	
	Sulfur oxidation protein SoxCD, sulfur dehydrogenase subunit	1.8.2.6	
Virulence/ fitness			
Toxin production	Putative RTX toxin/ hemolysin		
	Transcriptional activator HlyU		

403

404 High metabolic activity, revealed by a series of functional categories, was observed in all genera. In
 405 the “Translation” category, genes encoding ribosomal proteins, elongation and initiation factors, and
 406 tRNA ligases were overexpressed for all seven genera. In the “Precursor metabolite and energy
 407 production and conversion (Energy)” category, genes encoding ATP synthase and enzymes involved
 408 in glycolysis/neoglucogenesis and the TCA cycle were systematically present, as were genes encoding
 409 proteins of the respiratory chains (e.g. cytochrome-c and cytochrome oxidases). In the “Transcription
 410 and RNA metabolism (RNA)” category, genes encoding the various subunits of the RNA polymerase
 411 were overexpressed, as was the transcription housekeeping sigma factor RpoD (**Supplementary
 412 Table 6**).

413 Functions important for stress adaptation and resistance to host defenses varied over the course of
414 pathogenesis according to the bacterial genera considered (**Table 1**). “Stress response” genes encoding
415 cold-shock proteins and the ribosome-associated translation inhibitor RaiA were overexpressed by all
416 genera. The gene encoding the stress response RNA polymerase Sigma factor RpoS was
417 overexpressed in members of the Oceanospirillaceae family (*Amphritea Marinobacterium*,
418 *Marinomonas*, and *Oceanospirillum*). In the “redox homeostasis and oxidative stress” category,
419 peroxiredoxin genes were overexpressed by all genera, along with additional genus-specific functions
420 related to fighting oxidative stress (superoxide dismutases, catalases, and peroxidases) (**Table 1**). In
421 addition, all genera except *Oceanospirillum* overexpressed genes that included several nitrite and
422 nitrate reductases (classified in “Precursor metabolite and energy production and conversion”),
423 hydroxylamine reductases (classified in “Nitrogen compound metabolism”), and nitric-oxide reductase
424 components (classified in “Stress response/ Nitrosative stress”), all of which could contribute to
425 tolerance/resistance to reactive nitrogen species (RNS). RNS, like ROS, are important effectors of
426 oyster immunity [38].

427 “Cell defense”, “Metal homeostasis” and “Virulence/ fitness” categories were more specifically
428 encoded by a subset of bacterial genera. In the “Cell defense” category, multidrug resistance efflux
429 pumps with a TolC component and detoxifying enzymes were overexpressed in some genera. For
430 instance, an outer membrane channel protein TolC was overexpressed up to 16-fold ($\log_2(\text{EF}) \geq 4$) in
431 *Pseudoalteromonas* (**Supplementary Table 6**). Genes encoding Rhodanese, an enzyme that detoxifies
432 cyanide, were overexpressed in *Marinobacterium*, *Pseudoalteromonas*, and *Vibrio*. Several arsenate
433 reductase genes were also overexpressed in *Marinobacterium*, *Marinomonas*, and *Pseudoalteromonas*.
434 Genes encoding formaldehyde dehydrogenase, an aldehyde-detoxifying enzyme, were overexpressed
435 in the Oceanospirillaceae. Notably, no cell defense genes were overexpressed in *Arcobacter*. In the
436 “Metal homeostasis” category, 23 of 33 overexpressed genes were involved in iron homeostasis.
437 These genes are mainly expressed by *Marinobacterium* and *Marinomonas*. In *Vibrio*, the only up-
438 regulated gene of the category is annotated as a cobalt/magnesium transporter. In the “Virulence /
439 fitness” category, a small number of overexpressed genes corresponded to putative toxins encoded by

440 *Arcobacter*, *Oceanospirillum*, *Pseudoalteromonas*, and *Vibrio*. This has to be completed by genes
441 from the “Protein export and secretion system” category, which are important for both virulence and
442 interbacterial competitions. Genes encoding the general protein export system (i.e. the “Sec” system of
443 Gram-negative bacteria [39, 40]) were overexpressed by all genera, while type VI secretion systems
444 were overexpressed specifically in *Vibrio* (e.g. *V. crassostreae*) and *Marinomonas*.

445 Finally, a global increase in the “Sulfur compound metabolism” category was observed in
446 *Marinobacterium* (**Figure 5**). Overexpressed proteins of this category were also found in other genera
447 (**Table 1** and **Supplementary Table 6**). Interestingly, these overexpressed proteins corresponded to
448 enzymes that were (mostly non-redundantly) encoded by five different genera. Altogether, these
449 enzymes constitute several sulfur metabolic pathways (**Figure 6**).

450 These results highlight functional complementation at the level of the bacterial community, and a
451 metabolic adaptation to the within-host environment, both of which are likely important for successful
452 oyster colonization.

468 The bacterial consortium associated with POMS was remarkably similar in three different susceptible
469 oyster families and two different environments. All diseased oyster families harbored similar
470 microbial communities, with only a few exceptions. Remarkably, the oyster-associated microbial
471 communities were conserved across infectious environments, although the Mediterranean and Atlantic
472 ecosystems are likely to host diverse bacterial communities.

473 Until recently, only members of the *Vibrio* genus had been repeatedly associated with POMS. These
474 studies used culture-based approaches to investigate oyster-associated bacterial communities (Lemire
475 et al., 2015; Bruto et al., 2018). *Vibrio* species associated with POMS were characterized by key
476 virulence factors that are required to weaken oyster cellular defenses (Rubio et al., 2019; Piel et al.,
477 2019). Members of the *Arcobacter* genus had also been associated with POMS-diseased oysters [41,
478 42], but the role of this genus in pathogenesis was not investigated more deeply due to limitations of
479 culture-based techniques [43]. In the present study, we extended the core colonizing microbiota to five
480 bacterial genera. The discovery of the contributions of these genera, which are responsible for up to
481 40% of the bacterial transcriptional activity observed in POMS, provides new insights into the
482 pathogenesis of POMS. Altogether, our results strongly suggest that a colonizing core microbiota,
483 rather than specific bacterial pathogens, operates as a functional unit of pathogenesis. Together with
484 OsHV-1, these bacteria form the POMS pathobiota. POMS secondary bacteremia may resemble
485 periodontitis in humans, in which the evolution of the disease is characterized by the development of a
486 pathogenic consortium comprising a limited number of species [44, 45].

487 We used metatranscriptomics to unveil the functions of the microbiota in relation to POMS. Bacterial
488 metatranscriptomics from host tissues is technically challenging (due to the low proportion of bacterial
489 transcripts in the host samples), but it provides functional information that is thought to more
490 accurately portray the role of the microbiota in health and disease states [46]. Accordingly, gene
491 expression profiling has proven highly successful in advancing the understanding of the dynamics of
492 disease-associated microbial populations [47]. In the case of POMS, by linking functional genes to the
493 bacterial genera which encode them, we found a remarkably consistent relationship between the
494 structure of bacterial communities (through 16S metabarcoding) and the functions expressed by

495 bacterial genera in the communities (through metatranscriptomics), further supporting our hypothesis
496 of a pathobiota composed of at least five key genera.

497 Among functions overexpressed in the diseased oyster-associated microbiota, we first identified core
498 functions which were overrepresented in all dominant genera (**Table 1**). In addition to housekeeping
499 functions with expression levels that reflect the rapid growth of the genus (e.g. translation and
500 transcription, central metabolism), we found functional redundancy in two important categories
501 required for adaptation to the within-host environment: stress response and redox homeostasis.

502 We found a systematic and important overproduction of various cold shock proteins in the “Stress
503 response” functional category (**Supplementary Table 6**). In the diseased oyster microbiota, these
504 proteins may be important to sustain the high level of transcription and translation we observed [48,
505 49]. Redox homeostasis was another core function. Fighting oxidative stress in the host can be crucial
506 to survival and multiplication within the host. Thus, bacteria have many lines of defense against
507 oxidative damage [50]. Key enzymes and proteins involved in redox homeostasis and oxidative stress
508 response were overexpressed in several of the five genera, including superoxide dismutases (which
509 catalyze the dismutation of superoxide in hydrogen peroxide) and catalases and peroxidases (which
510 destroy hydrogen peroxide and other toxic peroxide compounds). All five genera overexpressed
511 redoxin, a small redox protein which plays important roles in maintaining an intracellular reducing
512 environment, and also acts as a cofactor in intracellular oxidoreductase reactions. Other proteins
513 important for fighting oxidative stress were specific to one or more given genera. For example,
514 NAD(P) reductase (which contributes to the homeostasis of reduced NAD(P)H, an important source of
515 reducing potential) was enriched in *Amphitrea*, *Marinobacterium*, *Marinomonas*, and
516 *Oceanospirillum*, four members of the Oceanospirillaceae family. Glutamate – cysteine ligase (which
517 is responsible for the production of Glutathione, an important antioxidant compound, and is also
518 involved in detoxifying reactions) was overexpressed in *Amphitrea*, *Oceanospirillum*, and
519 *Pseudoalteromonas*. Earlier functional studies have identified the AhpCF complex detoxifying
520 enzyme alkylhydroperoxydase as a key determinant of resistance to ROS and thus oyster

521 colonization in *Vibrio* [51]. In the diseased oyster microbiota, this enzyme was over-produced in
522 *Marinomonas* and *Oceanospirillum*. All of the successful genera, except *Oceanospirillum*, also
523 expressed enzymes required for tolerance/resistance to RNS (**Table 1**). ROS and RNS are major
524 players at the oyster-microbiota interface (Destoumieux-Garzon et al., 2020), thus it is not surprising
525 that successful colonizers express ROS detoxifying enzymes during oyster colonization. In summary,
526 each genus overexpressed several proteins involved in stress response, especially oxidative stress,
527 although each genus had its own repertoire of proteins.

528 We found that the bacterial community associated with POMS also expresses a series of genus-
529 specific (variable) functions. These functions include adhesion, cell defense, cell motility, metal
530 homeostasis, natural competence, quorum sensing, sulfur metabolism, transport, and virulence (**Table**
531 **1**). The observation that functions of interest may be contributed by a single or multiple bacterial
532 genera within a microbial community has also been made in the human gut [46, 52]. Interestingly, a
533 function expressed by one genus can be useful for the community as a whole. Functional
534 complementarity within the oyster-associated microbiota may therefore contribute to synergy between
535 genera.

536 Evidence of cross-benefits at the community level in the oyster microbiota was found in the form of
537 overexpressed genes related to iron homeostasis (**Supplementary Table 6**). The capacity to scavenge
538 iron from the environment and the host is a well-known determinant of pathogenicity [53, 54]. Except
539 for *Vibrio*, all genera overexpressed functions related to iron acquisition (e.g. iron siderophore
540 production and transport, which enable the chelation and uptake of iron). However, in the consortium,
541 only *Marinomonas* and *Marinobacterium* expressed siderophore-production enzymes, while other
542 members of the microbiota expressed the receptors for iron-siderophore uptake. Similarly, virulence
543 factors (such as secreted RTX family toxins) were expressed by *Vibrio* and *Arcobacter*, while the
544 Type 6 Secretion System (T6SS) was expressed by *Vibrio* and *Marinomonas*. T6SS expressed by
545 *Vibrio* has been shown to contribute to the suppression of oyster cellular defenses [21, 55]. Therefore,
546 their expression by a few genera is likely beneficial to the whole pathobiota.

547 The best example of cross-benefits, however, is the “Sulfur compound metabolism” functional
548 category. Marine *Arcobacter* species that can oxidize sulfide to elemental sulfur, producing sulfur
549 filaments, have been previously identified [56]. In our consortium, corresponding sulfide
550 dehydrogenase enzymatic activity (E.C. 1.8.2.3) was encoded by *Arcobacter* only, while other sulfur
551 compound metabolic pathways were associated with four other genera (mostly *Marinomonas* and
552 *Marinobacterium*) (**Figure 6**). This suggests that sulfur cycling is a property of the colonizing core
553 microbiota as a whole, and not of a single genus. A similar association between *Arcobacter* and
554 *Marinobacter* species has been described in biogeochemical cycling, specifically in the environment
555 of natural gas wells [57]. We believe that such interdependence and cooperation within microbial
556 communities allows these communities to meet metabolic requirements and mount an efficient stress
557 response, which are key determinants of their success. We further believe that this helps to explain the
558 conserved pathobiota structure associated with POMS across distinct environments and oyster genetic
559 backgrounds.

560

561 **Supplementary Materials**

562 Supplementary Table 1: Total raw reads (R1+R2) at each stage of biocomputing, after sequencing and
563 removal of rRNA reads (eukaryotic and bacterial), oyster reads, and viral reads.

564 Supplementary Table 2: List of functional categories defined in bacterial metatranscriptomics.

565 Supplementary Table 3: Complete list of contigs identified in bacterial metatranscriptomics, with
566 annotations and expression value in TPM. Taxons in red correspond to assignment at taxonomic ranks
567 other than the genus level.

568 Supplementary Table 4: Absolute abundance of Operational Taxonomic Units (*ie.* clusters) and their
569 corresponding taxonomic affiliations in susceptible and resistant oyster families confronted with two
570 different infectious environments. Susceptible oyster families are S_{F11} , S_{F14} , and S_{F15} ; resistant oyster
571 families are R_{F21} , R_{F23} , and R_{F48} . A indicates the Atlantic infectious environment, M the Mediterranean

572 infectious environment. T₀, T₆, T₁₂, T₂₄, T₄₈, T₆₀, and T₇₂ indicate sampling times (in hours) over
573 the course of experimental infection. R₁, R₂, R₃ indicate the results of each replicate (Excel file).

574 Supplementary Table 5: Frequencies of bacterial taxa that change significantly in abundance over the
575 course of each experimental infection (Atlantic or Mediterranean) in susceptible and resistant oyster
576 families. The change in abundance of bacterial taxa between initial and final time points was
577 determined using DESeq2 with the adjusted p-value < 0.05. Susceptible oyster families are SF₁₁,
578 SF₁₄, and SF₁₅; resistant oyster families are RF₂₁, RF₂₃, and RF₄₈. A indicates the Atlantic
579 infectious environment, M the Mediterranean infectious environment. T₀, T₆, T₁₂, T₂₄, T₄₈, T₆₀
580 and T₇₂ indicate sampling times (in hours) over the course of experimental infection. R₁, R₂, R₃
581 indicate the results of each replicate (Excel file).

582 Supplementary Table 6: List of the overexpressed (enriched) proteins in the seven main genera found
583 to colonize diseased oysters. A protein was considered enriched when it had a log₂(EF) ≥ 1 with a p-
584 value ≤ 0.05 (Student's t-test) in at least two conditions.

585 Supplementary Figure 1: Schematic representation of the steps of metatranscriptomic data analysis.

586 Supplementary Figure 2: Rarefaction curves of the sub-sampled 16S rDNA dataset (10,000 reads per
587 sample) for susceptible and resistant oysters in (a) the Atlantic infectious environment (AS_{F11}, AS_{F14},
588 AS_{F15} and AR_{F21}, AR_{F23}, AR_{F48}) and (b) the Mediterranean infectious environment (MS_{F11}, MS_{F14},
589 MS_{F15} and MR_{F21}, MR_{F23}, MR_{F48}). T₀, T₆, T₁₂, T₂₄, T₄₈, T₆₀, and T₇₂ indicate sampling times (in
590 hours) over the course of experimental infection. Results are shown in triplicate for each time point.

591 Supplementary Figure 3: Microbiota modification as analysed using 16S rDNA metabarcoding in
592 susceptible and resistant oyster families confronted with two different infectious environments.
593 Susceptible oyster families (S_{F11}, S_{F14} and S_{F15}) and resistant oyster families (R_{F21}, R_{F23} and R_{F48})
594 confronted with (a) Atlantic or (b) Mediterranean infectious environments. Significant changes in
595 abundance (up or down; DESeq2, p-value < 0.05) between the initial and the final time point of the
596 infection were much greater for each taxonomic rank (from the phylum to the OTU rank) for
597 susceptible oyster families than for resistant oyster families. Data for AS_{F11} and AR_{F21} were extracted

598 from [20].

599 Supplementary Figure 4: Heatmaps of bacterial communities that changed significantly in abundance
600 over the course of infection in resistant oysters (R_{F11} , R_{F14} , R_{F15}) in the Atlantic and Mediterranean
601 infectious environments. Analyses were performed at the genus level. Only genera with a relative
602 proportion greater than 2% in at least one sample are shown. Increased color intensity (blue) indicates
603 increased relative abundance of the genus.

604

605 **END NOTES**

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617

618 **Author contributions**

619 J.D.L., B.P., A.J. and G.M. designed experiments. B. P., J.D.L, A.L., J.M.E., Y.G., L.D. and G.M.
620 performed oyster experiments. J.D.L., A.L., E.T., C.C and G.M. performed microbiota analyses.
621 J.D.L. and A.L. performed qPCR analyses. A.J. and X.L performed the metatranscriptomic
622 experiments. A.J. and S.M. analyzed the metatranscriptomic data. J.D.L., A.L., A.J, S.M. and G.M.
623 interpreted results. J.D.L., A.L., A.J., D. D. G. and G.M. wrote the manuscript, which has been
624 reviewed and approved by all authors.

625

626 **Conflict of interest statement.** There are no conflicts of interest. This manuscript represents original
627 results, and has not been submitted elsewhere for publication.

628

629 REFERENCES

630

- 631 1. EFSA: P. o. A. H. W. Oyster mortality. *EFSA Journal* 2015, 13(4122-n/a).
- 632 2. Paul-Pont I, Dhand NK, Whittington RJ: Influence of husbandry practices on OsHV-1
633 associated mortality of Pacific oysters *Crassostrea gigas*. *Aquaculture* 2013, 412:202-214.
- 634 3. Martenot C, Oden E, Travaille E, Malas JP, Houssin M: Detection of different variants of
635 Ostreid Herpesvirus 1 in the Pacific oyster, *Crassostrea gigas* between 2008 and 2010. *Virus*
636 *Res* 2011, 160(1-2):25-31.
- 637 4. Renault T, Moreau P, Faury N, Pepin JF, Segarra A, Webb S: Analysis of clinical ostreid
638 herpesvirus 1 (Malacoherpesviridae) specimens by sequencing amplified fragments from
639 three virus genome areas. *J Virol* 2012, 86(10):5942-5947.
- 640 5. Segarra A, Pepin JF, Arzul I, Morga B, Faury N, Renault T: Detection and description of a
641 particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of
642 Pacific oysters, *Crassostrea gigas*, in France in 2008. *Virus Res* 2010, 153(1):92-99.
- 643 6. Peeler EJ, Reese RA, Cheslett DL, Geoghegan F, Power A, Thrush MA: Investigation of
644 mortality in Pacific oysters associated with Ostreid herpesvirus-1 muVar in the Republic of
645 Ireland in 2009. *Prev Vet Med* 2012, 105(1-2):136-143.
- 646 7. Lynch SA, Carlsson J, Reilly AO, Cotter E, Culloty SC: A previously undescribed ostreid herpes
647 virus 1 (OsHV-1) genotype detected in the pacific oyster, *Crassostrea gigas*, in Ireland.
648 *Parasitology* 2012, 139(12):1526-1532.
- 649 8. EFSA PoAHW: Oyster mortality. *EFSA Journal* 2015, 13(6):4122-n/a.
- 650 9. Abbadi M, Zamperin G, Gastaldelli M, Pascoli F, Rosani U, Milani A, Schivo A, Rossetti E,
651 Turolla E, Gennari L *et al*: Identification of a newly described OsHV-1 microvar from the North
652 Adriatic Sea (Italy). *J Gen Virol* 2018, 99(5):693-703.
- 653 10. Burioli EAV, Prearo M, Houssin M: Complete genome sequence of Ostreid herpesvirus type 1
654 microVar isolated during mortality events in the Pacific oyster *Crassostrea gigas* in France
655 and Ireland. *Virology* 2017, 509:239-251.
- 656 11. Burioli EAV, Prearo M, Riina MV, Bona MC, Fioravanti ML, Arcangeli G, Houssin M: Ostreid
657 herpesvirus type 1 genomic diversity in wild populations of Pacific oyster *Crassostrea gigas*
658 from Italian coasts. *J Invertebr Pathol* 2016, 137:71-83.
- 659 12. Azéma P, Lamy JB, Boudry P, Renault T, Travers MA, Dégremont L: Genetic parameters of
660 resistance to *Vibrio aestuarianus*, and OsHV-1 infections in the Pacific oyster, *Crassostrea*
661 *gigas*, at three different life stages. *Genetics Selection Evolution* 2017, 49:1-16.
- 662 13. Le Roux F, Wegner KM, Polz MF: Oysters and Vibrios as a Model for Disease Dynamics in Wild
663 Animals. *Trends Microbiol* 2016, 24(7):568-580.
- 664 14. Pernet F, Barret J, Le Gall P, Corporeau C, Dégremont L, Lagarde F, Pépin JF, Keck N: Mass
665 mortalities of Pacific oysters *Crassostrea gigas* reflect infectious diseases and vary with
666 farming practices in the Mediterranean Thau lagoon, France. *Aquaculture Environment*
667 *Interactions* 2012, 2(3):215-237.
- 668 15. Pernet F, Barret J, Marty C, Moal J, Le Gall P, Boudry P: Environmental anomalies, energetic
669 reserves and fatty acid modifications in oysters coincide with an exceptional mortality event.

- 670 *Marine Ecology Progress Series* 2010, 401:129-146.
- 671 16. Pernet F, Tamayo D, Fuhrmann M, Petton B: Deciphering the effect of food availability,
672 growth and host condition on disease susceptibility in a marine invertebrate. *J Exp Biol* 2019,
673 222(Pt 17).
- 674 17. Petton B, Pernet F, Robert R, Boudry P: Temperature influence on pathogen transmission and
675 subsequent mortalities in juvenile Pacific oysters *Crassostrea gigas*. *Aquaculture Environment*
676 *Interactions* 2013, 3(3):257-273.
- 677 18. Bruto M, James A, Petton B, Labreuche Y, Chenivresse S, Alunno-Bruscia M, Polz MF, Le Roux
678 F: *Vibrio crassostreae*, a benign oyster colonizer turned into a pathogen after plasmid
679 acquisition. *ISME J* 2017, 11(4):1043-1052.
- 680 19. Lemire A, Goudenege D, Versigny T, Petton B, Calteau A, Labreuche Y, Le Roux F: Populations,
681 not clones, are the unit of vibrio pathogenesis in naturally infected oysters. *ISME J* 2015,
682 9(7):1523-1531.
- 683 20. de Lorgeril J, Lucasson A, Petton B, Toulza E, Montagnani C, Clerissi C, Vidal-Dupiol J,
684 Chaparro C, Galinier R, Escoubas JM *et al*: Immune-suppression by OsHV-1 viral infection
685 causes fatal bacteraemia in Pacific oysters. *Nat Commun* 2018, 9(1):4215.
- 686 21. Rubio T, Oyanedel D, Labreuche Y, Toulza E, Luo X, Bruto M, Chaparro C, Torres M, de
687 Lorgeril J, Haffner P *et al*: Species-specific mechanisms of cytotoxicity toward immune cells
688 determine the successful outcome of *Vibrio* infections. *Proc Natl Acad Sci U S A* 2019,
689 116(28):14238-14247.
- 690 22. de Lorgeril J, Petton B, Lucasson A, Perez V, Stenger PL, Degremont L, Montagnani C,
691 Escoubas JM, Haffner P, Allienne JF *et al*: Differential basal expression of immune genes
692 confers *Crassostrea gigas* resistance to Pacific oyster mortality syndrome. *BMC Genomics*
693 2020, 21(1):63.
- 694 23. Petton B, Boudry P, Alunno-Bruscia M, Pernet F: Factors influencing disease-induced
695 mortality of Pacific oysters *Crassostrea gigas*. *Aquaculture Environment Interactions* 2015,
696 6(3):205-222.
- 697 24. Pfaffl MW: A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic*
698 *Acids Res* 2001, 29(9):e45.
- 699 25. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glockner FO: Evaluation of
700 general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-
701 based diversity studies. *Nucleic Acids Res* 2013, 41(1):e1.
- 702 26. Escudie F, Auer L, Bernard M, Mariadassou M, Cauquil L, Vidal K, Maman S, Hernandez-
703 Raquet G, Combes S, Pascal G: FROGS: Find, Rapidly, OTUs with Galaxy Solution. *Bioinformatics*
704 2017, 34(8):1287-1294.
- 705 27. Magoc T, Salzberg SL: FLASH: fast length adjustment of short reads to improve genome
706 assemblies. *Bioinformatics* 2011, 27(21):2957-2963.
- 707 28. Mahe F, Rognes T, Quince C, de Vargas C, Dunthorn M: Swarm: robust and fast clustering
708 method for amplicon-based studies. *PeerJ* 2014, 2:e593.
- 709 29. Rognes T, Flouri T, Nichols B, Quince C, Mahe F: VSEARCH: a versatile open source tool for
710 metagenomics. *PeerJ* 2016, 4:e2584.
- 711 30. Langmead B, Salzberg SL: Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012,
712 9(4):357-359.
- 713 31. Rosani U, Shapiro M, Venier P, Allam B: A Needle in A Haystack: Tracing Bivalve-Associated
714 Viruses in High-Throughput Transcriptomic Data. *Viruses* 2019, 11(3).
- 715 32. Buchfink B, Xie C, Huson DH: Fast and sensitive protein alignment using DIAMOND. *Nat*
716 *Methods* 2015, 12(1):59-60.
- 717 33. Huson DH, Albrecht B, Bagci C, Bessarab I, Gorska A, Jolic D, Williams RBH: MEGAN-LR: new
718 algorithms allow accurate binning and easy interactive exploration of metagenomic long
719 reads and contigs. *Biol Direct* 2018, 13(1):6.
- 720 34. Anders S, Pyl PT, Huber W: HTSeq--a Python framework to work with high-throughput
721 sequencing data. *Bioinformatics* 2015, 31(2):166-169.

- 722 35. team RC: R: a language and environment for statistical computing. In.: R Foundation for
723 Statistical Computing; 2013.
- 724 36. Love MI, Huber W, Anders S: Moderated estimation of fold change and dispersion for RNA-
725 seq data with DESeq2. *Genome Biol* 2014, 15(12):550.
- 726 37. McMurdie PJ, Holmes S: phyloseq: an R package for reproducible interactive analysis and
727 graphics of microbiome census data. *PLoS One* 2013, 8(4):e61217.
- 728 38. Destoumieux-Garzon D, Canesi L, Oyanedel D, Travers MA, Charriere GM, Pruzzo C, Vezzulli L:
729 Vibrio-bivalve interactions in health and disease. *Environ Microbiol* 2020.
- 730 39. Smets D, Loos MS, Karamanou S, Economou A: Protein Transport Across the Bacterial Plasma
731 Membrane by the Sec Pathway. *Protein J* 2019, 38(3):262-273.
- 732 40. Beckwith J: The Sec-dependent pathway. *Res Microbiol* 2013, 164(6):497-504.
- 733 41. Lasa A, di Cesare A, Tassistro G, Borello A, Gualdi S, Furones D, Carrasco N, Cheslett D,
734 Brechon A, Paillard C *et al*: Dynamics of the Pacific oyster pathobiota during mortality
735 episodes in Europe assessed by 16S rRNA gene profiling and a new target enrichment next-
736 generation sequencing strategy. *Environ Microbiol* 2019, 21(12):4548-4562.
- 737 42. Lokmer A, Mathias Wegner K: Hemolymph microbiome of Pacific oysters in response to
738 temperature, temperature stress and infection. *ISME J* 2015, 9(3):670-682.
- 739 43. Rahman FU, Andree KB, Salas-Masso N, Fernandez-Tejedor M, Sanjuan A, Figueras MJ,
740 Furones MD: Improved culture enrichment broth for isolation of Arcobacter-like species from
741 the marine environment. *Sci Rep* 2020, 10(1):14547.
- 742 44. Yost S, Duran-Pinedo AE, Teles R, Krishnan K, Frias-Lopez J: Functional signatures of oral
743 dysbiosis during periodontitis progression revealed by microbial metatranscriptome analysis.
744 *Genome Med* 2015, 7(1):27.
- 745 45. Lamont RJ, Hajishengallis G: Polymicrobial synergy and dysbiosis in inflammatory disease.
746 *Trends Mol Med* 2015, 21(3):172-183.
- 747 46. Heintz-Buschart A, Wilmes P: Human Gut Microbiome: Function Matters. *Trends Microbiol*
748 2018, 26(7):563-574.
- 749 47. Murray JL, Connell JL, Stacy A, Turner KH, Whiteley M: Mechanisms of synergy in
750 polymicrobial infections. *J Microbiol* 2014, 52(3):188-199.
- 751 48. Phadtare S: Unwinding activity of cold shock proteins and RNA metabolism. *RNA Biol* 2011,
752 8(3):394-397.
- 753 49. Phadtare S, Severinov K: RNA remodeling and gene regulation by cold shock proteins. *RNA*
754 *Biol* 2010, 7(6):788-795.
- 755 50. Staerck C, Gastebois A, Vandeputte P, Calenda A, Larcher G, Gillmann L, Papon N, Bouchara
756 JP, Fleury MJJ: Microbial antioxidant defense enzymes. *Microb Pathog* 2017, 110:56-65.
- 757 51. Vanhove AS, Rubio TP, Nguyen AN, Lemire A, Roche D, Nicod J, Vergnes A, Poirier AC,
758 Disconzi E, Bachere E *et al*: Copper homeostasis at the host vibrio interface: lessons from
759 intracellular vibrio transcriptomics. *Environ Microbiol* 2016, 18(3):875-888.
- 760 52. Schirmer M, Franzosa EA, Lloyd-Price J, Mclver LJ, Schwager R, Poon TW, Ananthakrishnan
761 AN, Andrews E, Barron G, Lake K *et al*: Dynamics of metatranscription in the inflammatory
762 bowel disease gut microbiome. *Nat Microbiol* 2018, 3(3):337-346.
- 763 53. Begg SL: The role of metal ions in the virulence and viability of bacterial pathogens. *Biochem*
764 *Soc Trans* 2019, 47(1):77-87.
- 765 54. Lemos ML, Balado M: Iron uptake mechanisms as key virulence factors in bacterial fish
766 pathogens. *J Appl Microbiol* 2020, 129(1):104-115.
- 767 55. Piel D, Bruto M, James A, Labreuche Y, Lambert C, Janicot A, Chenivesse S, Petton B, Wegner
768 KM, Stoudmann C *et al*: Selection of *Vibrio crassostreae* relies on a plasmid expressing a type
769 6 secretion system cytotoxic for host immune cells. *Environ Microbiol* 2019.
- 770 56. Wirsén CO, Sievert SM, Cavanaugh CM, Molyneaux SJ, Ahmad A, Taylor LT, DeLong EF, Taylor
771 CD: Characterization of an autotrophic sulfide-oxidizing marine *Arcobacter* sp. that produces
772 filamentous sulfur. *Appl Environ Microbiol* 2002, 68(1):316-325.
- 773 57. Evans MV, Panescu J, Hanson AJ, Welch SA, Sheets JM, Nastasi N, Daly RA, Cole DR, Darrah

774 TH, Wilkins MJ *et al*: Members of Marinobacter and Arcobacter Influence System
775 Biogeochemistry During Early Production of Hydraulically Fractured Natural Gas Wells in the
776 Appalachian Basin. *Front Microbiol* 2018, 9:2646.
777