



Microbiota Composition and Evenness Predict Survival Rate of Oysters Confronted to Pacific Oyster Mortality Syndrome

Camille Clerissi^{1,2*}, Julien de Lorgeril¹, Bruno Petton³, Aude Lucasson¹, Jean-Michel Escoubas¹, Yannick Gueguen¹, Lionel Dégremont⁴, Guillaume Mitta¹ and Eve Toulza^{1*}

¹ IHPE, Univ. Montpellier, CNRS, Ifremer, Univ. Perpignan Via Domitia, Perpignan, France, ² PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan, Perpignan, France, ³ Ifremer, LEMAR UMR 6539 (Université de Bretagne Occidentale, CNRS, IRD, Ifremer), Argenton-en-Landunvez, France, ⁴ Ifremer, RBE-SG2M-LGPMM, La Tremblade, France

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*Correspondence:

Camille Clerissi
camille.clerissi@ephe.psl.eu
Eve Toulza
eve.toulza@univ-perp.fr

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Pacific Oyster Mortality Syndrome (POMS) affects *Crassostrea gigas* oysters worldwide and causes important economic losses. Disease dynamic was recently deciphered and revealed a multiple and progressive infection caused by the *Ostreid herpesvirus* OsHV-1 μ Var, triggering an immunosuppression followed by microbiota destabilization and bacteraemia by opportunistic bacterial pathogens. However, it remains unknown if microbiota might participate to protect oysters against POMS, and if microbiota characteristics might be predictive of oyster mortalities. To tackle this issue, we transferred full-sib progenies of resistant and susceptible oyster families from hatchery to the field during a period in favor of POMS. After 5 days of transplantation, oysters from each family were either sampled for individual microbiota analyses using 16S rRNA gene-metabarcoding or transferred into facilities to record their survival using controlled condition. As expected, all oysters from susceptible families died, and all oysters from the resistant family survived. Quantification of OsHV-1 and bacteria showed that 5 days of transplantation were long enough to contaminate oysters by POMS, but not for entering the pathogenesis process. Thus, it was possible to compare microbiota characteristics between resistant and susceptible oysters families at the early steps of infection. Strikingly, we found that microbiota evenness and abundances of Cyanobacteria (Subsection III, family I), Mycoplasmataceae, Rhodobacteraceae, and Rhodospirillaceae were significantly different between resistant and susceptible oyster families. We concluded that these microbiota characteristics might predict oyster mortalities.

Keywords: holobiont, microbiome, metabarcoding, fitness, bacteria

INTRODUCTION

The farmed oyster *Crassostrea gigas* is heavily affected by the Pacific Oyster Mortality Syndrome (POMS) targeting juveniles (Barbosa Solomieu et al., 2015; Pernet et al., 2016). This disease is multifactorial and depends on water temperature (Petton et al., 2015), development stage (Azéma et al., 2017), and oyster diet (Pernet et al., 2019). It is also polymicrobial due to the

combined development of viral and bacterial infections (de Lorgeril et al., 2018). Different susceptibility levels were previously associated with oyster physiological status, genetic backgrounds (Dégremont et al., 2005; Samain et al., 2007; Wendling et al., 2017), in association with microbiota dysbiosis (Lokmer and Wegner, 2015).

Recently, holistic molecular approaches revealed the mechanism of POMS (de Lorgeril et al., 2018; Rubio et al., 2019). These studies showed that an infection by the *Ostreid herpesvirus* (OsHV-1 μ Var) is the critical step in the infectious process leading to an immune-compromised state by altering hemocyte physiology. This first process is followed by a microbiota destabilization which “opens the door” to bacterial pathogens (e.g., *vibrios*) that target hemocytes to induce their lysis. The infectious process is completed with subsequent bacteraemia, which is the ultimate step inducing oyster death.

So far, it is still unknown whether oyster microbial associates might influence disease development, and if microbiota characteristics might predict oyster mortalities. However, microbiota can play a role of physical barriers against pathogens. For example, it was suggested that part of the resident hemolymph bacteria may contribute to oysters protection by producing antimicrobial peptides (Desriac et al., 2014). Other studies highlighted protective effects of bacteria, such as secondary endosymbionts of aphids against parasitoid wasps (Oliver et al., 2003), or symbionts of frogs against pathogenic fungi (Woodhams et al., 2007). Furthermore, microbial associates might also stimulate immunity of their hosts (Wang et al., 2019), and thus indirectly limit pathogen development.

To tackle this issue, we used one resistant oyster family (R_{F21}) and two susceptible families (S_{F15} and S_{F32}) from a previous study (de Lorgeril et al., 2018). Pathogen-free oysters (reproduced and grown in bio-secured conditions) from these three full-sib families were placed for 5 days in the field during an infectious period (Figure 1). According to previous observations, 16°C was a relevant threshold to define the infectious period, as high mortality rates were observed above this temperature (Pernet et al., 2012; Petton et al., 2013; Dégremont et al., 2015). Moreover, 5 days were considered sufficient for oyster contamination by the causal agents of the disease (Petton et al., 2015), and allowed microbiota analyses before disease development and animal death. In both the hatchery (control) and after 5 days of transplantation in the field, oysters were sampled, and we analyzed oyster-associated bacterial communities using 16S rRNA gene-metabarcoding.

This study aimed at comparing bacteria content of resistant and susceptible families in order to possibly identify microbiota features associated with oyster mortality and/or resistance. According to the previous holistic study that used the resistant family R_{F21} and another susceptible family (S_{F11}) (de Lorgeril et al., 2018), we expected that susceptible families had (i) early microbiota destabilization (increase of species richness and microbiota dispersions, decrease of evenness), and (ii) possibly contained the opportunistic and/or pathogenic bacteria that participated to bacteraemia in the holistic study (e.g., *vibrios*).

MATERIALS AND METHODS

Full-sib *C. gigas* Oyster Families

In 2015, full-sib *C. gigas* oyster families were produced using a methodology that allowed the production of pathogen-free juveniles (de Lorgeril et al., 2018). Two oyster families (S_{F15} and S_{F32}) were produced using one female and one male sampled from wild populations in the Atlantic Ocean and the Mediterranean Sea, respectively (Figure 1). In addition, family R_{F21} was produced using genitors from a mass selective breeding program aiming to increase the resistance of *C. gigas* oysters against OsHV-1. It was performed by breeding disease survivors throughout four generations of selection (Dégremont et al., 2015). Thus, each family (cohort) corresponded to the offspring of a biparental reproduction (full-sib progenies). Conditioning, reproduction and larval breeding were performed as described previously (de Lorgeril et al., 2018). At the larval and post-larval stages, oysters were fed with the same diet as the genitors at a concentration between 1500–2000 $\mu\text{m}^3 \cdot \mu\text{l}^{-1}$ (Rico-Villa et al., 2009). Before experiments, all oyster families were maintained in controlled condition at the laboratory (Argenton-sur-Creuse, France) using seawater treated with UV, filtered through 1 μm mesh, and enriched with a bi-specific phytoplankton diet made of *Tisochrysis lutea* (CCAP 927/14) and *Chaetoceros muelleri* (CCAP 1010/3) (in equal biomass proportion) at a ratio equivalent to 6% of the oyster dry mass (Rico-Villa et al., 2009). Finally, all oysters remained free of any abnormal mortality.

Experimental Design

About 140 juveniles per oyster family were either kept in the controlled condition or placed in the field for 5 days. The field site was located within a oyster farm in the Atlantic Ocean during an infectious period (Figure 1). This infectious period was selected according to seawater temperatures (above 16°C). It was confirmed by the observed mortality rates, and the presence within some oysters of pathogens and/or opportunists previously described in the same region (Atlantic Ocean) (de Lorgeril et al., 2018). After 5 days of transplantation (July 2016), no mortality occurred and 14 individuals per family were flash frozen in liquid nitrogen and stored at -80°C . The remaining oysters were then transferred into the hatchery under controlled conditions to monitor the survival rates of the three families. The number of dead oysters was recorded at day 13 (i.e., 8 days after the end of transplantation). Similarly, 14 individuals per family (except 13 for S_{F32} , because DNA extraction failed for one sample) kept in the controlled condition were flash frozen in liquid nitrogen and stored at -80°C .

DNA Extraction, PCR and Sequencing

Frozen oysters were ground in liquid nitrogen in 50 ml stainless steel bowls using 20 mm diameter grinding balls (Retsch MM400 mill). The powders were stored at -80°C , and were then used for DNA extractions using the DNA from tissue Macherey-Nagel kit (reference 740952.250) according to the manufacturer's protocol. In order to improve DNA extractions, we added a crushing step, which consisted in an additional 12 min mechanical lysis

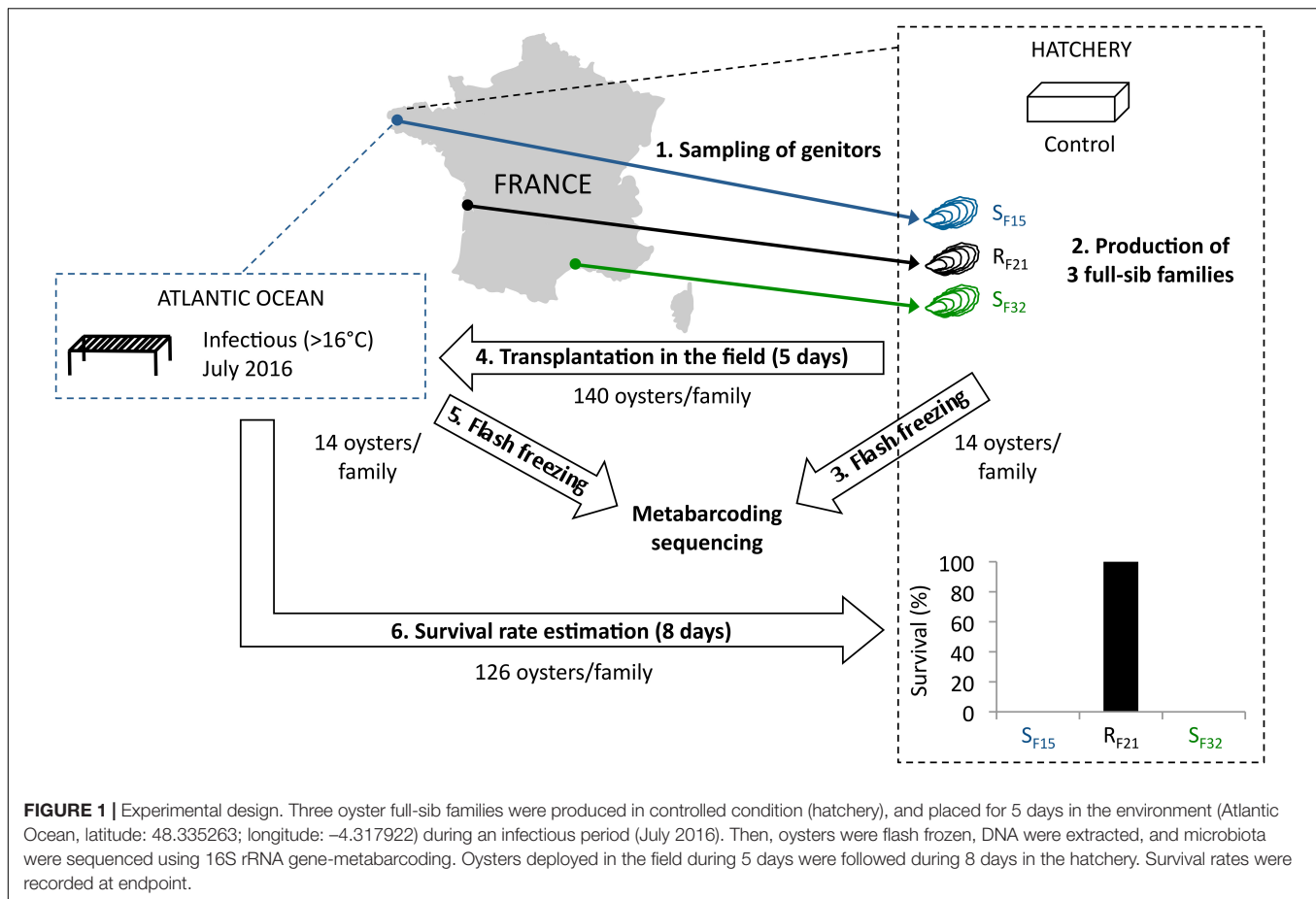


FIGURE 1 | Experimental design. Three oyster full-sib families were produced in controlled condition (hatchery), and placed for 5 days in the environment (Atlantic Ocean, latitude: 48.335263; longitude: -4.317922) during an infectious period (July 2016). Then, oysters were flash frozen, DNA were extracted, and microbiota were sequenced using 16S rRNA gene-metabarcoding. Oysters deployed in the field during 5 days were followed during 8 days in the hatchery. Survival rates were recorded at endpoint.

using zirconium beads before the 90 min enzymatic lysis in the presence of proteinase K. DNA concentration and quality were checked with Epoch microplate spectrophotometer (BioTek Instruments, Inc).

Then, the 16S rRNA gene of bacterial communities was amplified and sequenced using the variable V3V4 loops (341F: 5'-CCTACGGGNGGCWGCAG-3'; 805R: 5'-GACTACHVGGGTATCTAATCC-3') (Klindworth et al., 2013). Paired-end sequencing (250 bp read length) was performed at the McGill University (Génome Québec Innovation Centre, Montréal, Canada) on the MiSeq system (Illumina) using the v2 chemistry according to the manufacturer's protocol. Raw sequence data are available in the SRA database (BioProject ID PRJNA419907).

Quantification of Bacteria and OsHV-1

Quantification of OsHV-1, total bacteria 16S rDNA, and total *Vibrio* 16S rDNA was performed using quantitative PCR (qPCR). All amplification reactions were analyzed using a Roche LightCycler 480 Real-Time thermocycler (qPHD-Montpellier GenomiX platform, Montpellier University, France). A Labcyte acoustic automated liquid handling platform (ECHO) was used for pipetting into the 384-well plate (Roche). The total qPCR reaction volume was 1.5 μl and consisted of 0.5 μl DNA (40 $\text{ng}\cdot\mu\text{l}^{-1}$) and 1 μl

LightCycler 480 SYBR Green I Master mix (Roche) containing 0.5 μM PCR primer (Eurogentec SA). Virus-specific primer pairs targeted a DNA polymerase catalytic subunit (DP, ORF100, AY509253): 5'-ATTGATGATGTGGATAATCTGTG-3' and 5'-GGTAAATACCATTGGTCTTGTTC-3' (Davison et al., 2005). Total bacteria specific primer pairs were the 341F-805R primers targeting the variable V3V4 loops for bacterial communities (see above). Total *Vibrio* specific primer pairs were 5'-GGCGTAAAGCGCATGCAGGT-3' and 5'-GAAATTCTACCCCCCTCTACAG-3' (Mansergh and Zehr, 2014). qPCR reactions were performed with the following program: 95°C for 10 min, followed by 40 cycles of denaturation (95°C, 10 s), hybridization (60°C, 20 s) and elongation (72°C, 25 s) (de Lorgeril et al., 2018). After these PCR cycles a melting temperature curve of the amplicon was generated to verify the specificity of the amplification. Absolute quantification of OsHV-1 copies was calculated by comparing the observed C_q values to a standard curve generated from the DNA polymerase catalytic subunit amplification product cloned into the pCR4-TOPO vector. For total bacteria and total *Vibrio* 16S rDNA, we used the relative quantification calculated by the $2^{-\Delta\Delta C_q}$ method (Pfaffl, 2001) with the mean of the measured threshold cycle values of two reference genes: *Cg-BPI* (GenBank: AY165040, *Cg-BPI-F*: 5'-ACGGTACAGAACGGATCTACG-3'; *Cg-BPI-R*: 5'-AATCGTG GCTGACATCGTAGC-3'), and *Cg-actin* (GenBank: AF026063,

Cg-actin-F: 5'-TCATTGCTCCACCTGAGAGG-3'; Cg-actin-R: 5'-AGCATTTTCCTGTGGACAATGG-3').

Sequence Analyses

The FROGS pipeline (Find Rapidly OTU with Galaxy Solution) implemented into a galaxy instance was used to define Operational Taxonomic Units (OTU), and computed taxonomic affiliations (Escudié et al., 2017). Briefly, paired reads were merged using FLASH (Magoc and Salzberg, 2011). After denoising and primer/adapters removal with cutadapt (Martin, 2011), *de novo* clustering was performed using SWARM that uses a local clustering threshold, with aggregation distance $d = 3$ (Mahé et al., 2015). Chimera were removed using VSEARCH (*de novo* chimera detection) (Rognes et al., 2016). Particularly, this method divided each sequence into four fragments, and then looked for similarity with putative parents in the whole set of OTUs. We filtered the dataset for singletons and we annotated OTUs using Blast + against the Silva database (release 123, September 2015) to produce an OTU and affiliation table in standard BIOM format. Rarefaction curves of species richness were produced using the {phyloseq} R package, and the ggrare function (McMurdie and Holmes, 2013). In order to compare samples for alpha and beta diversity, we used the rarefy_even_depth function to subsample dataset to 5148 reads per sample excluding chloroplasts. The alpha diversity metrics (Chao1 and Shannon) were estimated at the OTU level with the estimate_richness function. Moreover, Pielou's measure of species evenness was computed using the diversity function in {vegan}. We also used phyloseq to obtain abundances at different taxonomic ranks (for genus and family) (tax_glom function). Because agglomerate of multi-affiliation and unknown taxa does not make sense at higher taxonomic ranks, we only kept taxa having a true annotation for each corresponding taxonomic rank. We computed Bray–Curtis dissimilarities to study beta diversity, i.e., distances between samples for OTU compositions (vegdist function, {vegan}).

Statistical and Multivariate Analyses

All statistical analyses were done using R v3.3.1 (R Development Core Team, 2008).

Principal coordinate analyses (pcoa, {vegan}) were computed to describe compositions of microbial communities between samples using Bray–Curtis dissimilarities (vegdist, {vegan}). Multivariate homogeneity of group dispersions was tested between microbial assemblages of resistant and susceptible oyster families using 999 permutations (permutest, {vegan}). We used DESeq2 (Love et al., 2014) (DESeq {DESeq2}) to identify OTUs having differential abundances between resistant and susceptible oyster families. Heatmaps of significant bacterial genera and families were computed using relative abundances and the heatmap.2 function ({gplots}).

We performed one-way ANOVA or non-parametric Kruskal–Wallis tests [when normality of residuals was rejected (Shapiro test)] to compare alpha diversity metrics of microbiota. When ANOVA or Kruskal–Wallis tests were significant, we then computed pairwise comparisons between group

levels (*post hoc* analyses) using pairwise-*t*-tests or Dunn tests, respectively.

For all analyses, the threshold significance level was set at 0.05. *P*-values were corrected for multiple comparisons using Benjamini and Hochberg's method (Benjamini and Hochberg, 1995) (p.adjust, {stats}).

RESULTS

Survival Rates and Disease Development

Full-sib progenies from one resistant (R_{F21}) and two susceptible (S_{F15} and S_{F32}) oyster families were produced and reared in bio-secured conditions (Argenton-sur-Creuse, France). About 140 pathogen-free oysters per family were transferred to the infectious environment for 5 days (Brest, Atlantic Ocean, temperature above 16°C) (Figure 1). 14 oysters per family were then flash frozen for individual bacterial microbiota analyses. The other 126 oysters per family were placed back in hatchery to monitor survival rates. As expected, all oysters from the two susceptible families died, whereas all oysters from the resistant family survived (Figure 1). This observation showed that oysters were contaminated during transplantation, and then developed the disease. Quantification of OsHV-1 and bacteria at day 5 post-transplantation showed that only three oysters from susceptible families displayed moderate to important viral infection, and that only one individual displayed both high viral infection and bacteraemia (SF15.I.R14) (Figure 2). These results suggested that 5 days of transplantation in the field during an infectious period were long enough for oyster contamination, but not for entering the pathogenesis process (except for three oysters).

The whole bacterial communities were sequenced using the 16S rRNA gene from 14 oysters per family and per condition (except 13 oysters for S_{F32} in hatchery) (i.e., 83 oyster-associated microbiota for control and infectious conditions). In average, each sample contained 21,423 sequences representing 1,075 OTUs (Supplementary Figure S1 and Supplementary Tables S1, S2). First, we searched for known opportunistic genera within microbiota of diseased oysters, i.e., already identified when the disease dynamic was described using R_{F21} and another susceptible family (S_{F11}) in the same region (Atlantic Ocean) (de Lorgeril et al., 2018). Nine out of ten previously identified genera were present in our dataset (Figure 3). Most were abundant for the oyster highly infected by OsHV-1 and displaying bacteraemia (SF15.I.R14) (Figures 2, 3A). Both *Vibrio* and *Psychromonas* genera were found within the three diseased oysters (Figure 3A). However, *Vibrio* occurred in the majority of healthy individuals as well (Figure 3B).

Microbiota Assemblages Before Diseased Development

Although 5 days of transplantation were long enough for oyster contamination, only three individuals entered the pathogenesis process as indicated by virus load. Thus, after discarding these three individuals, it was possible to compare microbiota characteristics of the other oysters between resistant and susceptible families before the onset of disease development.

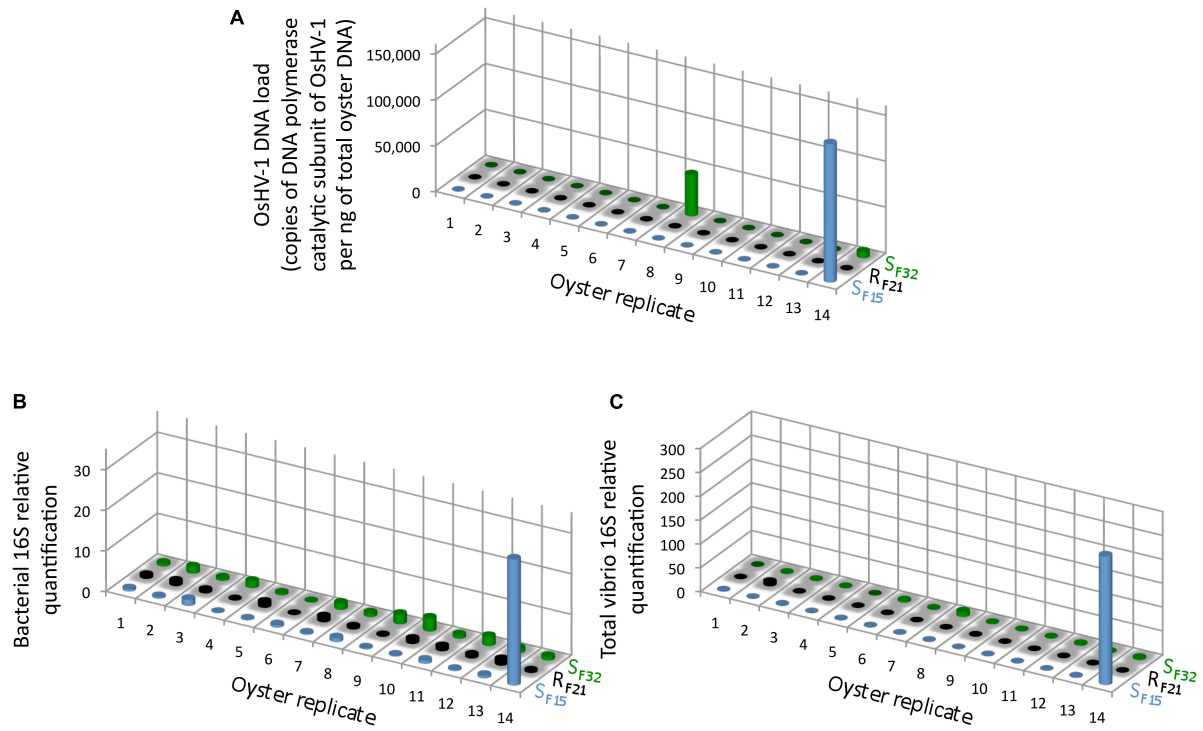


FIGURE 2 | OsHV-1 and bacterial colonization in oysters at day 5 post-transplantation in field condition during an infectious period. **(A)** The OsHV-1 load was quantified by qPCR and expressed as copies of DNA polymerase catalytic subunit of OsHV-1 per ng of total oyster DNA. Relative quantification of total bacteria **(B)**, and total *vibrios* **(C)** abundance were measured by qPCR ($n = 42$).

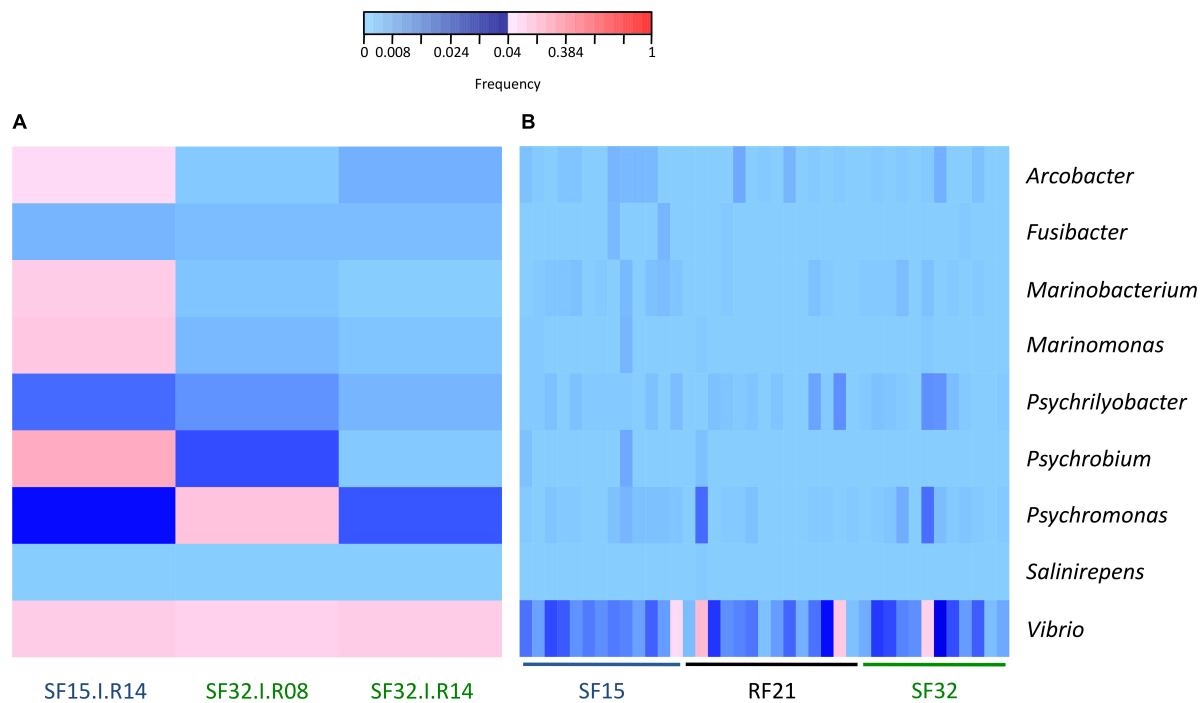
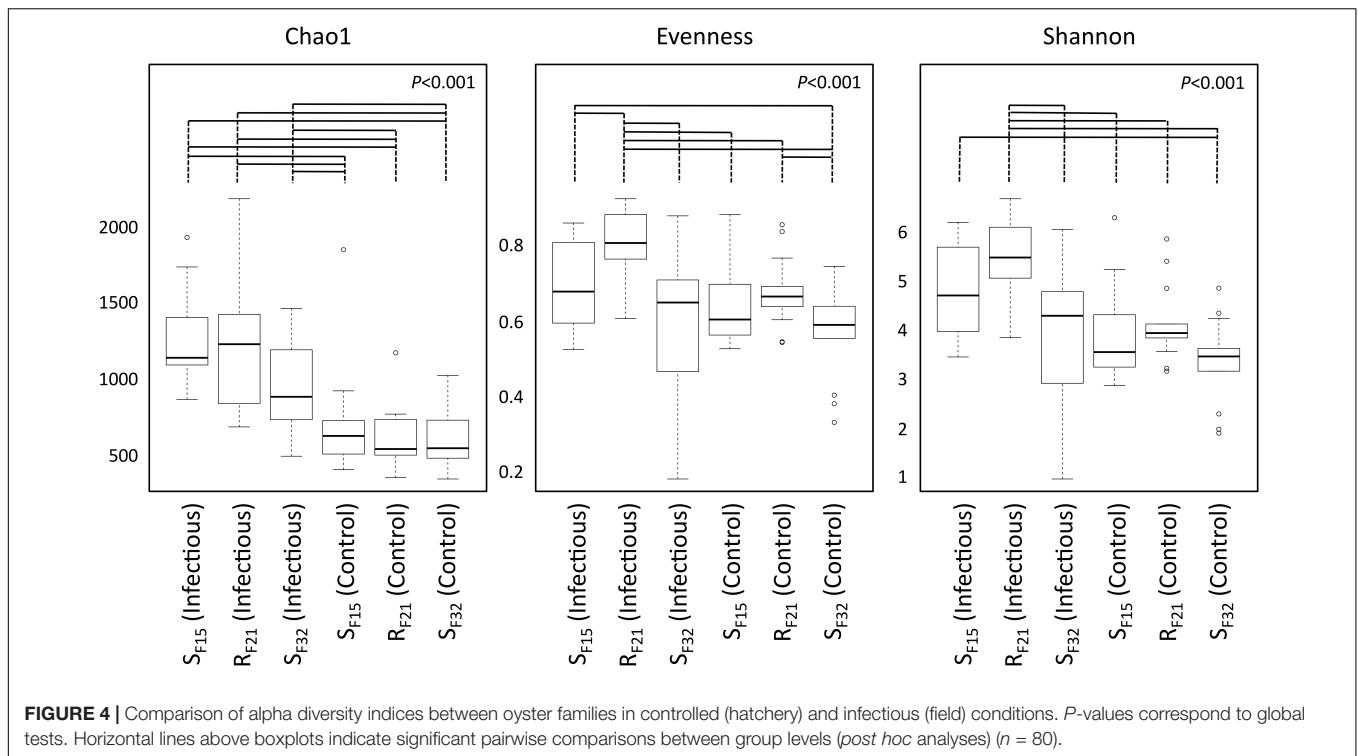


FIGURE 3 | Frequencies of known opportunistic bacterial genera associated with POMS. **(A)** Positive oysters for OsHV-1 detection (SF15.I.R14, SF32.I.R08, and SF32.I.R14). **(B)** Negative oysters for OsHV-1 detection for each family. These opportunistic genera were identified in a previous experimental infection (de Lorgeril et al., 2018). Frequencies above and below 4% are displayed in red and blue, respectively ($n = 42$).



First, we compared alpha diversity indices (Chao1, evenness and Shannon) between oyster families (Figure 4 and Supplementary Table S3). This analysis showed that Chao1 increased for all families between hatchery and the infectious environment. Moreover, evenness and Shannon indices increased only for the resistant family R_{F21} . Notably, R_{F21} had higher evenness than S_{F15} and S_{F32} during the infectious condition. Secondly, we computed a principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarities to describe microbiota compositions (Figure 5A). This ordination highlighted that oyster microbiota changed between hatchery and the infectious environment for the three oyster families. Unexpectedly, microbiota dispersion of R_{F21} was not lower than the susceptible families, and even showed higher dispersions for the infectious condition than in hatchery (Figure 5B).

Altogether, these results suggested that microbiota assemblages highly changed between hatchery and the infectious environment for the three families. Although we did not expect to find higher microbiota dispersion for R_{F21} , our analyses revealed that the resistant family had higher evenness than the susceptible families before the onset of disease development.

Different Bacteria Between Oyster Families in Hatchery and Infectious Environment

Because resistant and susceptible oyster families had different microbiota characteristics (evenness and dispersion) at the early steps of infection, we compared abundances of bacterial taxa within hatchery to identify putative pathogens, opportunists or mutualists that were consistently presents in microbiota

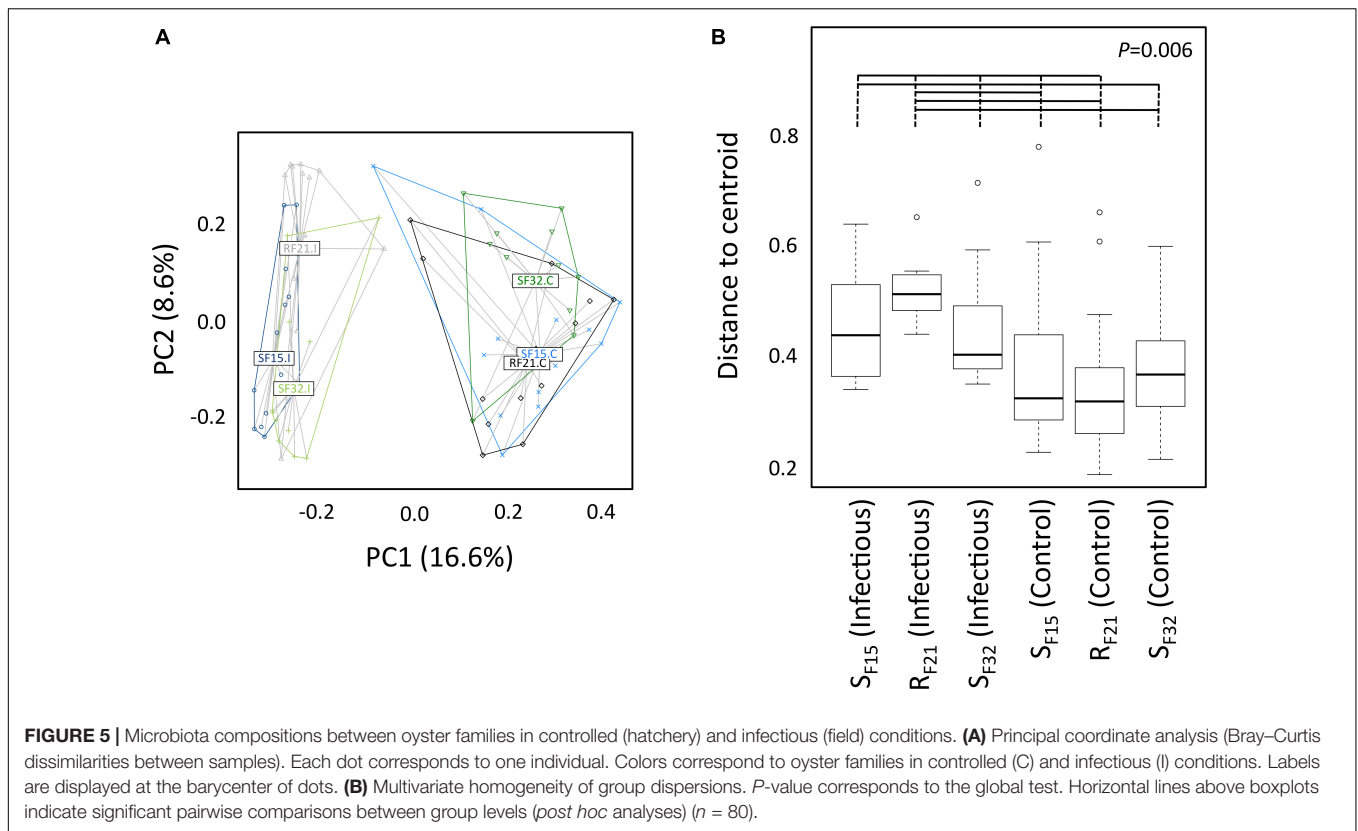
before transplantation. Abundances were compared at two taxonomic ranks, bacterial genera (Supplementary Table S4) and families (Supplementary Table S5). Two genera (*Colwellia* and *Photobacterium*) (Supplementary Figure S2) and four families (Anaplasmataceae, Colwelliaceae, Mycoplasmataceae, and Vibrionaceae) (Figure 6) showed high abundances (>4% in at least one sample) and were significantly different between resistant and susceptible oyster families.

We also compared bacterial taxa before disease development between resistant and susceptible oyster families that were placed in the infectious environment. Except for Mycoplasmataceae, significant bacterial taxa were not similar to hatchery-identified candidates (Figure 7 and Supplementary Figure S3). Four genera (*Phormidium*, *Pseudomonas*, *Thalassospira*, and *Umboniibacter*) (Supplementary Figure S3) and seven families (Cyanobacteria (Subsection I, family I), Cyanobacteria (Subsection III, family I), Mycoplasmataceae, Pseudomonadaceae, Rhodobacteraceae, Rhodospirillaceae, and Spirochaetaceae) (Figure 7) had high abundances (>4% in at least one sample) and were significantly different between resistant and susceptible oyster families.

DISCUSSION

Five Days of Transplantation Allowed Oyster Contamination, but Not Disease Development

Oysters were transplanted for 5 days in the field during an infectious period. According to previous observations



(Dégremont, 2011; Petton et al., 2015), this time period was considered sufficient for oyster contamination. We did not observe dead oysters when they were sampled, but all individuals of susceptible families died 8 days after they were placed back to laboratory tanks. This observation showed that oysters were contaminated during transplantation, and this exposure was sufficient for the further development of the complete pathogenesis process.

High abundances of OsHV-1 and total bacteria were previously observed for another susceptible family (S_{F11}) during disease development (de Lorgeril et al., 2018). In our study, most oysters (except three) did not exhibit high abundances of OsHV-1 or bacteria after 5 days of transplantation. After exclusion of these three oysters, we thus analyzed oyster microbiota before the onset of disease development (i.e., at the early steps of infection).

Microbiota Evenness Was Linked to Oyster Resistance During Transplantation

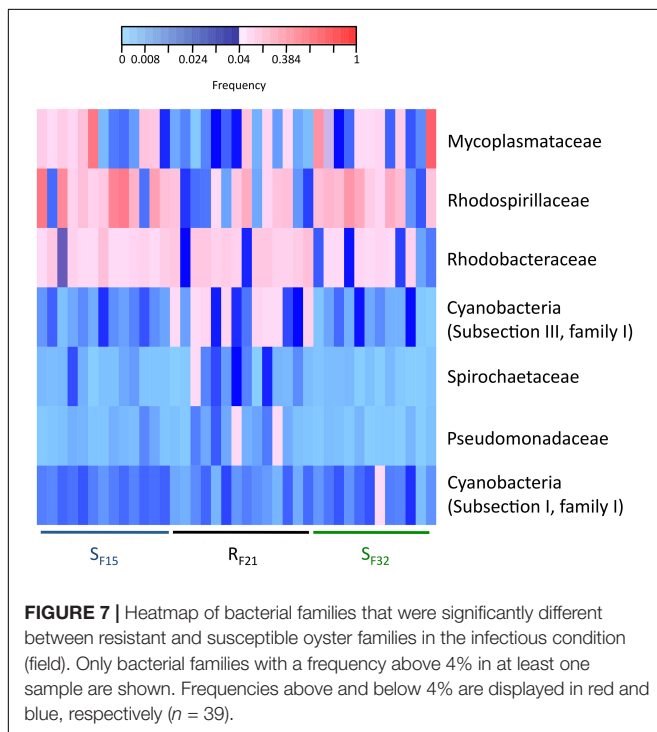
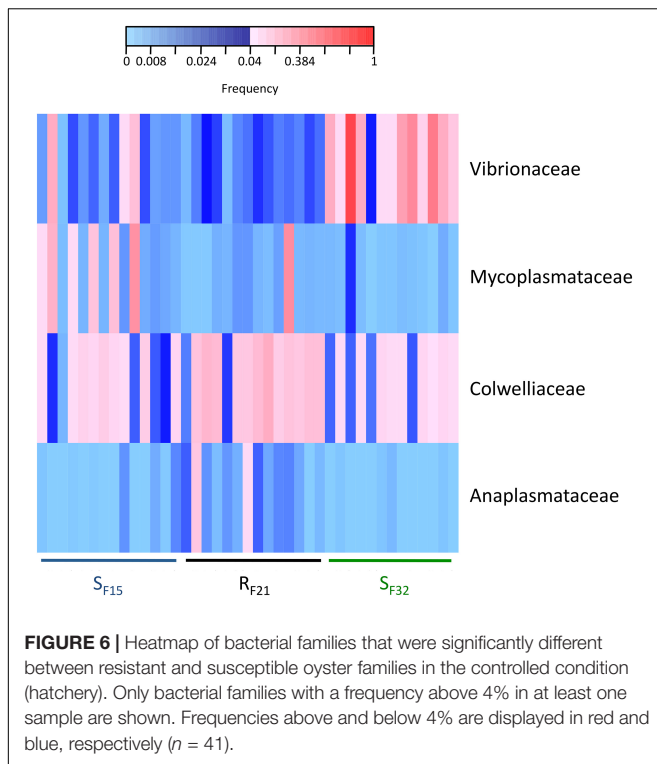
According to the previous holistic study (de Lorgeril et al., 2018), we expected to observe many modifications of microbiota for susceptible oyster families during transplantation, but not or few for the resistant one. In particular, we expected increase of alpha diversity indices (Chao1 and Shannon), and of microbiota dispersion for the susceptible families. Furthermore, we hypothesized that the resistant family might have stable microbiota, because this characteristic was linked to host

homeostasis in many studies (Faith et al., 2013; Rungrasamee et al., 2016; Zaneveld et al., 2017).

Unexpectedly, we found that alpha diversity indices and dispersion of microbiota highly changed for the three oyster families (susceptible as well as resistant) when they were transferred from hatchery to the infectious environment. As a consequence, stable microbiota was not a characteristic of the resistant family, suggesting either a neutral effect or that flexibility of microbiota might also be advantageous in a changing environment, such as proposed for *Nematostella vectensis* (Mortzfeld et al., 2016). Microbiota evenness was the only index that discriminated resistant and susceptible families. It highlighted that OTU abundances within resistant oysters were more equally distributed than within susceptible oysters. This index may possibly have important effects on oyster homeostasis, because it was already found to be positively linked to ecosystem productivity (Wilsey and Potvin, 2000; Zhang et al., 2012), functional stability (Balvanera et al., 2005; Wittebolle et al., 2009), and invasion resistance (Wilsey and Polley, 2002; De Roy et al., 2013).

Putative Opportunistic and/or Pathogenic Bacteria of Susceptible Oysters

Several resistant (e.g., R_{F21}) and susceptible (e.g., S_{F15} and S_{F32}) families were produced previously to describe POMS in the Atlantic Ocean (de Lorgeril et al., 2018). In this previous study,



a holistic approach combining metabarcoding, transcriptomic, qPCR and histology successfully described disease dynamic using a resistant (R_{F21}) and a susceptible (R_{F11}) family. In particular, after viral burst, several bacterial genera significantly contributed to the observed bacteraemia such as *Arcobacter*, *Cryomorphaceae*,

Fusibacter, *Marinobacterium*, *Marinomonas*, *Psychrilyobacter*, *Psychrobium*, *Psychromonas*, *Salinirepens*, and *Vibrio*. In our study, we thus analyzed if these genera were present in oyster microbiota. We particularly evaluated if their abundances were high within the three diseased oysters, but also significantly different between resistant and susceptible families (in hatchery and before the onset of disease development in the field). Most of these genera were identified within the oyster that displayed high abundances of OsHV-1 and bacteraemia (SF15.I.R14). *Psychromonas* occurred within the three diseased oysters, and might possibly be involved early in disease development, even if its pathogenic role was not demonstrated so far.

Furthermore, three bacterial families (Mycoplasmataceae, Rhodospirillaceae, and Vibrionaceae) were linked to susceptible oysters in hatchery and/or before disease development in the field, suggesting putative roles of opportunists or pathogens. Among Vibrionaceae, *Photobacterium* genus was mostly associated with S_{F32} in hatchery, and as well with low disease resistance oysters in a previous study (King et al., 2019). Among Rhodospirillaceae, *Thalassospira* genus was already identified in *C. gigas* oysters (Fernandez-Piquer et al., 2012). Even though Rhodospirillaceae were found in diseased tissues of *Platygyra carnosus* corals (Ng et al., 2015), there is no evidence that they may act as pathogens for oysters until now. Lastly, Mycoplasmataceae family was abundant in *C. virginica* oysters from Hackberry Bay (King et al., 2012), and decreased in oysters during experimental infections (de Lorgeril et al., 2018). However, it was intriguing to find in this study that Mycoplasmataceae abundances were significantly lower for resistant than susceptible oyster families in both hatchery and field conditions, at the early steps of infection. As a consequence, this family might have negative effects on oyster survival, but future studies should experimentally test its role within oyster microbiota.

Putative Beneficial Bacteria of Resistant Oysters

Some observations suggested that bacteria might protect oysters from pathogens. For example, *Aeromonas media* A199, *Phaeobacter gallaeciensis*, and *Pseudoalteromonas* sp. D41 improved survival of *C. gigas* against *Vibrio* strains when they were tested as probiotics (Gibson et al., 1998; Kesarcodi-Watson et al., 2012). In particular, the *Pseudoalteromonas* genus is known to produce a wide variety of biologically active secondary metabolites (Kalinovskaya et al., 2004; Bowman, 2007). Finding beneficial bacteria could be interesting and pave the way for prophylactic measures in oyster farming.

Here we identified three bacterial families significantly associated with resistant oysters: Colwelliaceae, Cyanobacteria (Subsection III, family I), and Rhodobacteraceae. Among Colwelliaceae, the *Colwellia* genus was already found within *C. gigas* microbiota (Madigan et al., 2014). In this study, this family and genus were significantly different between resistant and susceptible oysters in hatchery. However, some susceptible oysters also contained high abundances of these bacteria, and they were not significantly different between resistant and susceptible oyster families during transplantation.

Altogether, these observations thus suggested a limited role of Colwelliaceae in POMS. Moreover, although Rhodobacteraceae was associated to juvenile oyster disease (Boettcher, 2005), this bacterial family was commonly associated with oysters (Asmani et al., 2016; Takenaka et al., 2017; Arahall et al., 2018). Strikingly, Cyanobacteria (Subsection III, family I) had low abundances in all susceptible oyster microbiota before disease development, and were abundant in most resistant oysters. Although cyanobacteria had a negative effect on other marine invertebrates such as scleractinian corals (Meyer et al., 2016), they were already observed at high relative abundances within oyster microbiota (Chauhan et al., 2014). Particularly, they were abundant in the digestive gland, connective tissue, mantle, and gonad of oysters (Avila-Poveda et al., 2014). Because cyanobacteria persisted in oyster tissues without signs of alterations, a possible endosymbiotic relationship was even proposed (Avila-Poveda et al., 2014). Notably, cyanobacteria were also negatively correlated to culturable *Vibrio* abundance in free-living microbial communities (Turner et al., 2009). Hence, cyanobacteria might play a role of barrier against pathogens from the genus *Vibrio*. In particular, photosynthetic activity of cyanobacteria could inhibited pathogen growth through the production of reactive oxygen species, such as observed for *Plasmodium* infection in *Anopheles gambiae* (Cirimotich et al., 2011).

To conclude, we placed oysters from resistant and susceptible families in field condition for 5 days during a period in favor of POMS. The analyses of oyster microbiota at the early steps of infection revealed differences between resistant and susceptible oyster families. These differences suggested that both the structure (evenness) and the composition (putative opportunists, pathogens, mutualists) might predict oyster mortalities. Future studies should test the role of putative pathogens (Mycoplasmataceae and Rhodospirillaceae), but also of putative symbionts, such as Cyanobacteria (Subsection III, family I) and Rhodobacteraceae within *C. gigas* microbiota. In particular, these studies should evaluate the possibility of using Cyanobacteria (Subsection III, family I) as probiotics for oyster farming.

DATA AVAILABILITY STATEMENT

The datasets generated during the current study are available in the Sequence Read Archive repository under BioProject ID PRJNA419907.

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AUTHOR CONTRIBUTIONS

CC, JL, J-ME, YG, LD, GM, and ET were involved in the study concept and design. BP was involved in the generation and maintaining of all animals used in this study. JL, BP, J-ME, YG, GM, and ET were involved in the collection of samples. CC, JL, AL, and ET were involved in data acquisition and analysis. CC and ET drafted the manuscript. All authors contributed to critical revisions and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2020.00311/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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