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Impact of epizootics on mussel farms: Insights into microbiota composition of *Mytilus* species

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Abstract :

Outbreaks of marine mussel mortality on French farms could have different aetiologies. One of them implies Vibrio splendidus strains. Beyond the involvement of this pathogen, there is considerable evidence that diseases often result from interactions between several microbes and the host. In this study, we explored the bacterial communities associated with mussel species and the surrounding water collected from a mussel farm affected by mortalities. The microbiota of Mytilus edulis, Mytilus galloprovincialis and their hybrids displayed an abnormal abundance of Proteobacteria, in particular the genera Vibrio, Cobetia and Arcobacter. Despite the dysbiosis, the Mediterranean mussel showed a different microbiota profile with a higher richness and presence of the phylum Bacteroidetes. Bipartite network analyses at the level of bacteria families confirmed this finding and showed that the microbiomes of M. edulis and the hybrids tended to cluster together. In addition, injection of mussels with the virulent V. splendidus induced less mortality rate in M. galloprovincialis compared to the other Mytilus sp. suggesting a better resistance of the Mediterranean mussel to infection.

Our findings point to a probable aetiology of pathobiome-mediated disease in mussels. To fully understand this phenomenon, more knowledge is needed on the roles of pathobiotic systems and their development during disease establishment.

Keywords : Pathobiome, mussel mortalities, opportunistic bacteria, core microbiome

1. Introduction

Over the last two decades, European mussel production has been declining. Among the putative causes of this trend, the spread of diseases, algal blooms, lack of spat, predation and low profitability have been mentioned (Avdelas et al., 2021). Indeed, since 2014, mass mortality events have been recorded in blue mussel stocks in France (Bechemin et al., 2014) and since 2016 in the Netherlands (Capelle et al., 2021). While no cause could explain this phenomena in the Netherlands (Capelle et al., 2021), the involvement of pathogens in mortalities has been demonstrated in French mussel farms (Ben Cheikh et al., 2017, 2016; Benabdelmouna et al., 2018; Oden et al., 2016).

As filter-feeding organisms, marine mussels are constantly exposed to a variety of microorganisms including pathogenic bacteria that can threaten their lives. In addition to external microbes, like all macroorganisms, mussels interact with the microorganisms they harbour, i.e. their microbiota. This dialogue between the host and its microbiota can help maintain their respective integrity, as shown e.g. in leeches (Kikuchi and Graf, 2007; Tasiemski et al., 2015). In fact, advances in analytical techniques over the last few decades have favoured the expansion of studies on microbial communities, particularly in the human model,

highlighting their importance for their host (Amato, 2016; Bäckhed et al., 2005; Martin et al., 2009; Mushegian and Ebert, 2016). Considered the "last organ" in humans (Baquero and Nombela, 2012), a large body of evidence has revealed the beneficial contribution of the microbiota to basic biological processes such as digestive, metabolic and immune functions (Callens et al., 2016; Sirisinha, 2016; Wang et al., 2017) and its involvement in the eco-evolutionary dynamics of their host (Amato, 2016; Kohl et al., 2018; Macke et al., 2017; Moeller et al., 2016; Murall et al., 2017). However, like other ecosystems, the composition of the microbiota can be modulated by several factors such as antibiotics and pathogens leading to an altered community structure or dysbiosis (Clark et al., 2015; Costello et al., 2012; Lamont and Hajishengallis, 2015; Martin et al., 2009). In case of dysbiosis, the mutual harmony between bacteria is disrupted and the healthy balanced microbiota turns into a disease-inducing pathobiota (Pitlik and Koren, 2017; Tungland, 2018; Wang et al., 2017).

The large number of findings in the human model has sparked research interest in other organisms including bivalves. Numerous studies have investigated microbiota structure in oysters, mussels and to a lesser extent clams, under the influence of factors such as host genotype, environment and pathogens (for review see Paillard et al., 2022). The structure of the microbiota can be divided into at least two types of communities, namely a stable community of resident bacteria within a host population ("the core microbiome") and a variable microbial community that depends on the surrounding environment (Santibáñez et al., 2022). Data on the role of the core microbiome are limited, although some authors suggest a contribution to essential host biological functions (Lemanceau et al., 2017). Furthermore, the balance between the core and variable microbiome is still unclear, especially as the dynamics of host-associated communities are directly affected by abiotic and biotic factors. For example, elevated water temperature appears to be a major determinant and strongly influences the composition of the microbiota in mussels and oysters by also favouring pathogens such as *Vibrio* sp. (Li et al.,

2019, 2018; Lokmer and Wegner, 2015). In addition, water pollution by nanoparticles, nanoplastics or chemicals can affect the diversity of the microbiota as has been demonstrated in *Mytilus galloprovincialis* (Auguste et al., 2020, 2019; Balbi et al., 2020). Besides abiotic factors, microbiota imbalance has been linked to diseases (King et al., 2019). Indeed, infection of *Crassostrea gigas* by OsHV-1 virus induced dysbiosis with subsequent bacteraemia by opportunistic bacteria (Lorgeril et al., 2018). Similarly, field studies on oysters affected by mortalities have shown signs of microbiota disruption (Lasa et al., 2019).

For mussels, only a few studies have explored microbiota dynamics in interaction with pathogens. Li et al. (2019) reported the reduction in microbial diversity of *M. coruscus* haemolymph after exposure to *Vibrio cyclitrophicus*. Likewise, we recently demonstrated that experimental infection with *V. splendidus* reduces the diversity of the *M. edulis* microbiota and favours pathobiontic bacteria (Ben Cheikh and Travers, 2022). However, the response of the mussel microbiota during disease outbreaks remains unknown.

The aim of this study is to provide new insights into the microbiota of mussels experiencing mortality outbreaks on French farms. For this purpose, the bacterial communities associated with *M. edulis*, *M. galloprovincialis* and their hybrids, grown in the same environment, and the surrounding water were analysed. The differences between the microbial communities found in the different species were assessed using ecological network methods to cluster microbiota and link the obtained classification to the type of sampled mussels. The susceptibility of the different types of mussels to infection was then explored by experimental infection with *V. splendidus* strains.

2. Material and methods

2.1. Mussel collection

45 farmed adults of *Mytilus* sp. (*M. edulis*, *M. galloprovincialis* and hybrid *M. edulis* x *galloprovincialis*, 15 individuals from each taxon, size between 4 and 5 cm) were collected from a mussel farm affected by massive mortalities (Bay of Lannion, France 48°44 44.8 N, 3°35 24.9 W) in September 2018 (mean temperature = 17°C). The animals were immediately transported to the laboratory and randomly dispatched in different temperature-controlled (16°C) aerated water tanks filled with seawater sampled from the corresponding site (salinity = 35 PSU). Mussels were taken at random from two different tanks for microbiota analysis or for experimental infection. Seawater from the same site was also sampled for microbiota analyses.

2.2. Microbiota analysis

After cleaning, mussels were removed from their shells, flash frozen in liquid nitrogen and stored at -80°C (15 individuals/species). Serial filtrations of 5L of seawater were performed using 8 µm and 5 µm porosity (WhatmanTM NucleoporeTM Polycarbonate membrane filter, 47 mm diameter, UK). The resulting filtered seawater was centrifuged for 15 min at 11000 g. The membranes and bacterial pellets were stored at -80°C.

DNA extraction, amplicon library construction and Illumina MiSeq sequencing were performed by Biofidal (Vaulx-en-Velin, France). DNA was extracted from frozen mussels ground in liquid nitrogen and from bacteria pellet using Mag Bind® Universal Pathogen DNA Kit (Omega BIO TEK, Georgia, USA) and from the different filters using Quick DNA/RNA MagBead kit (Zymo Research, California, USA) according to the manufacturer's protocol. DNA concentration and quality were checked with the QuantiFluor® dsDNA System (Promega, France). The 16S rRNA gene of bacterial communities was amplified using the 5X HOT BIOAmp ® BlendMaster Mix (Biofidal) and the 341F: CCTACGGGNGGCWGCAG (17 pb) and 805R:

GACTACHVGGGTATCTAATCC (21 pb) primers (designed by Biofidal) targeting the variable V3-V4 loops (Klindworth et al., 2013). After purification of the PCR products by SPRIselect reagent kit (Beckman Coulter), a second amplification was performed using index adapter oligos (P5/P7). The purified PCR products were normalized to obtain an equimolar library and subsequently pooled. Negative controls and ZymoBIOMICS microbial community standards were included during the library preparation (Supplemental Figure S1).Amplicons were sequenced on the MiSeq platform with V3 reagents producing 2 X 300 bp paired-end reads.

After demultiplexing, primer sequence adapters were trimmed from the reads using cutadapt software version 1.12 (Martin, 2011). Paired-end sequence reads were collapsed into so-called pseudoreads using sequence overlap with USEARCH version 9.2 (Edgar, 2010). After chimera and singletons removing, filtered pseudoreads were aligned to the RDP database version 11.5 (Cole et al., 2014) with Snap-aligner version 1.0 beta.23 (Zaharia et al., 2011) to identify the taxonomy. Finally, USEARCH was used to create the OTU-table and to calculate alpha diversity. Principal coordinate analyses (PCoA) were performed via xlstat using the Bray–Curtis distance matrix. All FastQ files were deposited in SRA-NCBI under the project accession number PRJNA923705.

2.3. Host-microbiota network

To analyse the structure of the host-microbiota association dataset, we used methods borrowed from the analysis of bipartite ecological networks. First, we aggregated data on microbial taxa at the family level and transformed the microbial family abundance table into presence/absence data (i.e., all abundances > 0 were set to 1). We then modelled the obtained bipartite network of hosts by microbial families using a latent block model (LBM) (Govaert and Nadif, 2008; Leger et al., 2015). The clustering obtained with LBMs is indeed more useful than the one obtained with modularity-search algorithms (Newman, 2006; Traag et al., 2019) as blocks can

identify densely connected as well as sparsely connected regions of the network. We tested the correlation between the obtained clustering of hosts and their classification among species using a method described in Massol et al. (2021): we computed the normalised mutual information (NMI) index between the two classifications of the hosts (by host type and by LBM block); we tested the significance of NMI using randomizations of the bipartite network through the configuration model (Strona et al., 2014).

2.4. Experimental infection

Two strains of *V. splendidus*, the virulent 10/068 1T1 and the non-virulent 12/056 M24 T1 were used for experimental infection by injection as described in previous studies (Ben Cheikh et al., 2017, 2016). Briefly, bacteria were grown overnight in Luria Bertani NaCl 20 $g.L^{-1}$ at 22°C with constant agitation (80 rpm) and resuspended in filtered sterile seawater (FSSW, OD_{600nm} = 1, 2.10^8 CFU mL^{-1}) after double washing (FSWW) /centrifugation (1200 g for 10 min). Anesthetized mussels (2–3 h in magnesium chloride solution at 50 $g.L^{-1}$, 1/4: v/v seawater/freshwater) were intramuscularly injected with 100 µL of bacterial suspension or FSSW for negative controls. The animals were transferred to tanks (3 replicate tanks, 10 mussels per tank) filled with 2 L of UV-treated and filtered seawater supplemented with 50 mL of phytoplankton (*Isochrysis galbana*) and maintained under static conditions at 16°C with aeration. Mortality was monitored over 8 days with removing of newly dead mussels from the tanks.

2.5. Statistical analyses

SigmaPlot 12 (Systat Software Inc., Chicago, IL) and R version 4.0.5 (R Core Team, 2021) were used for statistical analysis. A comparison of OTUs between mussel types and/or seawater was performed. Values were tested for normality (Shapiro-Wilk test) then a one-way ANOVA was performed. Pairwise comparisons between groups were assessed by post-hoc analyses (with Holm-Sidak correction). Statistical significance was accepted for $p < 0.05$.

3. Results

3.1. Microbial structure in mussels and seawater

The 48 samples (15 *M. edulis*, 15 *M. galloprovincialis*, 15 hybrid and 3 seawater samples) sequenced using the 16S rRNA gene generated 4 842 875 raw reads ranging from 70 302 to 126 377 reads per sample with an average of 100 893 reads (Supplemental Table S1). After Chimera filtering, unique pseudoreads averaged 38 597 reads.

The clustering of reads in OTUs revealed a higher richness in the Mediterranean mussel *M. galloprovincialis* (significant difference with *M. edulis*, ANOVA $p < 0.05$) and in seawater with a mean absolute abundance of 80 486 ± 3 214 OTUs and 80 367 ± 808 OTUs respectively. The blue mussel *M. edulis* and the hybrids had a mean absolute abundance of 68 068 ± 2 322 and 70 637 ± 2 783 OTUs respectively (Figure 1 A). Alpha diversity metrics were higher in *M. galloprovincialis* compared to the other two mussel species and seawater (non-significant data, supplemental Figure S2A). Beta diversity analysis revealed dissimilarities between mussels and seawater (Supplemental Figure S2B). The compositional structure at the phylum level indicated a similarity between *M. edulis* and the hybrids (Figure 1 B) with a predominance of Proteobacteria (relative abundance r.a. of 92.3% to 99.4% in *M. edulis* and 89.9% to 99.9% in hybrids) and the presence of Bacteroidetes (r.a. from 0.3% to 5.5% in *M. edulis* and from 0 to 5.4% in hybrids). Other rare phyla with an abundance > 1 were detected in some hybrids, e.g., Firmicutes (replicates 2, 6 and 7) and Tenericutes (replicates 1 and 13). Mediterranean mussels harboured a lower proportion of Proteobacteria (r.a. from 47.3% to 99.6%) and a higher proportion of Bacteroidetes (r.a. from 0 to 23.5%). OTUs belonging to Tenericutes, Verrucomicrobia, Fusobacteria and Cyanobacteria were also identified in some mussels with r.a. > 1. The composition of the surrounding seawater was similar to that of the mussels with a notable r.a. of Bacteroidetes (mean 21.1% ± 6.4%) and the predominance of Proteobacteria (mean 77.4% ± 6.9%).

A comparative analysis of the most abundant families (Top 30) and genera revealed a more specific layout for each group although a similar bacterial community was detected in most samples (Figure 2, Supplemental Table S2). Overall, Vibrionaceae, Halomonadaceae, Pseudoalteromonadaceae and Campylobacteraceae were the dominant families in the blue mussel and hybrids (mean r.a. 5-35%) with notably the genera *Vibrio, Cobetia, Pseudoalteromonas* and *Arcobacter* but also unclassified Epsilonproteobacteria. Similarly, the Mediterranean mussel harboured Vibrionaceae (*Vibrio*), Pseudoalteromonadaceae (*Pseudoalteromonas*) and unclassified Epsilonproteobacteria but also a higher proportion of Campylobacteraceae (*Arcobacter*) and Flavobacteriaceae (especially *Tenacibaculum* and *Flavobacterium*) (mean r.a. 18.2% and 6.8% respectively). In contrast, in addition to unclassified Gammaproteobacteria, the prevailing taxa (r.a. up to 5%) in seawater samples were the families Rhodobacteraceae, Oceanospirillaceae, Pseudoalteromonadaceae, Crocinitomicaceae and Flavobacteriaceae and the genera *Sulfitobacter*, *Oceanospirillum*, *Pseudoalteromonas*. The majority of *Vibrio* identified in mussels have been affiliated to the *V. Splendidus* group (Supplemental Table S3).

3.2. Association between microbial family structure and mussel taxa

The LBM inferred 5 clusters of hosts and 4 clusters of bacterial families (Figure 3A). Assignment of host individuals to clusters resulted in probabilities of membership at least as high as 88.3%; by contrast, a few bacterial families were only assigned to a particular cluster with a probability of 50.8% (Supplemental Figure S3). The first cluster of hosts consisted in 2 samples, one of *M. galloprovincialis* and one hybrid, which harboured high numbers of bacterial families (223 families on average). The second cluster of hosts consisted in 20 samples (1 hybrid, 7 *M. edulis* and 12 *M. galloprovincialis*) with an average of 141.9 bacterial families per sample. The third cluster included only 4 samples (one replicate of *M. galloprovincialis* together with the three seawater samples) with an average of 125.5 families per sample. The

fourth cluster of hosts consisted in 16 samples (7 *M. edulis*, 1 *M. galloprovincialis*, 8 hybrids) with an average of 109 families per sample. The fifth cluster of hosts consisted in 6 samples (1 *M. edulis*, 5 hybrids) with an average of 69 families per sample. The four clusters of bacterial families harboured 56, 57, 89 and 176 families, respectively, and were found on average in 45.46, 30.44, 13.44 and 2.63 host samples respectively. Probabilities of host-bacterial family interactions can be found in Supplemental Table S4.

The classification of the hosts (48 samples) according to their LBM cluster was significantly congruent with their classification based on sample type (mussel taxon or seawater sample, i.e., 4 groups) according to the NMI (NMI = 0.381, p value < 0.0001 when tested against 10,000 randomizations of the bipartite network). Indeed, a graphical representation of the two classifications evince that *M. galloprovincialis* projects almost completely onto LBM cluster 2 while LBM clusters 4 and 5 project mostly onto *M. edulis* and hybrids (Figure 3B). Seawater samples coincide almost perfectly with LBM cluster 3.

3.3. Susceptibility of mussels to pathogens

To assess the susceptibility of mussel populations to bacterial infection, *M. edulis*, *M. galloprovincialis* and hybrids were injected with two strains of *V. splendidus* of contrasting virulence. Exposure of mussels to avirulent strain 12/056 M24 T1 induced limited mortalities in all mussel groups, ranging from 3.3% ± 3.3% in hybrids to 13.3% ± 3.3% in *M. edulis* at day 8 (Figure 4). In contrast, injection of the virulent strain 10/068 1T1 induced higher mortalities with different rates in the mussel species. The first mortalities appeared 24h post-injection and increased progressively until stabilization on day 8. Hybrids and *M. edulis* had different patterns at the beginning of the infection (16.6% ± 12% for *M. edulis* and 46.6% ± 6.6% for hybrids on day 1) but reached 76.6% of mortality at the end of the experiment while *M. galloprovincialis* had lower mortalities (23.3% ± 3.3% on day 1 and 46.6% ± 12% on day 8). No or negligible mortality was observed in controls.

4. Discussion

Since 2014, mass mortalities have been reported in French mussel farms. While *M. edulis, M. galloprovincialis* and their hybrids represent the main farmed species, the epizootics seem to mostly affect the blue mussel (Bechemin et al., 2014). In general, investigations of mussel disease have so far been limited to the detection and isolation of known pathogens from moribund animals such as *V. splendidus* (Oden et al., 2016), combined with experimental infection in the laboratory (Ben Cheikh et al., 2017, 2016). In this study, we provide an overview of the bacterial communities associated with three mussel species and the surrounding water sampled from a farm affected by mortality epidemics.

4.1. The microbiota of the three *Mytilus* sp. is dysbiotic

According to previous studies, the microbiota of healthy marine bivalves, and mussels in particular is characterized by the presence of different phyla with a predominance of Proteobacteria but also other bacteria belonging to Cyanobacteria, Firmicutes, Planctomycetes, Bacteroidetes, Actinobacteria, Verrucomicrobia, Tenericutes and Chloroflexi (Ben Cheikh and Travers, 2022; Musella et al., 2020). Here, the analysis of whole animal microbiota showed a severe dysbiosis marked by an abnormal dominance of Proteobacteria (Fig. 1, mean r.a. ranging from 86.5% to 96.1%). Microbiota imbalance has been linked to mortality events and infectious diseases in bivalves. Field analysis of the Pacific oyster *C. gigas* showed that animals experiencing mortality outbreaks at different life stages displayed signs of dysbiosis resulting in a change in the diversity and composition of the microbiota (Lasa et al., 2019). Similarly, a study on Unionida freshwater mussel during mass mortality events revealed bacterial invasion and shifts in the bacterial microbiome (Richard et al., 2021). For marine mussels, there are no data available to our knowledge on animal microbiota during mortality events. However, we have recently reported a disruption of the blue mussel microbiota during experimental infection with *V. splendidus* and demonstrated the implication of some opportunistic pathobiontic

bacteria in the infection (Ben Cheikh and Travers, 2022). Thus, even if the causal origin of mussel mortalities in France still not clear, we can establish a link with pathogenic bacteria, especially as they are detected in *Mytilus* tissues. Our analysis highlighted the genera *Vibrio*, *Cobetia* and *Arcobacter* as the predominant bacteria in mussel species (Fig. 2). The abundance of *Vibrio* sp. in the microbiota of mussels is not surprising as these genera are naturally occurring in marine environments and certain strains are often associated with outbreaks of mortality marine bivalves (Travers et al., 2015). In our study, we mainly detected strains belonging to the *V. Splendidus* clade which includes pathogens of bivalves such as *V. splendidus*, *V. tasmaniensis* and *V. crassostreae* (Ben Cheikh et al., 2016; Bruto et al., 2017; Duperthuy et al., 2011) but also less studied strains showing virulence for some marine organisms (e.g. *V. toranzoniae* and *V. kanaloae*) and environmental species without pathogenic potential (e.g. *V. gigantis* and *V. atlanticus*) (Lasa et al., 2017; Romalde et al., 2014). In accordance with our findings, different studies have reported the presence of *V. splendidus*-related strains in different French mussel farms affected by mortalities (Bechemin et al., 2014; Charles et al., 2020a; Oden et al., 2016). However, these studies are restrictive as they are based on the isolation of culturable bacteria of interest and are therefore not representative of the animal's microflora. In addition to *V. splendidus* strains, *Francisella halioticida* has been recently described as potentially involved in blue mussel mortality (Cano et al., 2022; Charles et al., 2020b). The implication *F. halioticida* in mussel disease is not confirmed in our study since this strain was detected at very low proportions in some individuals of blue mussels and hybrids (data not shown). In contrast, metagenomic analyses showed the prevalence of bacteria of the genus *Cobetia* and *Arcobacter*. While knowledge of *Cobetia* strains is limited, several reports described the dominance of *Arcobacter* sp. in oysters and mussels in a context of disease suggesting them as opportunistic bacteria (Ben Cheikh and Travers, 2022; Li et al., 2019; Lokmer and Wegner, 2015; Lorgeril et al., 2018). Nevertheless, the potential role of these

bacteria needs further investigation, as some species (e.g. *A. cryaerophilus, A. ellisii, A. nitrofigilis*) have virulence genes and the ability to colonise and enter human cells (Ferreira et al., 2016; Levican et al., 2013).

4.2. Microbiota of *M. galloprovincialis* differs from *M. edulis* and hybrids

The analysis of microbiomes using a latent block model identified four clusters of bacteria families and five clusters of host individuals (Fig. 3A). Since this clustering proved to be congruent with the one obtained from host types, with a significantly high NMI, this suggests that microbiota are different between mussel species. Indeed, host clusters 4 and 5 belong almost exclusively to *M. edulis* and hybrids, while *M. galloprovincialis* individuals are almost all found in host cluster 2. The differences in bacteria family richness between clusters thus confirm that *M. galloprovincialis* is associated to a richer microbiome than both *M. edulis* and hybrids. The microbial cluster 3 is more associated with host cluster 2 than with host clusters 4 and 5 (probabilities of interactions: 36.6% vs. 18.1% and 8.7%; Supp. Table S4). Therefore, it probably contains some of the bacterial families peculiar to *M. galloprovincialis*. Despite these differences, the latent block model also uncovered a potential list of bacterial families that could constitute the 'core microbiome' of all sampled mussels, i.e., microbial cluster 1. This group of 56 bacterial families comprises quite common families such as Aeromonadaceae, Enterobacteriaceae, Pseudomonadaceae, Rickettsiaceae and Vibrionaceae (Supp. Table S5). In particular, this cluster contains the three families comprising the three genera described in Fig. 2 (Halomonadaceae, Campylobacteraceae and Vibrionaceae).

The shared core microbiota among the three mussel species confirms that the surrounding environment plays a major role in shaping the composition of the microbiome. In agreement with our findings, previous reports have shown that the microbiota of co-cultivated oysters and mussels displayed a high degree of similarity (Pierce and Ward, 2019; Vezzulli et al., 2018). However, host genetics also appears to be a driving factor in the mussel microbiota given the

dissimilarity between the microbial communities associated with *M. galloprovincialis* and the other two species. The specific microbiome of the Mediterranean mussel may confer fitness advantages to the host including resistance and survival from disease as demonstrated in other organisms (Kueneman et al., 2016; Madison et al., 2022; Zackular et al., 2013). Compared to *M. edulis* and hybrids, *M. galloprovincialis* showed a lower abundance of *Cobetia* and *Arcobacter* species. In addition, despite the high prevalence of *V. Splendidus* strains, the Mediterranean mussel survived experimental infection with the virulent *V. splendidus* 10/068 1T1 better (Fig. 4). Resistance of *M. galloprovincialis* to infectious diseases has been previously reported. Fuentes et al. (2002) described higher warm season mortality of *M. galloprovincialis* x *M. edulis* hybrids than *M. galloprovincialis* individuals. Similarly, Benabdelmouna et al. (2018) suggested a higher susceptibility in *M. edulis* relative to *M. galloprovincialis* at the spat stage, which is consistent with field mortalities. Thus, the microbiome of *M. galloprovincialis* may be related to the efficiency of defence mechanisms and resistance to infectious diseases. Characterization of host-microbiota dynamics at the spatial and temporal scales will be crucial to understanding the roles of the microbiome in maintaining host homeostasis.

5. Conclusions

In this study, we provide for the first time an overview of the microbiota of *M. edulis*, *M. galloprovincialis* and their hybrids in the context of mortalities in French mussel farm. We reported dysbiosis of the microbiota in the three mussel species and a difference between bacterial communities in seawater and mussel tissues but also between mussel species. Among the pathobiontic bacteria detected, members of *Vibrio*, *Cobetia* and *Arcobacter* genera were the most abundant suggesting their involvement in mussel disease. In addition, *M. galloprovincialis* showed a different microbiota profile and was also more resistant to infection by *V. splendidus*. Thus, we can speculate on the link between the microbiota of Mediterranean mussels and their resistance to infectious disease. Further research is needed to understand the nature of the

interactions of mussel microbiota, including the pathobiome, with their host, which may ultimately help to manage and monitor host health to prevent mortalities in mussel farms.

CRediT authorship contribution statement

Yosra Ben Cheikh: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization, supervision. **François Massol**: Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Visualization. **Nathalie Giusti-Petrucciani**: Investigation. **Marie-Agnès Travers**: Conceptualization, Writing - Review & Editing.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare no conflicts of interest.

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Figure legends

Figure 1. Total OTUs and the relative abundance of bacterial phyla in mussels and seawater. (A) total OTUs presented by box-and-whisker plots showing high, low, and median values, with lower and upper edges of each box denoting first and third quartiles, respectively and the mean with a cross. Edu = *M. edulis*, gallo = *M. galloprovincialis*, edu x gallo = *M. edulis* x *galloprovincialis* and SW = seawater. * indicates significant difference $p<0.05$ (ANOVA, Holm-Sidak test); (B) relative abundance of bacterial communities at the phylum level. Replicates are labelled with the numbers 1 to 15 for animals and 1 to 3 for seawater.

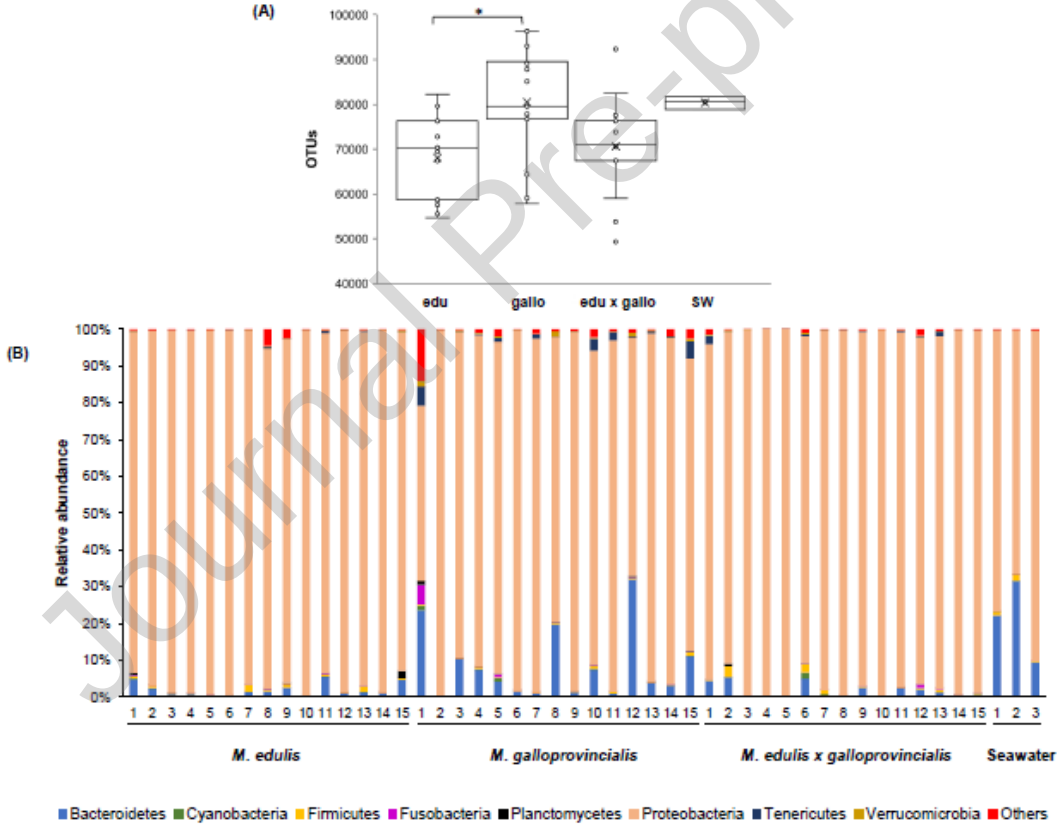


Figure 2. The most abundant bacterial family and genera in *Mytilus* sp. and seawater. (A-D) heatmaps revealing the top 30 bacterial families (%); (E-H) Bacterial genera with a mean relative abundance >1. Replicates are labelled with the numbers 1 to 15 for animals and 1 to 3 for seawater.

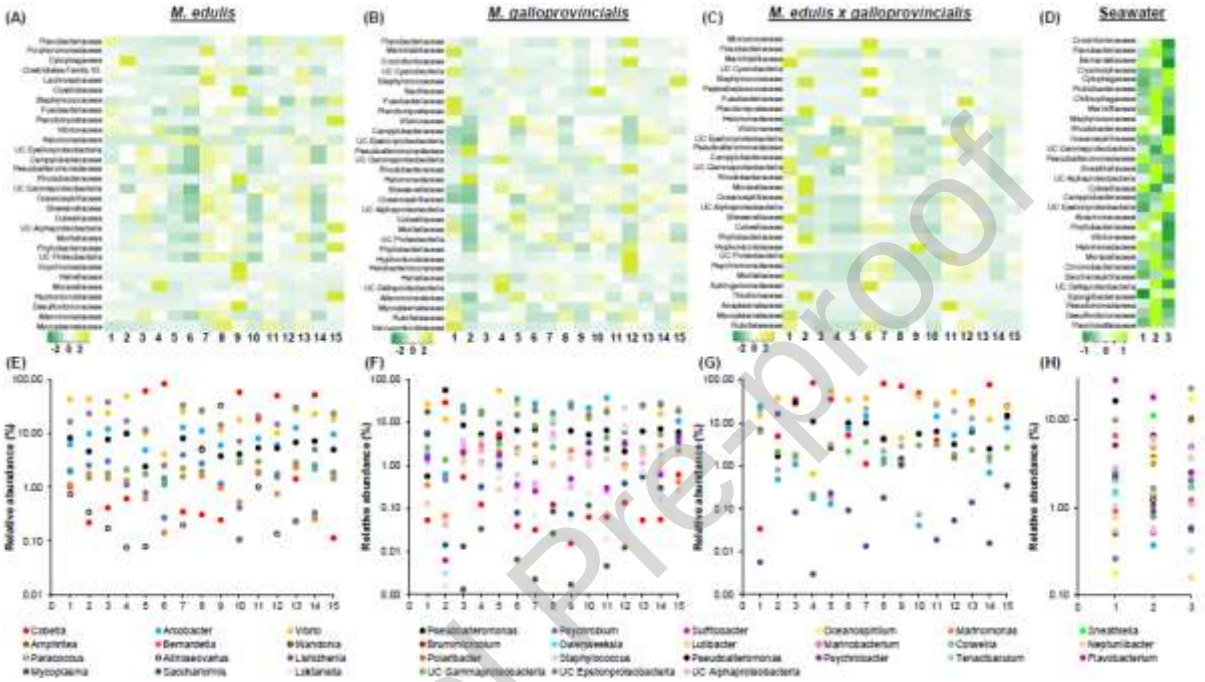


Figure 3. Clustering of hosts and bacterial families. (A) Representation of the association between hosts (rows) and bacterial families (columns) as coloured vs. white rectangles. Colours are associated to the four different host types (purple: *M. edulis*, blue: *M. galloprovincialis*, green: hybrids, yellow: seawater). The solid black lines (vertical and horizontal) delimit the clustering obtained using the latent block model, e.g. hosts (rows) enclosed by the same horizontal lines belong to the same host cluster. (B) Alluvial plot linking the individual hosts within the five host clusters (from cluster 1 at the bottom to cluster 5 at the top, on the left-hand side) with their host type (on the right-hand side).

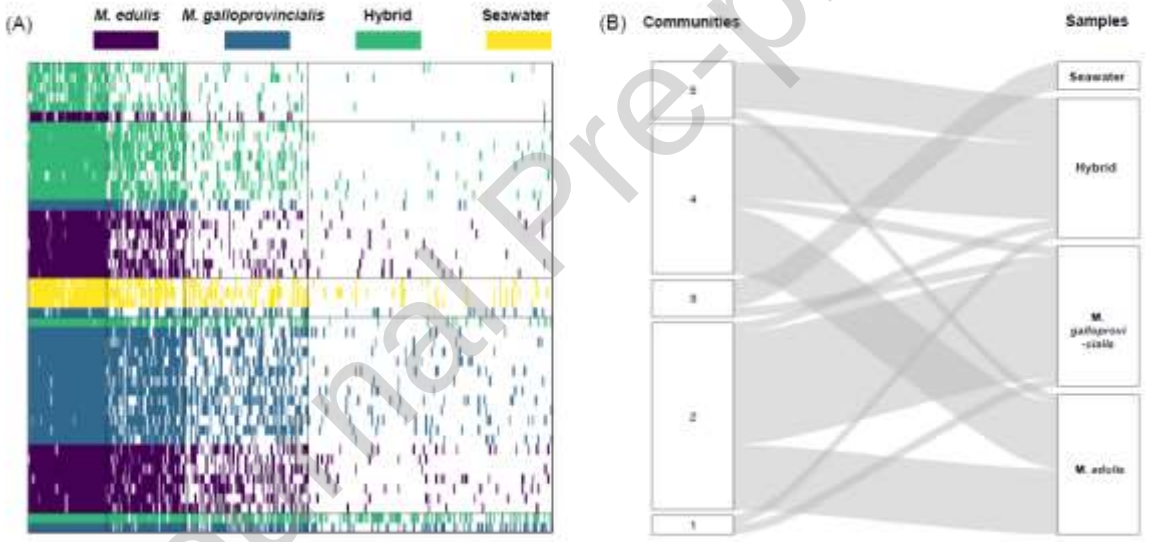
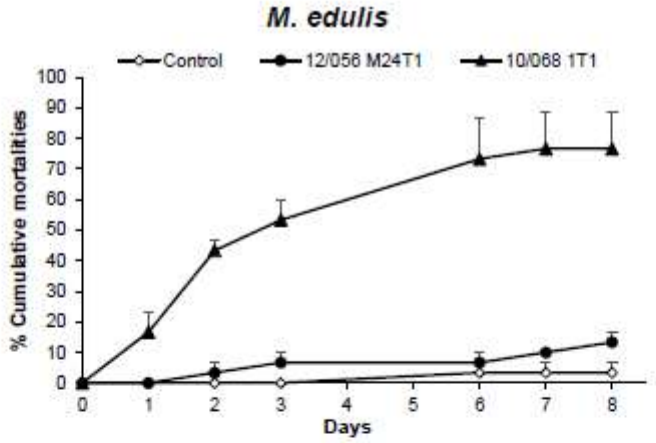
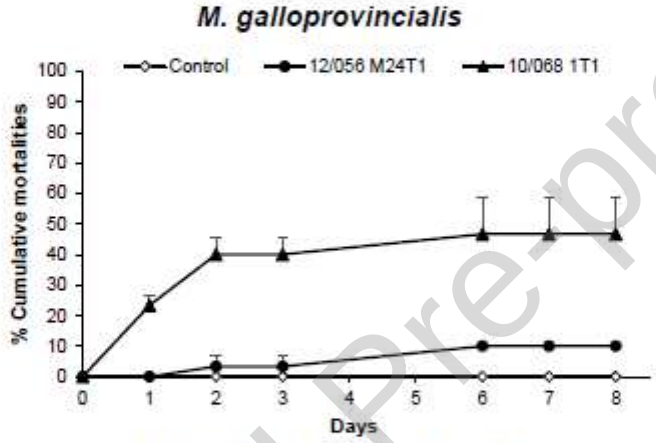
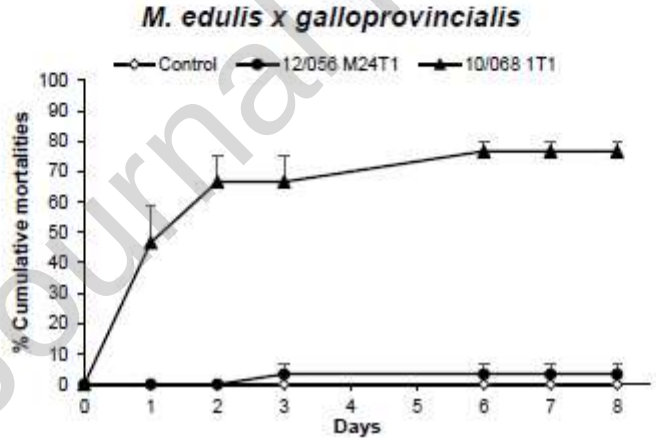


Figure 4. Resistance of *Mytilus* sp. to *V. splendidus* infection. Mussels were intramuscularly injected with the pathogenic *V. splendidus* 10/068 1T1 or with the non-virulent *V. splendidus* 12/056 M24 T1 or with FSSW (control). Curves are mean of cumulative mortalities in triplicate tanks (10 mussels/tank) ± SEM.







Supplemental Figure S1. Relative abundance of ZymoBIOMICS microbial standards at the genus level. ZymoBIOMICS kit theoretically contains 8 bacterial strains: *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enterica*, *Lactobacillus fermentum*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus subtilis*.

Supplemental Figure S2. **Alpha and beta diversity in mussels and seawater.** (A) alpha diversity expressed as Chao1 and Shannon index. Data are the mean ± SEM (n=15 for mussels, n=3 for seawater); (B) Beta diversity calculated using Principal Component Analysis (PCoA) applied on Bray-Curtis distance matrix.

Supplemental Figure S3. Uncertainty of cluster assignments. Violin plots representing the distribution of probabilities of assignment of each host (left panel) and bacterial family (right panel) to its LBM cluster (i.e. the highest among all the probabilities of cluster memberships for each host and family). The white circle indicates the value of the median of the distribution.

Supplemental Table S1. Number of pseudoreads in mussels and seawater before and after chimera filtering.

Supplemental Table S2. Mean relative abundance of the top 30 bacterial families identified in mussels and seawater, n = 15 for mussels and n = 3 for seawater. Data are percentage ± SEM.

Supplemental Table S3. Mean absolute abundance of *Vibrio*, *Cobetia* and *Arcobacter* species identified in mussels, n = 15 for mussels. Data are mean ± SEM. Only mean abundance > 100 OTUs in at least one mussel species is considered.

Supplemental Table S4. Matrix summarizing the estimated probabilities of association between hosts and bacterial families according to the LBM clusters they belong to. For instance, associations between a random host from host cluster 3 and a random family from microbial cluster 4 occur with a probability of 7.88%.

Supplemental Table S5. Inventory of the bacterial families found in each of the four latent block model clusters for bacteria.

CRediT authorship contribution statement

Yosra Ben Cheikh: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization, supervision. **François Massol**: Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Visualization. **Nathalie Giusti-Petrucciani**: Investigation. **Marie-Agnès Travers**: Conceptualization, Writing - Review & Editing.