

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

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

15 Outbreaks of marine mussel mortality on French farms could have different aetiologies. One of 16 them implies Vibrio splendidus strains. Beyond the involvement of this pathogen, there is 17 considerable evidence that diseases often result from interactions between several microbes and 18 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 19 20 of *Mytilus edulis*, *Mytilus galloprovincialis* and their hybrids displayed an abnormal abundance 21 of Proteobacteria, in particular the genera Vibrio, Cobetia and Arcobacter. Despite the 22 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 23 24 bacteria families confirmed this finding and showed that the microbiomes of *M. edulis* and the 25 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. 26 27 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.

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

32 **1. Introduction**

33 Over the last two decades, European mussel production has been declining. Among the putative 34 causes of this trend, the spread of diseases, algal blooms, lack of spat, predation and low 35 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 36 37 2016 in the Netherlands (Capelle et al., 2021). While no cause could explain this phenomena in 38 the Netherlands (Capelle et al., 2021), the involvement of pathogens in mortalities has been 39 demonstrated in French mussel farms (Ben Cheikh et al., 2017, 2016; Benabdelmouna et al., 40 2018; Oden et al., 2016).

As filter-feeding organisms, marine mussels are constantly exposed to a variety of 41 42 microorganisms including pathogenic bacteria that can threaten their lives. In addition to external microbes, like all macroorganisms, mussels interact with the microorganisms they 43 44 harbour, i.e. their microbiota. This dialogue between the host and its microbiota can help 45 maintain their respective integrity, as shown e.g. in leeches (Kikuchi and Graf, 2007; Tasiemski 46 et al., 2015). In fact, advances in analytical techniques over the last few decades have favoured 47 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., 48 49 2009; Mushegian and Ebert, 2016). Considered the "last organ" in humans (Baquero and 50 Nombela, 2012), a large body of evidence has revealed the beneficial contribution of the 51 microbiota to basic biological processes such as digestive, metabolic and immune functions 52 (Callens et al., 2016; Sirisinha, 2016; Wang et al., 2017) and its involvement in the eco-53 evolutionary dynamics of their host (Amato, 2016; Kohl et al., 2018; Macke et al., 2017; 54 Moeller et al., 2016; Murall et al., 2017). However, like other ecosystems, the composition of 55 the microbiota can be modulated by several factors such as antibiotics and pathogens leading 56 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).

60 The large number of findings in the human model has sparked research interest in other 61 organisms including bivalves. Numerous studies have investigated microbiota structure in 62 oysters, mussels and to a lesser extent clams, under the influence of factors such as host 63 genotype, environment and pathogens (for review see Paillard et al., 2022). The structure of the 64 microbiota can be divided into at least two types of communities, namely a stable community 65 of resident bacteria within a host population ("the core microbiome") and a variable microbial 66 community that depends on the surrounding environment (Santibáñez et al., 2022). Data on the 67 role of the core microbiome are limited, although some authors suggest a contribution to 68 essential host biological functions (Lemanceau et al., 2017). Furthermore, the balance between 69 the core and variable microbiome is still unclear, especially as the dynamics of host-associated 70 communities are directly affected by abiotic and biotic factors. For example, elevated water 71 temperature appears to be a major determinant and strongly influences the composition of the 72 microbiota in mussels and oysters by also favouring pathogens such as Vibrio sp. (Li et al., 73 2019, 2018; Lokmer and Wegner, 2015). In addition, water pollution by nanoparticles, 74 nanoplastics or chemicals can affect the diversity of the microbiota as has been demonstrated 75 in Mytilus galloprovincialis (Auguste et al., 2020, 2019; Balbi et al., 2020). Besides abiotic 76 factors, microbiota imbalance has been linked to diseases (King et al., 2019). Indeed, infection 77 of Crassostrea gigas by OsHV-1 virus induced dysbiosis with subsequent bacteraemia by 78 opportunistic bacteria (Lorgeril et al., 2018). Similarly, field studies on oysters affected by 79 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.

86 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 87 88 with *M. edulis*, *M. galloprovincialis* and their hybrids, grown in the same environment, and the 89 surrounding water were analysed. The differences between the microbial communities found in 90 the different species were assessed using ecological network methods to cluster microbiota and 91 link the obtained classification to the type of sampled mussels. The susceptibility of the 92 different types of mussels to infection was then explored by experimental infection with V. 93 splendidus strains.

94 **2. Material and methods**

95 **2.1. Mussel collection**

45 farmed adults of Mytilus sp. (M. edulis, M. galloprovincialis and hybrid M. edulis x 96 97 galloprovincialis, 15 individuals from each taxon, size between 4 and 5 cm) were collected 98 from a mussel farm affected by massive mortalities (Bay of Lannion, France 48°44 44.8 N, 99 $3^{\circ}35$ 24.9 W) in September 2018 (mean temperature = 17° C). The animals were immediately 100 transported to the laboratory and randomly dispatched in different temperature-controlled 101 $(16^{\circ}C)$ aerated water tanks filled with seawater sampled from the corresponding site (salinity = 102 35 PSU). Mussels were taken at random from two different tanks for microbiota analysis or for 103 experimental infection. Seawater from the same site was also sampled for microbiota analyses.

104 **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.

110 DNA extraction, amplicon library construction and Illumina MiSeq sequencing were performed 111 by Biofidal (Vaulx-en-Velin, France). DNA was extracted from frozen mussels ground in liquid 112 nitrogen and from bacteria pellet using Mag Bind® Universal Pathogen DNA Kit (Omega BIO 113 TEK, Georgia, USA) and from the different filters using Quick DNA/RNA MagBead kit (Zymo 114 Research, California, USA) according to the manufacturer's protocol. DNA concentration and 115 quality were checked with the QuantiFluor® dsDNA System (Promega, France). The 16S 116 rRNA gene of bacterial communities was amplified using the 5X HOT BIOAmp ® 117 BlendMaster Mix (Biofidal) and the 341F: CCTACGGGNGGCWGCAG (17 pb) and 805R: 118 GACTACHVGGGTATCTAATCC (21 pb) primers (designed by Biofidal) targeting the 119 variable V3-V4 loops (Klindworth et al., 2013). After purification of the PCR products by 120 SPRIselect reagent kit (Beckman Coulter), a second amplification was performed using index 121 adapter oligos (P5/P7). The purified PCR products were normalized to obtain an equimolar 122 library and subsequently pooled. Negative controls and ZymoBIOMICS microbial community 123 standards were included during the library preparation (Supplemental Figure S1). Amplicons 124 were sequenced on the MiSeq platform with V3 reagents producing 2 X 300 bp paired-end 125 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.

135

2.3. Host-microbiota network

136 To analyse the structure of the host-microbiota association dataset, we used methods borrowed 137 from the analysis of bipartite ecological networks. First, we aggregated data on microbial taxa 138 at the family level and transformed the microbial family abundance table into presence/absence 139 data (i.e., all abundances > 0 were set to 1). We then modelled the obtained bipartite network 140 of hosts by microbial families using a latent block model (LBM) (Govaert and Nadif, 2008; 141 Leger et al., 2015). The clustering obtained with LBMs is indeed more useful than the one 142 obtained with modularity-search algorithms (Newman, 2006; Traag et al., 2019) as blocks can 143 identify densely connected as well as sparsely connected regions of the network. We tested the 144 correlation between the obtained clustering of hosts and their classification among species using 145 a method described in Massol et al. (2021): we computed the normalised mutual information 146 (NMI) index between the two classifications of the hosts (by host type and by LBM block); we 147 tested the significance of NMI using randomizations of the bipartite network through the 148 configuration model (Strona et al., 2014).

149

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

153 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). 154 155 Anesthetized mussels (2-3 h in magnesium chloride solution at 50 g.L⁻¹, 1/4: v/v 156 seawater/freshwater) were intramuscularly injected with 100 µL of bacterial suspension or 157 FSSW for negative controls. The animals were transferred to tanks (3 replicate tanks, 10 158 mussels per tank) filled with 2 L of UV-treated and filtered seawater supplemented with 50 mL 159 of phytoplankton (Isochrysis galbana) and maintained under static conditions at 16°C with 160 aeration. Mortality was monitored over 8 days with removing of newly dead mussels from the 161 tanks.

162 **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.

168 **3. Results**

169 **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.

174 The clustering of reads in OTUs revealed a higher richness in the Mediterranean mussel *M*.

175 galloprovincialis (significant difference with *M. edulis*, ANOVA p < 0.05) and in seawater with

a mean absolute abundance of $80\,486 \pm 3\,214$ OTUs and $80\,367 \pm 808$ OTUs respectively. The

177 blue mussel *M. edulis* and the hybrids had a mean absolute abundance of $68\ 068 \pm 2\ 322$ and 178 70 637 \pm 2 783 OTUs respectively (Figure 1 A). Alpha diversity metrics were higher in M. 179 galloprovincialis compared to the other two mussel species and seawater (non-significant data, 180 supplemental Figure S2A). Beta diversity analysis revealed dissimilarities between mussels and 181 seawater (Supplemental Figure S2B). The compositional structure at the phylum level indicated 182 a similarity between *M. edulis* and the hybrids (Figure 1 B) with a predominance of 183 Proteobacteria (relative abundance r.a. of 92.3% to 99.4% in M. edulis and 89.9% to 99.9% in 184 hybrids) and the presence of Bacteroidetes (r.a. from 0.3% to 5.5% in M. edulis and from 0 to 185 5.4% in hybrids). Other rare phyla with an abundance > 1 were detected in some hybrids, e.g., 186 Firmicutes (replicates 2, 6 and 7) and Tenericutes (replicates 1 and 13). Mediterranean mussels 187 harboured a lower proportion of Proteobacteria (r.a. from 47.3% to 99.6%) and a higher 188 proportion of Bacteroidetes (r.a. from 0 to 23.5%). OTUs belonging to Tenericutes, 189 Verrucomicrobia, Fusobacteria and Cyanobacteria were also identified in some mussels with 190 r.a. > 1. The composition of the surrounding seawater was similar to that of the mussels with a 191 notable r.a. of Bacteroidetes (mean $21.1\% \pm 6.4\%$) and the predominance of Proteobacteria 192 (mean 77.4% \pm 6.9%).

193 A comparative analysis of the most abundant families (Top 30) and genera revealed a more 194 specific layout for each group although a similar bacterial community was detected in most 195 samples (Figure 2, Supplemental Table S2). Overall, Vibrionaceae, Halomonadaceae, 196 Pseudoalteromonadaceae and Campylobacteraceae were the dominant families in the blue 197 mussel and hybrids (mean r.a. 5-35%) with notably the genera Vibrio, Cobetia, 198 Pseudoalteromonas and Arcobacter but also unclassified Epsilonproteobacteria. Similarly, the 199 harboured Vibrionaceae Mediterranean mussel (Vibrio), Pseudoalteromonadaceae 200 (Pseudoalteromonas) and unclassified Epsilonproteobacteria but also a higher proportion of 201 Campylobacteraceae (Arcobacter) and Flavobacteriaceae (especially Tenacibaculum and

202 Flavobacterium) (mean r.a. 18.2% and 6.8% respectively). In contrast, in addition to 203 unclassified Gammaproteobacteria, the prevailing taxa (r.a. up to 5%) in seawater samples were 204 families Rhodobacteraceae, Oceanospirillaceae, Pseudoalteromonadaceae, the 205 Crocinitomicaceae and Flavobacteriaceae and the genera Sulfitobacter, Oceanospirillum, 206 *Pseudoalteromonas.* The majority of *Vibrio* identified in mussels have been affiliated to the V. 207 Splendidus group (Supplemental Table S3).

208 **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). 209 210 Assignment of host individuals to clusters resulted in probabilities of membership at least as 211 high as 88.3%; by contrast, a few bacterial families were only assigned to a particular cluster 212 with a probability of 50.8% (Supplemental Figure S3). The first cluster of hosts consisted in 2 213 samples, one of *M. galloprovincialis* and one hybrid, which harboured high numbers of 214 bacterial families (223 families on average). The second cluster of hosts consisted in 20 samples 215 (1 hybrid, 7 *M. edulis* and 12 *M. galloprovincialis*) with an average of 141.9 bacterial families 216 per sample. The third cluster included only 4 samples (one replicate of *M. galloprovincialis* 217 together with the three seawater samples) with an average of 125.5 families per sample. The 218 fourth cluster of hosts consisted in 16 samples (7 *M. edulis*, 1 *M. galloprovincialis*, 8 hybrids) 219 with an average of 109 families per sample. The fifth cluster of hosts consisted in 6 samples (1 220 *M. edulis*, 5 hybrids) with an average of 69 families per sample. The four clusters of bacterial 221 families harboured 56, 57, 89 and 176 families, respectively, and were found on average in 222 45.46, 30.44, 13.44 and 2.63 host samples respectively. Probabilities of host-bacterial family 223 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.

231

3.3. Susceptibility of mussels to pathogens

232 To assess the susceptibility of mussel populations to bacterial infection, M. edulis, M. 233 galloprovincialis and hybrids were injected with two strains of V. splendidus of contrasting 234 virulence. Exposure of mussels to avirulent strain 12/056 M24 T1 induced limited mortalities 235 in all mussel groups, ranging from $3.3\% \pm 3.3\%$ in hybrids to $13.3\% \pm 3.3\%$ in *M. edulis* at day 236 8 (Figure 4). In contrast, injection of the virulent strain 10/068 1T1 induced higher mortalities 237 with different rates in the mussel species. The first mortalities appeared 24h post-injection and 238 increased progressively until stabilization on day 8. Hybrids and M. edulis had different patterns 239 at the beginning of the infection (16.6% \pm 12% for *M. edulis* and 46.6% \pm 6.6% for hybrids on 240 day 1) but reached 76.6% of mortality at the end of the experiment while *M. galloprovincialis* 241 had lower mortalities $(23.3\% \pm 3.3\%$ on day 1 and 46.6% \pm 12% on day 8). No or negligible 242 mortality was observed in controls.

4. Discussion

244 Since 2014, mass mortalities have been reported in French mussel farms. While M. edulis, M. 245 galloprovincialis and their hybrids represent the main farmed species, the epizootics seem to 246 mostly affect the blue mussel (Bechemin et al., 2014). In general, investigations of mussel 247 disease have so far been limited to the detection and isolation of known pathogens from 248 moribund animals such as V. splendidus (Oden et al., 2016), combined with experimental 249 infection in the laboratory (Ben Cheikh et al., 2017, 2016). In this study, we provide an 250 overview of the bacterial communities associated with three mussel species and the surrounding 251 water sampled from a farm affected by mortality epidemics.

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

253 According to previous studies, the microbiota of healthy marine bivalves, and mussels in 254 particular is characterized by the presence of different phyla with a predominance of 255 Proteobacteria but also other bacteria belonging to Cyanobacteria, Firmicutes, Planctomycetes, 256 Bacteroidetes, Actinobacteria, Verrucomicrobia, Tenericutes and Chloroflexi (Ben Cheikh and 257 Travers, 2022; Musella et al., 2020). Here, the analysis of whole animal microbiota showed a 258 severe dysbiosis marked by an abnormal dominance of Proteobacteria (Fig. 1, mean r.a. ranging 259 from 86.5% to 96.1%). Microbiota imbalance has been linked to mortality events and infectious 260 diseases in bivalves. Field analysis of the Pacific oyster C. gigas showed that animals 261 experiencing mortality outbreaks at different life stages displayed signs of dysbiosis resulting 262 in a change in the diversity and composition of the microbiota (Lasa et al., 2019). Similarly, a 263 study on Unionida freshwater mussel during mass mortality events revealed bacterial invasion 264 and shifts in the bacterial microbiome (Richard et al., 2021). For marine mussels, there are no 265 data available to our knowledge on animal microbiota during mortality events. However, we 266 have recently reported a disruption of the blue mussel microbiota during experimental infection 267 with V. splendidus and demonstrated the implication of some opportunistic pathobiontic 268 bacteria in the infection (Ben Cheikh and Travers, 2022). Thus, even if the causal origin of 269 mussel mortalities in France still not clear, we can establish a link with pathogenic bacteria, 270 especially as they are detected in *Mytilus* tissues. Our analysis highlighted the genera Vibrio, 271 Cobetia and Arcobacter as the predominant bacteria in mussel species (Fig. 2). The abundance 272 of Vibrio sp. in the microbiota of mussels is not surprising as these genera are naturally 273 occurring in marine environments and certain strains are often associated with outbreaks of 274 mortality marine bivalves (Travers et al., 2015). In our study, we mainly detected strains 275 belonging to the V. Splendidus clade which includes pathogens of bivalves such as V. 276 splendidus, V. tasmaniensis and V. crassostreae (Ben Cheikh et al., 2016; Bruto et al., 2017;

277 Duperthuy et al., 2011) but also less studied strains showing virulence for some marine 278 organisms (e.g. V. toranzoniae and V. kanaloae) and environmental species without pathogenic 279 potential (e.g. V. gigantis and V. atlanticus) (Lasa et al., 2017; Romalde et al., 2014). In 280 accordance with our findings, different studies have reported the presence of V. splendidus-281 related strains in different French mussel farms affected by mortalities (Bechemin et al., 2014; 282 Charles et al., 2020a; Oden et al., 2016). However, these studies are restrictive as they are based 283 on the isolation of culturable bacteria of interest and are therefore not representative of the 284 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 285 286 et al., 2020b). The implication F. halioticida in mussel disease is not confirmed in our study 287 since this strain was detected at very low proportions in some individuals of blue mussels and 288 hybrids (data not shown). In contrast, metagenomic analyses showed the prevalence of bacteria 289 of the genus Cobetia and Arcobacter. While knowledge of Cobetia strains is limited, several 290 reports described the dominance of Arcobacter sp. in oysters and mussels in a context of disease 291 suggesting them as opportunistic bacteria (Ben Cheikh and Travers, 2022; Li et al., 2019; 292 Lokmer and Wegner, 2015; Lorgeril et al., 2018). Nevertheless, the potential role of these 293 bacteria needs further investigation, as some species (e.g. A. cryaerophilus, A. ellisii, A. 294 nitrofigilis) have virulence genes and the ability to colonise and enter human cells (Ferreira et 295 al., 2016; Levican et al., 2013).

296

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 302 all found in host cluster 2. The differences in bacteria family richness between clusters thus 303 confirm that *M. galloprovincialis* is associated to a richer microbiome than both *M. edulis* and 304 hybrids. The microbial cluster 3 is more associated with host cluster 2 than with host clusters 4 305 and 5 (probabilities of interactions: 36.6% vs. 18.1% and 8.7%; Supp. Table S4). Therefore, it 306 probably contains some of the bacterial families peculiar to *M. galloprovincialis*. Despite these 307 differences, the latent block model also uncovered a potential list of bacterial families that could 308 constitute the 'core microbiome' of all sampled mussels, i.e., microbial cluster 1. This group of 309 56 bacterial families comprises quite common families such as Aeromonadaceae, 310 Enterobacteriaceae, Pseudomonadaceae, Rickettsiaceae and Vibrionaceae (Supp. Table S5). In 311 particular, this cluster contains the three families comprising the three genera described in Fig. 312 2 (Halomonadaceae, Campylobacteraceae and Vibrionaceae).

313 The shared core microbiota among the three mussel species confirms that the surrounding 314 environment plays a major role in shaping the composition of the microbiome. In agreement 315 with our findings, previous reports have shown that the microbiota of co-cultivated oysters and 316 mussels displayed a high degree of similarity (Pierce and Ward, 2019; Vezzulli et al., 2018). 317 However, host genetics also appears to be a driving factor in the mussel microbiota given the 318 dissimilarity between the microbial communities associated with M. galloprovincialis and the 319 other two species. The specific microbiome of the Mediterranean mussel may confer fitness 320 advantages to the host including resistance and survival from disease as demonstrated in other 321 organisms (Kueneman et al., 2016; Madison et al., 2022; Zackular et al., 2013). Compared to 322 M. edulis and hybrids, M. galloprovincialis showed a lower abundance of Cobetia and 323 Arcobacter species. In addition, despite the high prevalence of V. Splendidus strains, the 324 Mediterranean mussel survived experimental infection with the virulent V. splendidus 10/068 325 1T1 better (Fig. 4). Resistance of *M. galloprovincialis* to infectious diseases has been previously 326 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

334 In this study, we provide for the first time an overview of the microbiota of *M. edulis*, *M.* 335 galloprovincialis and their hybrids in the context of mortalities in French mussel farm. We 336 reported dysbiosis of the microbiota in the three mussel species and a difference between 337 bacterial communities in seawater and mussel tissues but also between mussel species. Among 338 the pathobiontic bacteria detected, members of Vibrio, Cobetia and Arcobacter genera were the 339 most abundant suggesting their involvement in mussel disease. In addition, M. galloprovincialis 340 showed a different microbiota profile and was also more resistant to infection by V. splendidus. 341 Thus, we can speculate on the link between the microbiota of Mediterranean mussels and their 342 resistance to infectious disease. Further research is needed to understand the nature of the 343 interactions of mussel microbiota, including the pathobiome, with their host, which may 344 ultimately help to manage and monitor host health to prevent mortalities in mussel farms.

345 **CRediT authorship contribution statement**

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

351 Data availability statement

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

Declaration of competing interest

355 The authors declare no conflicts of interest.

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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.



Bacteroidetes Cyanobacteria Firmicutes Fusobacteria Planctomycetes Proteobacteria Tenericutes Verrucomicrobia Others

- 601 Figure 2. The most abundant bacterial family and genera in Mytilus sp. and seawater. (A-D) heatmaps revealing the top 30 bacterial families
- 602 (%); (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.

603



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).



610

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.



615 Supplemental Figure S1. Relative abundance of ZymoBIOMICS microbial standards at the genus level.

616 ZymoBIOMICS kit theoretically contains 8 bacterial strains: Pseudomonas aeruginosa, Escherichia coli,

617 Salmonella enterica, Lactobacillus fermentum, Enterococcus faecalis, Staphylococcus aureus, Listeria 618 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.

622 Supplemental Figure S3. Uncertainty of cluster assignments. Violin plots representing the distribution of 623 probabilities of assignment of each host (left panel) and bacterial family (right panel) to its LBM cluster (i.e. 624 the highest among all the probabilities of cluster memberships for each host and family). The white circle 625 indicates the value of the median of the distribution.

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

627 **Supplemental Table S2.** Mean relative abundance of the top 30 bacterial families identified in mussels and 628 seawater, n = 15 for mussels and n = 3 for seawater. Data are percentage \pm SEM.

629 **Supplemental Table S3.** Mean absolute abundance of *Vibrio*, *Cobetia* and *Arcobacter* species identified in 630 mussels, n = 15 for mussels. Data are mean \pm SEM. Only mean abundance > 100 OTUs in at least one mussel 631 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%.

635 Supplemental Table S5. Inventory of the bacterial families found in each of the four latent block model636 clusters for bacteria.

637