Microbiota modification of *Mytilus edulis* larvae in response to the use of a new probiotic, the marennine, in aquaculture

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1. Introduction

- Blue Mussels (*Mytilus edulis*) production in hatcheries (Figure 1) is limited by the occurence of mass mortality events which are generally related to the presence of bacterial pathogens in the rearing system.
- Culture conditions in the rearing system can lead to the development of opportunistic pathogens, such as *Vibrio splendidus,* at a high density.
- Despite their effectiveness, antibiotics pose many problems in aquaculture (e.g. occurrence and transmission of antibiotics resistance in the food web, long-term inefficiency, etc…) and their use is now highly regulated worldwide.
- The use of probiotics such as marennine, a blue pigment produced by the diatom *Haslea ostrearia* (figure 2), could be a promising alternative to antibiotics in bivalve hatcheries.[1]

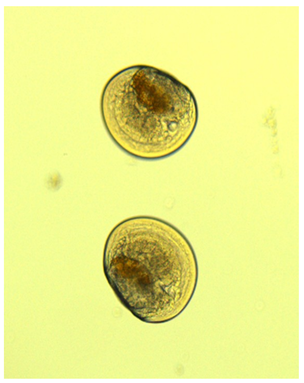


Figure 1. Blue mussel D-larvae (Latour ©)

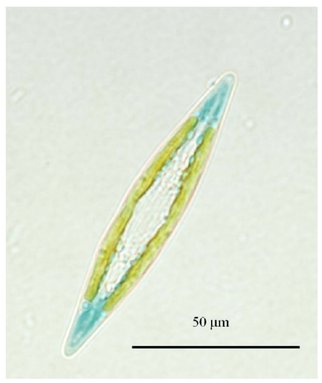
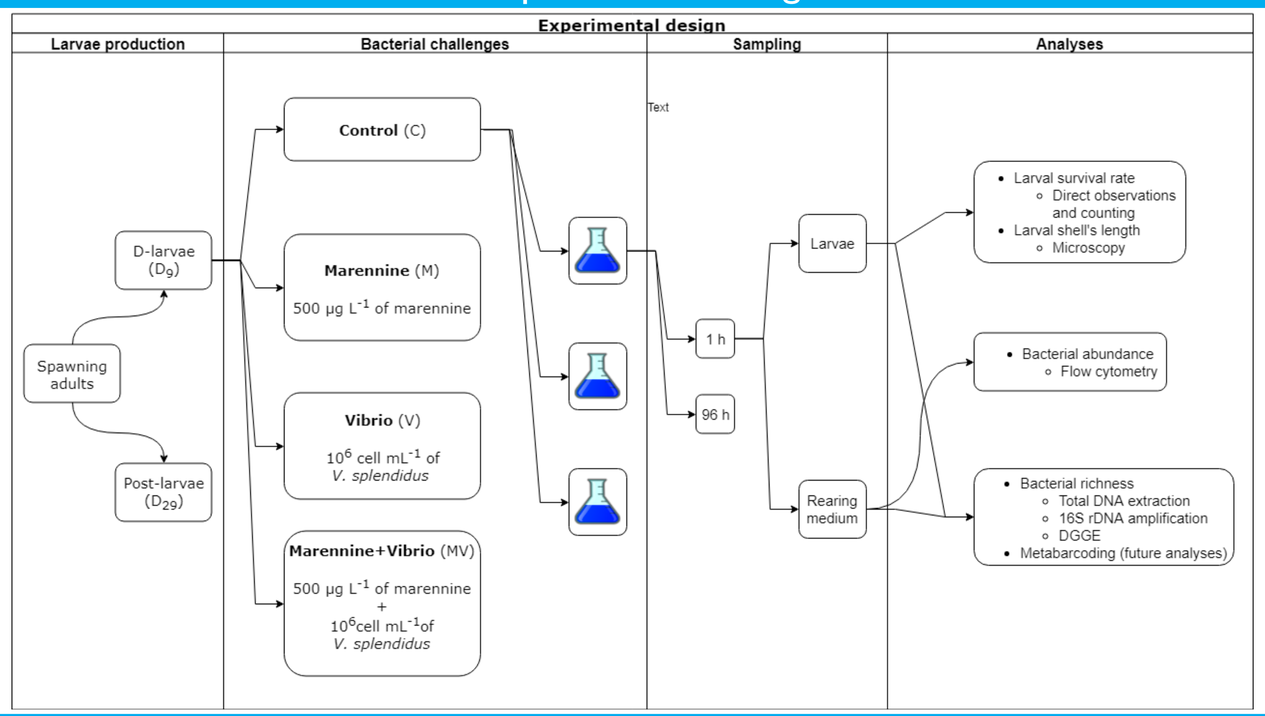


Figure 2. *Haslea ostrearia*[2]

2. Main objective of the study

Highlighting the potential protective effect of a new natural probiotic, the marennine, on *Mytilus edulis* larvae during bacterial challenges in relation to modification of the microbiota of the marennine-treated larvae

3. Experimental design



4.1. Larval survival and bacterial abundance

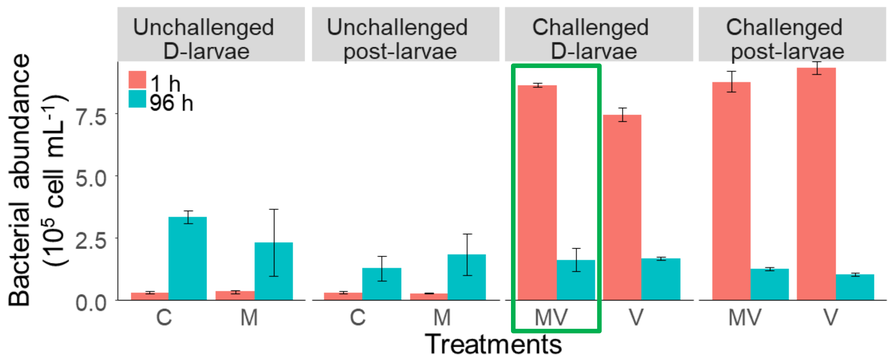


Figure 3. Bacterial abundance in the rearing medium after 1 h and 96 h of exposition of a) the unchallenged D-larvae, b) the challenged D-larvae against, c) the unchallenged post-larvae and d) the challenged post-larvae. Standard deviation is shown with error bars.

- Higher survival of marennine-treated D-larvae in presence of *V. splendidus* after 96 h of exposition compared to the control (C)
  - **MV: 91.1% ($p > 0.05$)**
  - V: 73.2% ($p < 0.01$)
- The presence of marennine did not affect the abundance of bacterial cells

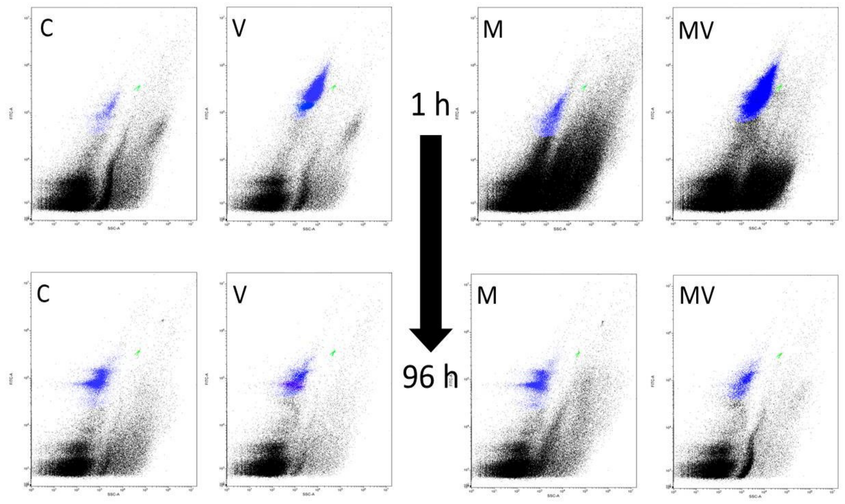


Figure 4. Cytograms obtained from the flow cytometry analyses for each treatments after 1 h and 96 h of exposition. The events in blue are considered as bacterial cells and the events in green are fluorescent beads (Fluoresbrite YG microsphere 1 µm, Polysciences) used as an internal standard used.

- The addition of *V. splendidus* at 500 µg L^{-1} is clearly visible after 1 h but this signature disapeared after 96 h of incubation suggesting an ingestion of the bacteria by the larvae

Marennine did not demonstrate a direct antibacterial effect when used during the bacterial challenges of both larval stages against *V. splendidus* suggesting its effect is "in the larvae"

4.2. Bacterial richness

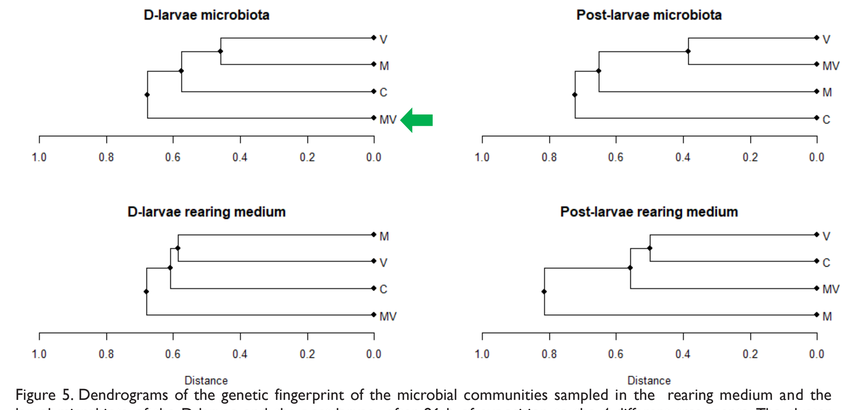


Figure 5. Dendrograms of the genetic fingerprint of the microbial communities sampled in the rearing medium and the larval microbiota of the D-larvae and the post-larvae after 96 h of exposition to the 4 different treatments. The cluster analyses were based on the Jaccard coefficient similarity and the dendrograms were constructed with UPGMA.

- The microbiota's genetic fingerprint from challenged D-larvae exposed to marennine was highly dissimilar (~70%)
- The presence of marennine highly modified the genetic fingerprints except for the post-larvae's microbiota

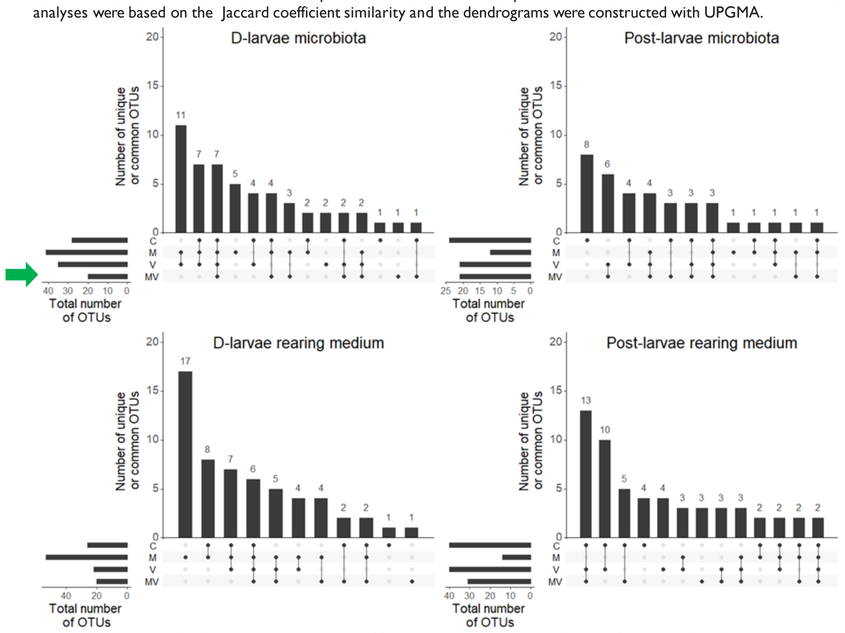


Figure 6. Numbers of unique, common and total OTUs between treatments for the D-larvae microbiota, the post-larvae microbiota, the D-larvae rearing medium and the post-larvae rearing medium..

- The number of unique OTUs increased with the presence of marennine (M) in the D-larvae microbiota (1 → 5) and rearing medium (0 → 17) compared to the control (C)
- The total number of OTUs in the challenged marennine-treated D-larvae microbiota (MV) and rearing medium decreased compared to the challenged D-larvae (V)

The presence of marennine modified the genetic fingerprint of both the rearing medium and the larvae microbiota regarding total number of OTUs and number of unique OTUs detected in each treatment

5. Conclusion

The presence of marennine in the rearing medium of the challenged D-larvae had a protective effect which is associated with a larval microbiota modification.

Metabarcoding analyses will enable us to investigate the latter larval microbiota modification.

References
1. Turcotte F, Mouget J-L, Genard B, Lemarchand K, Deschênes J-S, Tremblay R. 2016. Aquatic Living Resources 29:401.
2. Gastineau R, Turcotte F, Pouvreau JB, Morancais M, Fleurence J, Windarto E, Prasetiya FS, Arsad S, Jaouen P, Babin M, Coiffard L, Couteau C, Bardeau JF, Jacquette B, Leignel V, Hardivillier Y, Marcotte I, Bourgougnon N, Tremblay R, Deschenes JS, Badawy H, Pasetto P, Davidovich N, Hansen G, Dittmer J, Mouget JL. 2014. Mar Drugs 12:3161-3189.