Bacterial community structure of the marine diatom *Haslea ostrearia*

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Introduction

The marine diatom *Haslea ostrearia* produces a water-soluble blue-pigment named marennine of economic interest. But the lack of knowledge of the ecological conditions under which this microalga develops in its natural ecosystem (oyster ponds), more especially bacteria-*H. ostrearia* interactions, prevents any optimization of its culture in well-controlled conditions.

The present work has been intended to: i) characterize the structure of the bacterial community of *H. ostrearia* from oyster ponds in different localities of the French Atlantic coast; ii) describe the temporal dynamics of the bacterial community structure at the time scale of one culture cycle under laboratory conditions and after several subculturing steps.

A metabolic fingerprinting (untargeted approach) was aimed at assessing the global metabolic profile of *H. ostrearia* cultures, whether or not associated with the bacterial phycosphere.

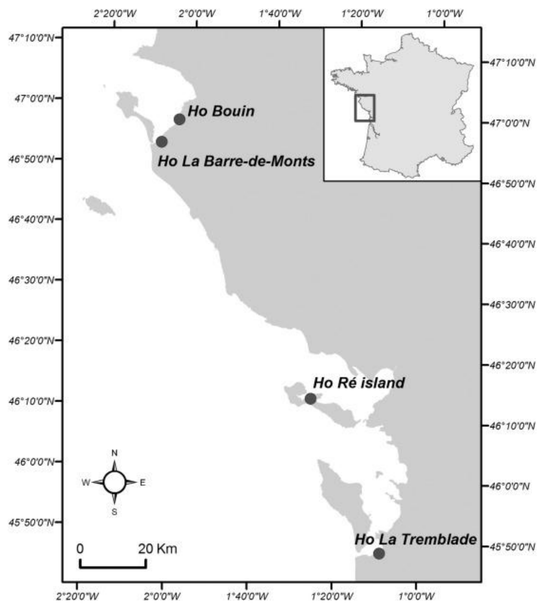

Fig. 1: Map of the French Atlantic coast showing oyster-pond locations where samples were collected to isolate *Haslea ostrearia*: Bouin, La Barre-de-Monts, Isle of Ré, and La Tremblade.

Materials and methods

Water and sediment samples were collected in 4 oyster farming localities (Fig. 1) more especially in oyster-ponds (Fig. 2), were bivalves are immersed for a fattening and ripening period before marketing.

In laboratory, six monospecific cultures of *H. ostrearia* were obtained by isolating a single cell of *H. ostrearia* from the raw samples. A first comparison of the bacterial community of the microalga was made before and after isolation of *H. ostrearia* cells (sediment vs. biofilm) by PCR-TTGE.

A second comparison with the six monospecific cultures was carried out between the biofilm (bacteria of the microalgal phycosphere, i.e., embedded in exopolysaccharides or epiphytic) and the supernatant (suspended cells in the culture medium) after separation of the two compartments by centrifugation and filtration.

A third comparison was undertaken for a same monospecific culture at various growth stages during a culture cycle (0, 3, 6, 9, 15, 30 days after inoculation) and for several subcultures (0, 3, 6, 9 months) sampled during the exponential growth stage.

Small soluble extracellular target compounds produced by the bacteria and *H. ostrearia* recovered from the culture medium in axenic and non-axenic *H. ostrearia* cultures were detected by high-resolution mass spectrometry (HRMS).

Fig. 2: Traditional oyster ponds in Marennes-Oléron region (IFREMER – LGPMM La Tremblade Laboratory).

Results

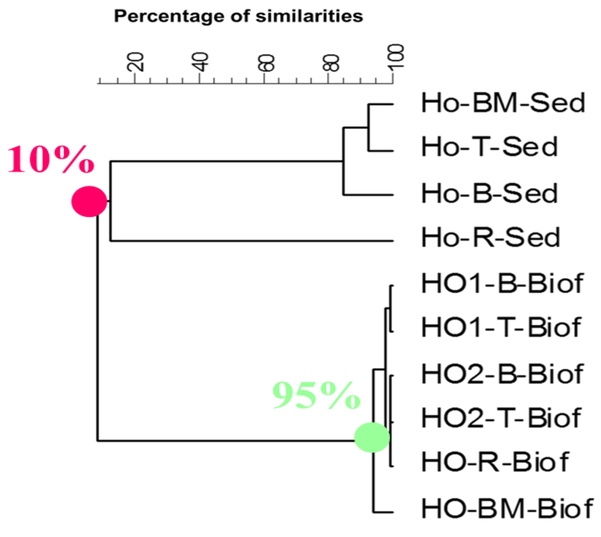

Fig. 3. Bacterial community structure of sediment samples (Sed) and after isolation of *H. ostrearia* (Biof) collected in oyster ponds from various localities (B: Bouin, BM: La Barre-de-Monts, R: Isle of Ré, T: La Tremblade).

- The structure of the bacterial community from the sediment compared to that of the biofilm after *H. ostrearia* isolation differed considerably (only 10% similarity between sediment and biofilm) (Fig. 3). This result demonstrates that the bacteria associated with *H. ostrearia* were specific to the microalga.
- A comparison of community structure of the bacteria recovered from the biofilm with those of the water column (WL), i.e. suspended bacterial cells (Fig. 4), revealed similarities that did not exceed 10%.
- With respect to the biofilm, the observed similarities in bacterial community structures exceeded 90% regardless of the geographic origin of the *H. ostrearia* isolates.

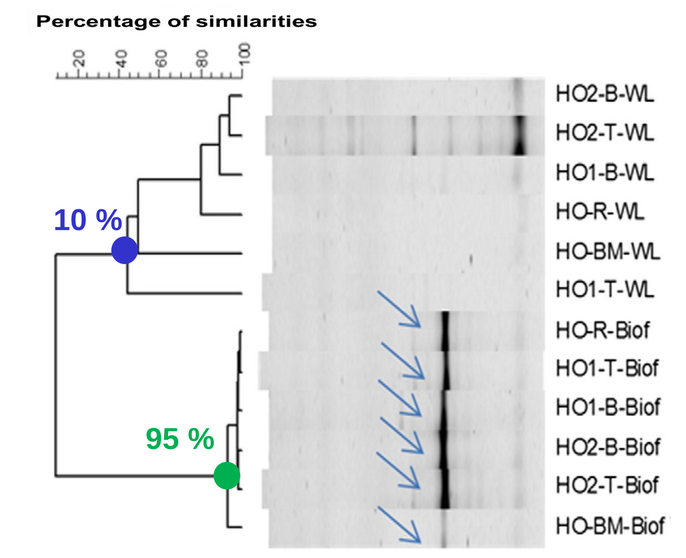

Fig. 4. TTGE analysis of the bacterial community structure from biofilm (Biof) and water column (WL) samples after isolation of *H. ostrearia* from various localities and subculturing in ES 1/3 medium under laboratory conditions (B: Bouin, BM: La Barre-de-Monts, R: Isle of Ré, T: La Tremblade). Arrows indicate the position of the band corresponding to the suspected chloroplastic and/or mitochondrial DNA of *H. ostrearia*.

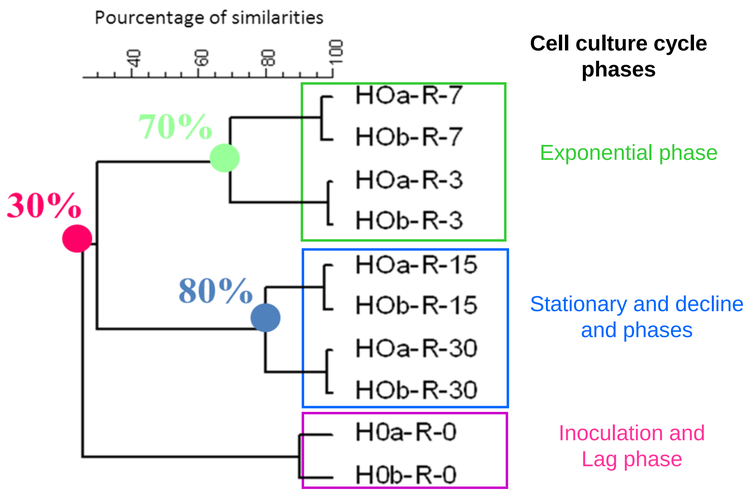

Fig. 5. Structure of the global bacterial community (suspended cells and biofilm cells) during a growth cycle of the *H. ostrearia* isolate Isle of Ré (HO-R) at days 0, 3, 7, 15 and 30. Letters a and b stand for the two experimental replicates.

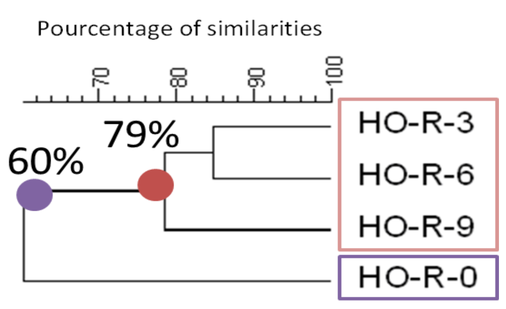

Fig. 6. TTGE analysis of the global bacterial community structure of Haslea ostrearia HO-R: Isle of Ré at the time of isolation (T-0) and after 3, 6 and 9 months of cultivation (T-3, T-6 and T-9)

- At the culture cycle scale (Fig 5), a marked evolution in the bacterial community could be observed. Three distinct clusters emerged, all of which were related to the algal growth stage: day 0 (initial bacterial community structure), days 3 and 7 (exponential phase), and days 15 and 30 (stationary phase and decline phase).
- A rather low similarity in the bacterial structure was observed between T0 and the subsequent subculturing 60% similarity for HO-R (Fig. 6). This result demonstrates that once *H. ostrearia* was isolated and cultivated under laboratory conditions, the bacterial community structure evolved, but afterwards, i.e. from 3 to 9 months, the bacterial community structure was fairly stable.

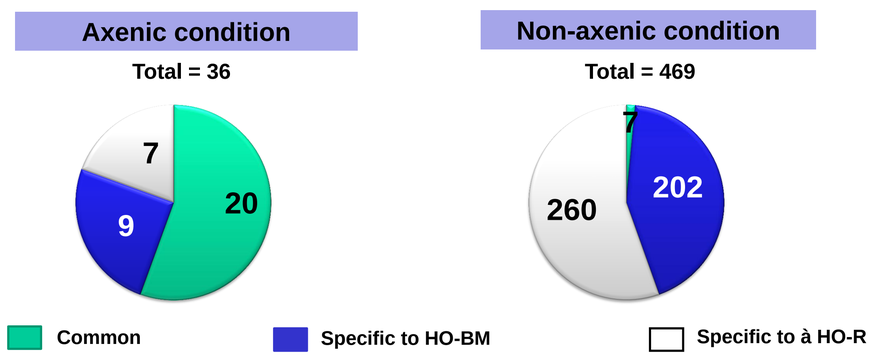

Fig. 7. Number of compounds recovered from HO-BM and HO-R isolate cultures in axenic and non-axenic conditions. "Total", "common" and "specific" refer to i) the total compounds recovered, ii) compounds shared by both isolates and iii) compounds specific to each isolate, respectively. All analyses were performed in triplicate.

- The number of total compounds was much lower in the so-called "axenic" *H. ostrearia* cultures, which account for only algal compounds (Fig 7), compared to the non-axenic cultures encompassing both algal and bacterial compounds
- Axenic conditions were associated with a high number of common compounds (20 out of 36) and a small number of compounds specific to each isolate (7 and 9 for HO-R and HO-BM, respectively). The opposite was exposed with non-axenic conditions, i.e. a low number of common compounds (7 out of 469),while 260 and 202 compounds were specific to HO-R and HO-BM, respectively.
- **For more details, see Mondeguer *et al.* poster.**

Conclusion

For the first time, this study has analyzed the bacterial ecosystem surrounding the marine diatom *H. ostrearia* and showed that this bacterial structure is specific to the geographic origin of the microalgal isolate. Under laboratory conditions, once *H. ostrearia* has been isolated from oyster ponds, the bacterial community structure was shown to be resilient over a 9-month subculturing despite structural changes at the culture time scale according to the growth stage. Similarly, the differences in bacterial structures of two *H. ostrearia* isolates (HO-R and HO-BM) gave rise to specific metabolomic profiles. These profiles were more distinct with non-axenic microalgae, i.e. with inclusion of their associated bacteria, than with axenic microalgae, thus suggesting reciprocal relationships between bacteria and *H. ostrearia* cells.

Moreover, in parallel of this first study, 36 bacterial strains have been isolated from the raw samples and cultured under appropriate conditions (Marine Agar 2216, 16°C). Afterwards, all isolated strains were tested in co-culture with *Haslea ostrearia* under controlled conditions. Different responses have been observed on algal biomass production, in comparison with a control (*Haslea* mono-culture). Maximal biomass, lag-time and μ_{max} have been modeled in order to select and identify bacteria species enhancing algal biomass. Publication of this second part of COSELMAR action 2.3 will be done in 2016-17.

Results presented here were published in Algal Research in April 2016

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