First metabolomic approach of the epiphytic bacteria-marine diatom *Haslea ostrearia* relationships

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Keys Words: *Haslea ostrearia*, marine diatom, co-culture microalgae, bacterial community, PCR-TTGE, High Resolution Mass Spectrometry, untargeted metabolomics

Introduction:

Haslea ostrearia produces a water-soluble, blue-green pigment, called marennine, with proven economic benefits (as a bioactive compound used to green oysters, which improves their market value). The structure of the bacterial community was analyzed by PCR-TTGE before and after the isolation of H. ostrearia cells recovered from 4 localities, to distinguish the relative part of the biotope and the biocenose and eventually to describe the temporal dynamic of the structure of the bacterial community at two time-scales. A non-targeted metabolomic investigation was undertaken on i) samples with the highest differences in genetic fingerprints and ii) bacteria-H. ostrearia co-cultures vs. microalgal monoculture.

4 Teams _ 2 Subjects: Biology & Chemistry

- **Thierry Lebeau** du laboratoire de planétologies et géodynamique de Nantes (Université de Nantes - CNRS)
  Skills and expertise: **Phytoremediation – Soil Ecology**

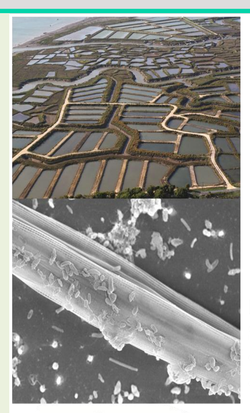
- **Vincent Turpin** du laboratoire « Mer Molécules Santé » (Université de Nantes)
  Skills & expertise: **Marine Ecology - Marine Environment**

- **Hervé Capiaux** de la plateforme d'Analyse Moléculaire Biodiversité-Environnement du département génie biologique (IUT de La Roche/Yon)
  Skills & expertise: Environmental Microbiology - **Molecular Biology**

- **Alexandra Lépinay** : du laboratoire « Mer Molécules Santé » et du laboratoire de planétologies et géodynamique de Nantes (Université de Nantes)
  Skills & expertise: **Microbiology - Cell Biology**

- **Florence Mondeguer** de l'Ifremer, Laboratoire Phycotoxines (Nantes)
  Skills & expertise: **Mass Spectrometry - Metabolomics**

1 Objective



The « Fine Claire verte »

PART 1

Better understand the ecology of the marine diatom

Microalgae-bacteria interactions
Study in:
- Natural environment
- Laboratory

PART 2

Improve growth / Biomass *H.o*

Co-cultivate *H.o* & bacteria

Light micrograph of *Haslea ostrearia*

2 Economic issues:
- Marennine, blue pigment: new molecule of great interest.
- Greening of oysters

Coselmar Project (*Philipp Hess**)
http://www.coselmar.fr/index.cfm?lng=en
Action 2.3: Growth interactions between microorganisms (*Régis Baron***)

Marennine accumulates predominantly in the apical regions of the cells and is composed of different molecules belonging to the same chemical family, whose structure remains unknown.
This molecular complex has demonstrated allelopathic, antioxidant, antibacterial, antiviral, and growth-inhibiting properties

Biology

Experimental design:

For metabolomics fingerprint exudates algae and / or bacteria of 25 samples more the blank experiment.

Microalgae strain of *Haslea ostrearia*								
	Sampling site: Ho Ré-island (46.22N; 1.45°W)				Sampling site: Ho Barre-de-Monts (46.90N; 2.11°W)			
	Monoculture		Co-culture (no axenic strain Ho)		Monoculture		Co-culture (no axenic strain Ho)	
Culture Conditions	With indigenous bacteria algae = no axenic	With antibiotic treatment = axenic	With bacteria effect + on growth (activator) = N ° 10	Bacteria with no effect on growth (inhibitory) = N ° 26	With indigenous bacteria algae = no axenic	With antibiotic treatmen t = axenic	With bacteria effect + on growth (activator) = N ° 10	Bacteria with no effect on growth (inhibitory) = N ° 26

Experiment number : n = 3
More: Control (culture medium ES1 / 3 without algae) n = 3

Material and Methods:

Algae and co culture bacteria_algae were grown in wells plate, six days at 3 · 10^3 cells mL−1

- Daily monitoring of algal growth (2 times per day) by measuring the fluorescence of chlorophyll (BMG LabTech: 440; 680 nm)
- After 6 days of culture (during the exponential growth stage), 200 µL of the culture supernatant were collected, filtered on 0.20-µm PTFE membrane filters and frozen at−80 °C prior to fingerprint acquisition.

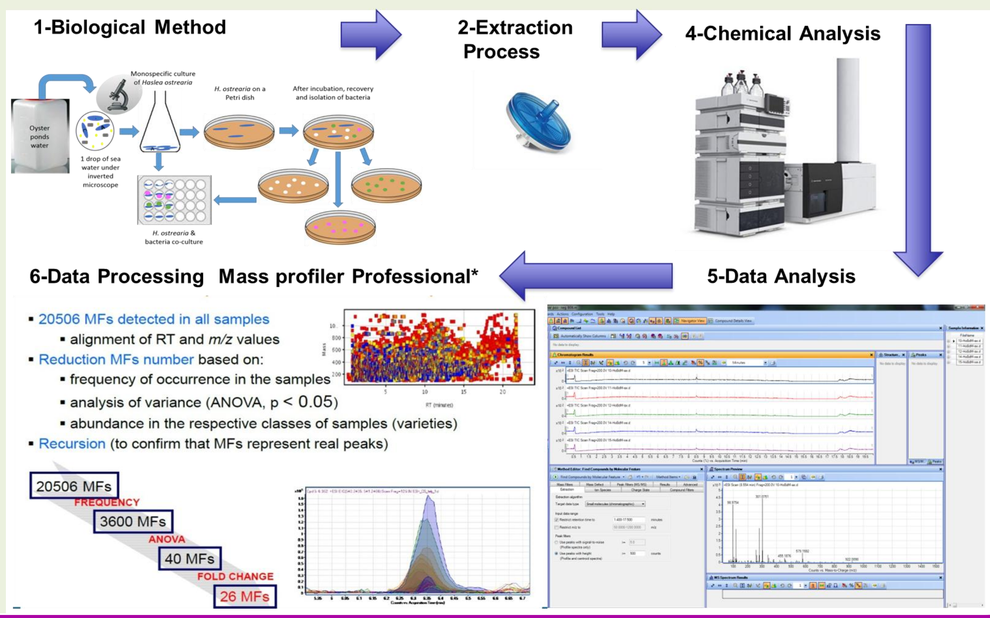
Chemistry

Untargeted metabolomic profiling UHPLC-ESI-QToF, through implementing a non-targeted analytical strategy via high resolution mass spectrometry (HRMS), was used to detect small soluble extracellular target compounds produced by the bacteria and *H. ostrearia* recovered from the culture medium. LC-TOF/MS analysis samples: aliquots (5 µL) of each sample from the supernatant of *H. ostrearia* cultures were separated on a Kinetex, 1.7-µm C18 100 Å (Phenomenex) column (150 × 2.1 mm) maintained at 40 °C, using an Agilent 1290 Infinity LC system with a gradient mobile phase (0.5 mL min−1) comprising 0.1% aqueous acetic acid (A) and acetonitrile containing 0.1% acetic acid (B). The gradient present was as follows: 5% B from0 to 2.4 min, increasing to 25% B from2.4 to 4.5 min, then raised to 30% B from4.5 to 11 min, finally reaching 100% B from11 to 14 min and held there until 16.5 min, followed by a decrease to 5% B until 20 min have elapsed and then maintained at 5% B until 25 min. The eluent was directly introduced into the mass spectrometer by an electrospray. Mass spectrometry was conducted on a 6540 UHD Q-TOF mass spectrometer (Agilent Technologies, Waldbronn, Germany) operating in positive and negative ion mode.

The capillary voltage, fragmentor voltage and skimmer were set at 3900, 150 and 60 V, respectively. The sheath gas was measured at 350 °C (12 mL min−1) and the drying gas at 175 °C (5 mL min−1) with a 43 Psi nebulizer. Nitrogen was used as the collision gas.

Mass spectra were acquired in a full scan analysis over an m/z range of 50–1700 using an extended dynamic range and a centroid mode of storage. The data station operating software was the Mass Hunter Workstation Software (B.06).

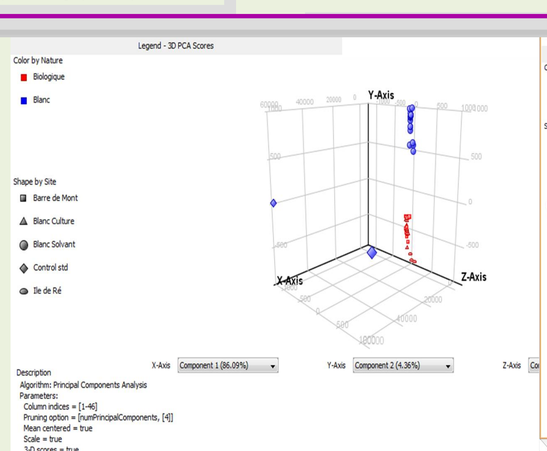
Metabolomics Workflow

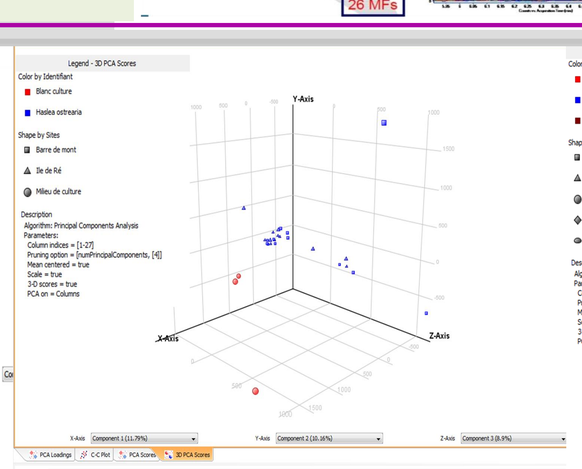


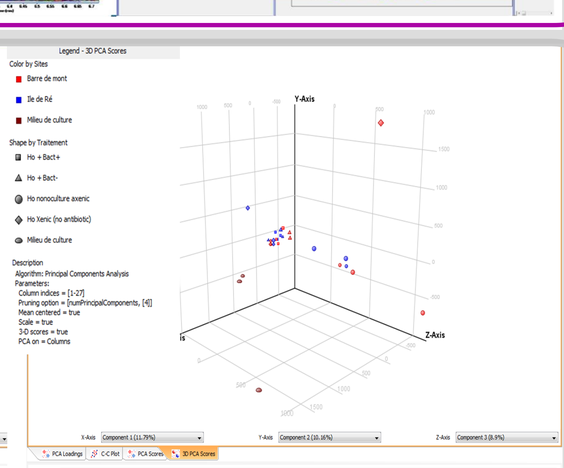
Statistical Best Results

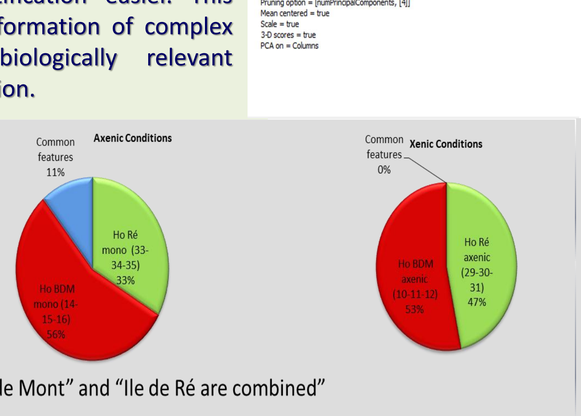
Q-TOF LC/MS metabolomic fingerprinting approach was used to:
- investigate differential metabolites of axenic (i.e. microalgae without their associated bacteria) vs. non axenic H. ostrearia cultures.
- focus on the specific metabolites of H. ostrearia monoculture vs. bacteria-H. ostrearia co-cultures.

The Agilent suite of data processing software makes feature finding, statistical analysis, and identification easier. This enables rapid transformation of complex raw data into biologically relevant metabolite information.









The sampling sites : "Barre de Mont" and "Ile de Ré are combined"

The sampling sites : "Barre de Mont" and "Ile de Ré are distinct

This comprehensive study analyzes and searches compounds of interest by focusing on the following comparisons:
1.Co-culture of bacteria-*H. ostrearia* by testing positive vs. negative bacterial effects on the microalgal growth
2."xenic" monoculture of *H. ostrearia* vs. co-culture with "positive" bacteria
3. "xenic" monoculture of *H. ostrearia* vs. co-culture with "negative" bacteria
HO-R and HO-BM were tested separately and the culture medium background was removed from analysis

1/ This Venn Diagram brings up the specific compounds in each treatments (4) on *Haslea ostrearia* of "Barre de Mont " (filter by frequency)

2/This Venn Diagram brings up the specific compounds in each treatment (4) on *Haslea ostrearia* of "Ile de Ré" (filter by frequency)

More informations with these References:

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