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Preliminary metabolomic approach on the bacterial structure community of *Haslea ostrearia*

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

Introduction:

*Haslea ostrearia (HO)*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. The differences in genetic fingerprints, more especially high between two H. ostrearia isolates from Ile de Ré and Barre de Mont (HO-R & HO-BDM) showed also the highest differences in the bacterial structure as the result of specific metabolomics profiles. The non-targeted metabolomic investigation showed that these profiles were more distinct in case of bacteria-alga associations than for the H. ostrearia 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**

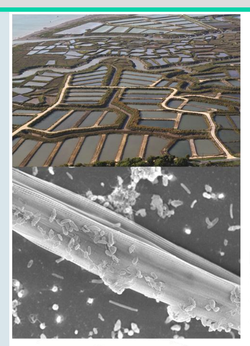
- **Vincent Turpin** du laboratoire « Mer Molécules Santé » (Université de Nantes)
  Skills & expertise: **Marine Ecology - Marine Environment**

- **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***)

** IFREMER, Laboratoire Phycotoxines*
***IFREMER Laboratoire Bioressources marines et bioraffinerie par hydrolyse enzymatique-*

In the marine diatom Haslea ostrearia, a water soluble blue pigment commonly called marennine, which accumulates predominantly in the apical regions of the cells.
Pigment structure are different molecules belonging to the same chemical family, but whose structure remains unknown have shown its 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 treatment = axenic	With bacteria effect + on growth (activator) = N ° 10	Bacteria with no effect on growth (inhibitory) = N ° 26
Experiment number	n = 3	n = 3	n = 3	n = 3	n = 3	n = 3	n = 3	n = 3
more: Control (culture medium ES1 / 3 without algae) n = 3								

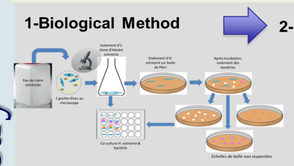
Material and Methods:

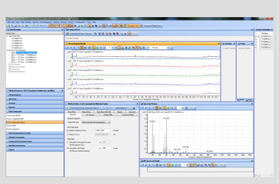
- Algae and co culture bacteria_algae were grown in wells plate, six days at 3 · 103 cells mL−1
- Daily monitoring of algal growth (2 times per day) by measuring the fluorescence of chlorophyll (BMG LabTech: 440; 680 nm)
- After 6 days of culture (during the exponential growth stage), 200 µL of the culture supernatant were collected, filtered on 0.20-µm PTFE membrane filters and frozen at−80 °C prior to fingerprint acquisition.

Chemistry

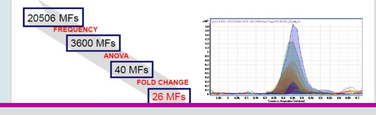
Metabolomics Workflow

1-Biological Method → 2-Extraction Process → 4-Chemical Analysis → 5-Data Analysis → 6-Data Processing Mass profiler Professional*





- 20506 MFs detected in all samples
  - alignment of RT and *m/z* values
- Reduction MFs number based on:
  - frequency of occurrence in the samples
  - analysis of variance (ANOVA, p < 0.05)
  - abundance in the respective classes of samples (varieties)
- Recursion (to confirm that MFs represent real peaks)



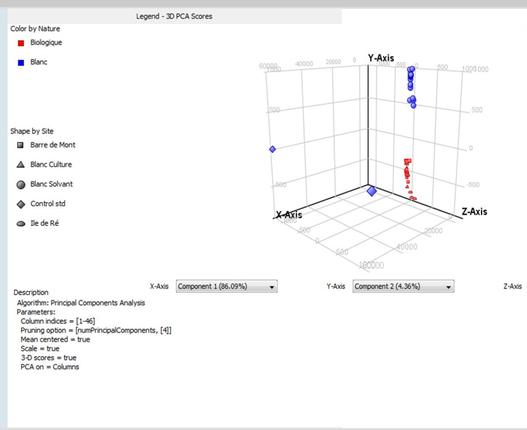
*Agilent Technologies

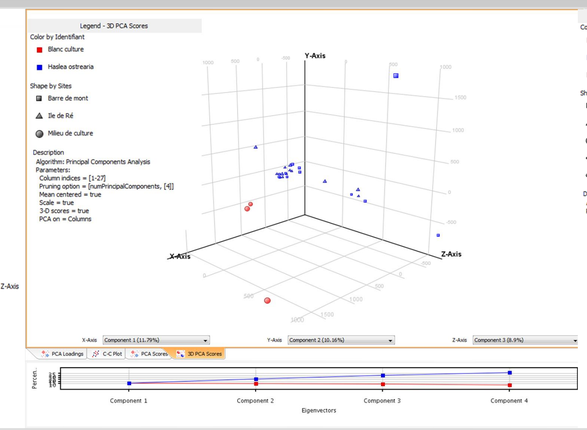
Untargeted metabolomic profiling UHPLC-ESI-QToF, through implementing a non-targeted analytical strategy via high resolution mass spectrometry (HRMS), was used to detect small soluble extracellular target compounds produced by the bacteria and H. ostrearia recovered from the culture medium.
LC-TOF/MS 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).

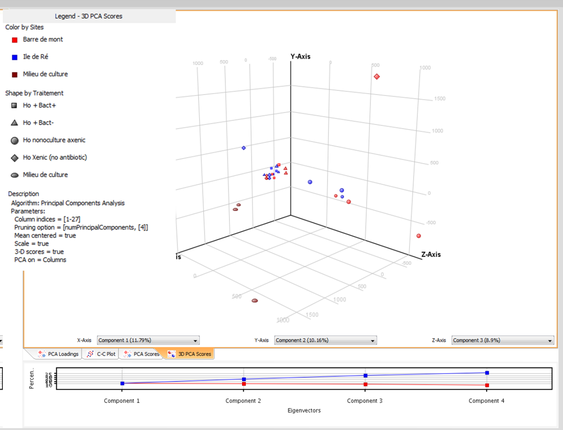
Best Results

Here we present a Q-TOF LC/MS metabolomic fingerprinting approach
- to investigate differential metabolites of axenic versus non axenic H. ostrearia cultures.
- to focus on the specific metabolites of a bacterial surrounding associated with the activation or inhibition of the microalga growing.

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.

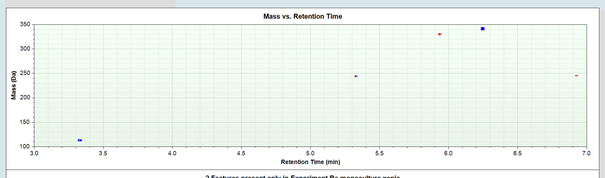


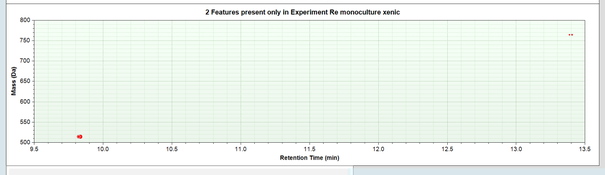




This comprehensive study analyzes and search compounds of interest by focusing on the following comparisons. HO-R and BM clones separated and the removing effect of ES medium 1/3 is done
1.Co-culture inhibitory HO-bacterium vs. activating co-culture HO-bacterium growth of HO
2.Monoculture xenic vs. HO-activating bacteria co-culture
3.Monoculture xenic vs. inhibiting HO-bacteria co-culture

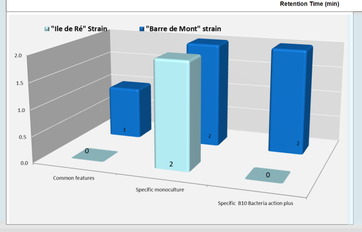
Statistical





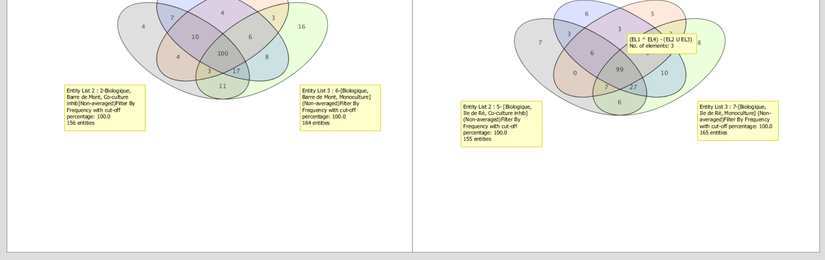
MarK	ID	Rt	Mass	Diff score	RMS	Loading	Corrélation
Control BDM: co-cult B10 action plus_Milieu	1	3.332	113.0837	99.999	6.45	0.458	1
	4	6.247	341.2563	99.939	7.66	0.457	0.99
Experiment: BDM monoculture	3	5.936	330.1802	100	6.29	-0.458	-0.99
	5	6.925	245.2349	100	5.74	-0.458	-0.99
Common	2	5.33	244.0982	99.978	6.38	-0.402	-0.87

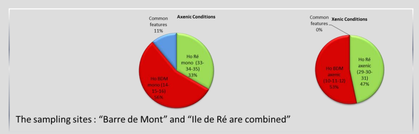
MarK	ID	Rt	Mass	Diff score	RMS	Loading	Corrélation
Control Ré: co-cult B10 action plus_Milieu							
Experiment: Ré monoculture	1	9.826	514.2674	99.995	7.63	-0.707	-1
	2	13.393	764.5218	100	7.29	-0.707	-1
Common							

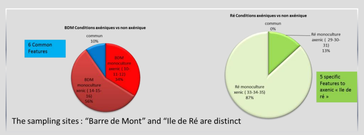


Fingerprinting by feature plots map
Processing data leads a project that contains sample files that are arranged in two groups:
- gr1: Control in Blue Co-culture of Ho and a growth promoter bacteria .
- Gr2: Experiment in red Mono culture of Ho without a growth promoter bacteria

Created method set to features (or compounds) finding, alignment, normalization, and filtering parameters.







More informations with these References:
Lepinay Alexandra, Capiaux Hervé, Turpin Vincent, Mondeguer Florence, Lebeau Thierry (2016). Bacterial community structure of the marine diatom Haslea ostrearia. Algal Research, 16, 418-426. http://doi.org/10.1016/j.algal.2016.04.011
R. Gastineau, N. Davidovich, G. Hansen, J. Rines, A. Wulff, I. Kaczmarska, G. Carrier, Halea ostrearia-like Diatoms: Biodiversity out of the Blue. In Advances in Botanical Re-search: sea plant (2014) pp. 441-465.
T. Lebeau, P. Gaudin, R. Moan, J.M. Robert, A new photobioreactor for continuous ma-rennin production with a marine diatom: influence of the light intensity and the immobilised-cell matrix (alginate beads or agar layer), Appl. Microbiol. Biotechnol. 59 (2002) 153-159.
V. Turpin, Etude des événements physico-chimiques et biologiques présidant à la prolifé-ration d'Haslea ostrearia (Simonsen) dans les claires ostréicoles de la région de Marennes-Oléron: implications dans la maîtrise du verdissement. PhD Thesis, University of Nantes (France), (1999) 208 pp.
F. Mondeguer, J.P. Antignac, Y. Guitton, F. Monteau, S. Le Borgne, P. Hess, Nouvelle stratégie de caractérisation non ciblée de type métabolomique au service de l'identification de composés bioactifs accumulés dans les mollusques bivalves, Spectra Analyse (2012) 24-33.

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