

Innovative metabolomic workflows to study growth kinetics dinoflagellate cysts revived from modern and ancient sediment.

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3 Teams:
 Biology, Chemistry & Metabolomics

- Raffaele Siano, Marie Latimier, Julien Quere, Experimental procedures, Growth curves, responses to P limitation, Dinoflagellate germination from ancient sediment, Conception and design of the study
- Manoëlla Sibat, Philipp Hess, LC-Mass spectrometry data acquisition
- Florence Souard; Yann Guitton, Data analysis workflows W4M 3.0 Galaxy online
- Nicolas Elie, Molecular Network (MetGem), Pierre-Marie Allard; Jean Luc Wolfender, Molecular Network (GNPS) & MetaboMaps Pipeline
- Florence Mondeguer, Experimental design - LC-MS/MS data analysis, Biostatistics (MPP) - Molecular Network, Data interpretations

Keywords: Paleocology; Dinoflagellates cyst; Harmful Algal Blooms (HAB); LC-QToF data processing, Metabolomics workflows; Statistics analysis; Network

Introduction : Aquatic ecosystems worldwide have been negatively affected by eutrophication, many of them driven by increasing nutrient inputs from untreated domestic sewage and industrial and agricultural wastewater. During their life cycle, *Alexandrium minutum* and *Scrippsiella donghaiensis* can produce resistant and revivable cysts that can accumulate in the sediments of the coastal marine environment for hundreds of years. As a working hypothesis, we presumed that these two genera have preserved their adaptation to such ecosystem changes in the form of cysts in the old sediments. From revived cysts, some cultures of toxic and non-toxic dinoflagellates (*A. minutum* and *S. donghaiensis*) were analyzed by an untargeted metabolomics approach by LC-HRMS to study the possible adaptive responses of phytoplankton to these modifications of coastal ecosystems. Strains of *S. donghaiensis* were obtained from sediments isotopically dated to 1986 (ancient) and 2006 (modern) while those of *A. minutum* dated from 1996 (ancient) and 2006 (modern). In total 84 samples were obtained, with each experimental condition (species, age, growth phase) being cultured in triplicate in phosphate depleted conditions. More specifically, two workflows have been used for univariate and multivariate modelling on:

- chemometrics platform, Agilent Mass Profiler Professional (MPP)
- collaborative portal dedicated to metabolomic data processing, analysis and annotation "Workflow4Metabolomics"
- molecular network

Hypothesis Metabolomic Approach

Phenotype untargeted approach

- Q1: Can metabolomics profiles show the inter- and intra- specific diversity of dinoflagellate in a same culture conditions?
- Q2: Can metabolomics profiles of different ages inform about the potential adaptation of a species to the environmental changes occurred across the last century?

Dinoflagellate germination from ancient sediments

Chemistry

Sample extraction lipophilic compounds

- The design: (~200000 cells / mL)
- A liquid-liquid extraction: + 2 mL of DCM and vortexing (x3). The 3 supernatants pooled in centrifuge tube. The total extracted volume dried under N2 at 40 °C
- The 100% methanol residue (1 mL) filtered with a NANOSEP MF centrifugal device (PALL, 0.2 µm)
- 3,500 µg, 30°, 4 °C and directly transferred into a vial
- Stored at -20 °C prior to UHPLC-HR-QTOF MS analysis

Samples cultures:
Scrippsiella donghaiensis
Alexandrium minutum

3,500 µg, 30°, 4 °C

Agilent 6550 (Funnel Q-TOF) LC/MS System

LC-QTOF-MS Analysis:

- Aliquots (5 µl)
- Column: (100 × 2.1 mm) Kinetex 2.6 µm XB C18 100Å-
- (A) 100% water; 95% acetonitrile / water (B), both 2 mM ammonium formate and 50 mM formic acid.
- Flow rate : 0.3 ml min⁻¹.
- Gradient: starting at 5% B during 2 min then rising from 5% B to 50% B in 6 min then raise to 75% B in 3.50 min to finally raise to 100% B and maintain at 100% B during 2 min, subsequently returned to initial condition in 0.50 min and re-equilibrate at 5% B during 4 min.
- Detection: full scan and auto MS/MS (+/- ion mode).
- Mass spectra: m/z range of 100-1700

Data processing

Agilent 1290 Infinity II UHPLC

2 Metabolomics Workflows tested

1st step from: Agilent Mass Profiler Professional (MPP) soft a chemometrics platform

A Q-TOF LC/MS metabolomic fingerprinting approach to investigate differential metabolites of Scrippsiella Donghaiensis versus Alexandrium Minutum cultures.

Specific metabolites can be identified between *Scrippsiella Donghaiensis* (green) and *Alexandrium Minutum* (red) cultures.

Different lipophilic metabolites produced during S. Donghaiensis growth.

2 Metabolomics Workflows tested

2nd step from: Collaborative portal Galaxy-W4M the Workflow4Metabolomics 3.0 Data Processing

The 2 workflows (MPP 1 & W4M) give the same trends but different and complementary relevant metabolite information. The two strains do not have the same distribution:

- Neither in the difference between "ancient" and "modern"
- Neither according to the different phases of growth

Multivariate analysis for species

Scrippsiella Donghaiensis vs Alexandrium Minutum

Multivariate analysis for "Age"

A. minutum strains of different ages cannot be differentiated by their metabolomics profiles

S. donghaiensis strains of different ages have coherent different metabolomics profiles

Pacman? or Start exploring the network with a desktop software for live Molecular Networking

The MetGem¹ new software provides two ways of overcoming GNPS weaknesses. With MetGem, you have a user-friendly, local software for MS/MS comparison and spectral network generation, without the requirement of several different software (FTP client, browser and molecular interaction networks visualizer).

¹Elie, N., Olvon, F., Grelier, G., Litaudon, M. and Toublou, D. (2018). HDISpec: A new desktop software for live Molecular Networking and t-SNE projections of MS/MS spectra. 11èmes Journées Scientifiques du Réseau Francophone de Métabolomique et Fluxomique (RM). 23-25 mai 2018, Liège, Belgique.

Network View

A representation of high-dimensional dataset MS/MS spectra, life cycle of *Alexandrium minutum* and *Scrippsiella donghaiensis*. The color scheme: *Scrippsiella Donghaiensis* (Ancient; Modern) and *Alexandrium Minutum* (Ancient; Modern) shows a great similarity between the two species. In blue the concept proof with 10 external standards.

t-SNE View

Recent technique known as t-distributed stochastic neighbor embedding (t-SNE) can provide a representation of high-dimensional datasets like MS/MS spectra

Compound Name	Formula	Mass	(M+H) ⁺
GYM-A	C32H45NO4	507,33486	508,3421
13-desMe-SPX C	C42H61NO7	691,44448	692,4521
PnTx-G	C42H63NO7	693,46045	694,4677
DTX-2-H2O	C44H68O13	804,46599	787,4623
OA	C44H68O13	804,46599	805,4745
AZA-3	C46H69NO12	827,48198	828,4893
DTX-1	C45H70O13	818,48164	819,4889
AZA-1	C47H71NO12	841,49763	842,5049
AZA-2	C48H73NO12	855,51328	856,5206
PTX2 NH4 ⁺	C47H70O14	858,47656	876,5104