MS-based lipidomics on diatom response to allelopathic chemicals from the red tide dinoflagellate (Karenia brevis)

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Data Type: experimental
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Project
» Waterborne chemical cues in the plankton: a systems biology approach (Plankton Chemical Cues)

Contributors	Affiliation	Role
Kubanek, Julia	Georgia Institute of Technology (GA Tech)	Principal Investigator
Fernandez, Facundo	Georgia Institute of Technology (GA Tech)	Co-Principal Investigator
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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Dataset Description

MS-based lipidomics on diatom response to allelopathic chemicals from the red tide dinoflagellate (Karenia brevis).

Manuscript currently in revision with Nature Scientific Reports contains the analyses of the processed data.

MS data files are also available from the Chorus Project.

Methods & Sampling

The MS instrument was operated in negative electrospray ionization mode with a capillary voltage of -2.0 kV and a sampling cone voltage of 30 V. The source temperature of 90 °C was maintained throughout the experiment. Nitrogen was used as a desolvation gas at 250 °C with a flow rate of 600 L/h. The mass spectrometer was calibrated across the 50-1200 Da mass range using a sodium formate solution. Leucine Enkephalin was infused at a flow rate of 2 µL/min and acquired as a lockmass correction. Run order was randomized and samples were acquired in duplicate. Pooled quality control samples were acquired after every twelfth sample injection to monitor instrumental drift and minimize batch effects.

The UPLC column was operated at 60 °C, while the autosampler tray was maintained at 5 °C. Mobile phase A contained water: acetonitrile (40:60) and mobile phase B contained 10% acetonitrile in 2-propanol. A flow rate of 300 µL/min was used with the following gradient: 0-1 min, 70% B; 1-3 min, 75% B; 3-6 min, 80% B; 6-10 min, 90% B; 10-14 min, 100% B. Both mobile phases included 10 mM ammonium formate (Sigma Aldrich, >99.995%) and 0.1% formic acid (Fluka Analytical) additives to improve peak shape and ionization efficiency. All solvents used were of LCMS grade and provided by OmniSolv (water, acetonitrile) or Honeywell (2-propanol).

Quantitative MS metabolomics data were acquired using a Waters Xevo G2 QTOF mass spectrometer.

Chromatographic separation was accomplished using a Waters Acquity UPLC quaternary solvent manager

system fitted with a Waters ACQUITY UPLC BEH C18 column (1.7-µm particle size, 2.1 × 50 mm), with an injection volume of 10 µL.

Data Processing Description

MS data were imported into Progenesis QI for chromatographic alignment, de-isotoping, adduct deconvolution, normalization, and peak picking. Peaks detected in the sample blanks at greater than 10% of the average sample intensity were removed as potential contaminants. The corresponding normalized intensities across each sample for every feature (m/z, retention time pair) were imported into Matlab for multivariate analysis.

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Data Files

File
MS_lipidomics.csv (Comma Separated Values (.csv), 259 bytes) MD5:ae41ab651d7cb55cdd8e03e58d2bce05 Primary data file for dataset ID 731223

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Parameters

Parameter	Description	Units
description	Description of the file package	dimensionless
file_size	Approximate file size	gigabytes (GB)
download_link	Link to download the file	dimensionless

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Instruments

Dataset-specific Instrument Name	Waters Acquity UPLC quaternary solvent manager system fitted with a Waters ACQUITY UPLC BEH C18 column
Generic Instrument Name	High-Performance Liquid Chromatograph
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	Waters Xevo G2 QTOF mass spectrometer
Generic Instrument Name	Mass Spectrometer
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

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Project Information

Waterborne chemical cues in the plankton: a systems biology approach (Plankton Chemical Cues)

Website: http://devwp.kubanek.biology.gatech.edu/red-tide-competition-and-metabolomics/

Coverage: Gulf of Mexico

Description from NSF award abstract:
Competition is a major force structuring communities, including the marine plankton. The release of compounds that inhibit competitors, a process known as allelopathy, is hypothesized to be important among phytoplankton, especially for species that compete poorly for resources yet form dense blooms. Ecological interactions involving the toxic red tide dinoflagellate *Karenia brevis* present an ideal system for understanding chemically mediated interactions. Blooms of this species occur frequently in accessible coastal areas of the Gulf of Mexico, causing massive fish kills and contaminating shellfish. The dramatic consequences of these blooms motivate the following questions. What strategies does this harmful alga use in competition with other phytoplankton? What lethal and sub-lethal effects are experienced by competitors? How do phytoplankton respond, resist, and detoxify their surroundings? What roles do chemical cues play in these interactions? How are different phytoplankton communities affected by allelopathy?

Previous studies have shown that *K. brevis* is allelopathic to several naturally co-occurring phytoplankton species, but compounds other than the known neurotoxic brevetoxins produced by *K. brevis* generally were responsible. This species produces allelopathic mixtures of unstable, 500-1000 Da organic compounds which cause reduced photosystem II activity and disrupt cell membranes of sensitive species, whereas some other competitors remain unaffected. Moreover, natural blooms of *K. brevis* were allelopathic to the competing diatom *Skeletonema grethae*. This species, in turn, appeared to influence the chemistry of *K. brevis*, reducing its allelopathic effects. Death is a rare outcome of *K. brevis* allelopathy; more subtle, non-lethal responses have predominated. Overall, environmental context may be critical for predicting what ecologically important chemical mediators are released into marine systems and the consequences of these compounds to plankton communities.

The project will:
1) Characterize the exudate metabolome among *K. brevis* samples of varying allelopathic potency. Exudates of *K. brevis* strains and natural bloom samples will be studied by mass spectrometry (MS) and nuclear magnetic resonance (NMR) metabolomics to pinpoint candidate chemical cues involved in competition. *Karenia brevis* protein expression will be examined by MS proteomics to test whether *K. brevis* up- or down-regulates key proteins involved in pathway networks in response to challenges by competitors.

2) Seek to understand sub-lethal metabolic impacts of exposure to allelopathy on target phytoplankton, by studying responses of phytoplankton to *K. brevis* allelopathy by MS-based metabolomics and proteomics. This work will provide an unbiased approach to determining molecular targets of allelopathy and allow testing of whether sub-lethal responses to allelopathy include suppressed fundamental cellular functioning and up-regulated pathways related to stress and detoxification.

3) Relate allelopathic sensitivity to metabolic responses in target phytoplankton, by comparing metabolomic and proteomic changes of sensitive versus resistant competitors to *K. brevis* allelopathy. The expectation is that more resistant species experience enhancement of detoxification pathways and more robust, unaffected cellular function relative to competitors most sensitive to allelopathy.

4) Determine how estuarine and off-shore phytoplankton differ in their physiological responses to allelopathy, because allelopathy may be more important for maintaining dense blooms in near-shore waters than in the initiation of blooms off-shore.

Phytoplankton blooms can be devastating to local economies and pose human health risks. The discovery of new chemically mediated interactions and metabolic responses in the marine plankton could eventually lead to prediction and control strategies to alleviate the harmful consequences of these blooms. Continued effort to characterize mixtures of allelopathic compounds and determine their effects on competing species could lead to biodegradable treatments for reducing phytoplankton or microbial growth in aquatic and terrestrial environments. This study builds on past successes, applying lessons learned from chemistry about ecological processes and using ecological insights to discover unique natural products with important biological functions.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1060300

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