NMR-based polar metabolomics on diatom response to allelopathic chemicals from the red tide dinoflagellate (Karenia brevis)

Website: https://www.bco-dmo.org/dataset/731159
Data Type: experimental
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Project
» Waterborne chemical cues in the plankton: a systems biology approach (Plankton Chemical Cues)

Contributors	Affiliation	Role
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Dataset Description

NMR-based polar metabolomics on diatom response to allelopathic chemicals from the red tide dinoflagellate (Karenia brevis).

The raw NMR spectral data are also available at: https://smartech.gatech.edu/handle/1853/59097

Methods & Sampling

Please see supporting information file associated with publication:
Poulson-Ellestad, Jones, Roy, Viant, Fernandez, Kubanek, Nunn (2014) Metabolomics and proteomics reveal impacts of chemically mediated competition on marine plankton. Proc. Natl. Acad. Sci. USA 111:9009-9014. doi:10.1073/pnas.1402130111

Spectra were collected on a Bruker Avance 500-MHz DRX NMR spectrometer equipped with a 5-mm broadband direct probe, with an excitation-sculpting gradient pulse program for water suppression as follows: 11-μs (90°) pulse, 22-μs (180°) pulse, and 2-ms (108°) shaped pulse (5). The spectral width was 5.5 kHz, with a relaxation delay of 1 s. For each sample, 256 scans were compiled to gather adequate signal for analysis.

Data Processing Description

Spectra were imported into MATLAB, version 7.12.0, and preprocessing was performed in NMRLab (6) and ProMetab 3.3 (7). Spectra were aligned to the chemical shift of the internal standard (TMSP) at 0.00 ppm, manually phased, and baseline corrected. Spectral regions around TMSP (−0.5 to 0.5 ppm), water (4.6–5.0 ppm for A. glacialis; 4.0–4.9 ppm for T. pseudonana), and residual methanol (3.3–3.5 ppm) were removed before noise filtering. For the A. glacialis experiment only in which a small number of K. brevis cells escaped from the dialysis tubing, we removed signals caused by K. brevis cell contamination (identified by their

presence in K. brevis blank culture extracts), to not confound intracellular K. brevis and T. pseudonana metabolites. These regions included the following: 1.41–1.42, 2.65– 2.67, 2.93–2.95, 2.98–3.02, and 4.15–4.40 ppm. Spectra were then binned (0.005 ppm), probabilistic quotient normalized (8) to account for slight differential dilution among samples, generalized log (glog) transformed to account for variance among signals within each sample reducing bias toward highly concentrated metabolites, and mean centered. The lambda values for glog optimization were obtained using a set of five quality control extracts generated with the above methods from a single large batch culture of each diatom species (9). The λ value for T. pseudonana was 9.8068 × 10−8, whereas a λ value of 5.7289 × 10−9 was used for A. glacialis samples.

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Data Files

File
NMR-based_polar_metabolomics.csv (Comma Separated Values (.csv), 421 bytes) MD5:508c78ebf8abdccc0693b998b79a96b5 Primary data file for dataset ID 731159

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Parameters

Parameter	Description	Units
description	Description of the file package	dimensionless
file_size	Approximate file size	megabytes (MB) or kilobytes (KB)
download_link	Link to download the file	dimensionless

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Instruments

Dataset-specific Instrument Name	Bruker Avance 500-MHz DRX NMR spectrometer
Generic Instrument Name	Nuclear Magnetic Resonance Spectrometers
Generic Instrument Description	Instruments that identify and quantify magnetically active chemical entities by subjecting a sample to orthogonal magnetic and electrical fields.

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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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