NMR-based metabolomics of red tide dinoflagellate Karenia brevis

Website: https://www.bco-dmo.org/dataset/720673
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 metabolomics of red tide dinoflagellate *Karenia brevis* exudates with effects on diatom *Asterionellopsis glacialis*.

This dataset includes the following files, packaged in a .zip file:

- archived_spectra.zip = the unprocessed ^{1}H NMR spectral data files underlying the publication "Variable allelopathy among phytoplankton reflected in red tide metabolome dataset."
- NMR_FolderContents-ArchivedSpectraDataset.xlsx = Excel file describing the contents of the spectra folder.
- README_ArchivedSpectraDataset_Harmful_Algae.doc = readme file describing the dataset.

Refer to the following paper for complete methodology:
Poulin RX, Poulson-Ellestad KL, Roy J, Kubanek J. 2018. Variable allelopathy among phytoplankton reflected in red tide metabolome. Harmful Algae 71:50-56. doi.org/10.1016/j.hal.2017.12.002

Methods & Sampling

K. brevis exudates were collected from five strains across three blocks and multiple replicates of each strain within each block, as described in published manuscript.

NMR spectral data were processed using NMRLab software version 0.99.0.0 in Matlab version R2013a (8.1.0.604). Spectra were aligned to the chemical shift of 0.00 ppm using TMSP, manually phased, and baseline-corrected to allow for accurate integration of spectral features. Spectral regions corresponding to solvent signals and unoccupied regions were removed to simplify multivariate analysis (TMSP: -5.0 – 0.5 ppm, residual DMSO: 2.46 – 2.53 ppm, methanol: 2.94 – 3.27 ppm, water: 3.40 – 4.40 ppm, and unoccupied low field region: 8.5 – 15.0 ppm). Spectral features were binned into 0.005 ppm bins, filtered to reduce the impact of noise, probabilistic quotient-normalized to remove the effects of differential dilution among samples, and generalized log (glog) transformed to raise sensitivity to low concentration metabolites within each sample. Glog optimization was conducted using a set of five quality control extracts separately generated from a single

batch of K. brevis culture, yielding λ = 1.3879 x 10-7.

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Data Files

File
NMR_K_Brevis.csv (Comma Separated Values (.csv), 223 bytes) MD5:ba579f53756b4d8bdc2117e6a9851029 Primary data file for dataset ID 720673

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Parameters

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

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Instruments

Dataset-specific Instrument Name	
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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