Laboratory results: Raw mass spectrometer data from a diatom (Thalassiosira pseudonana) grown under CO2-replete and CO2-stressed conditions; conducted in the Kustka lab at Rutgers from 2007-2013

Website: https://www.bco-dmo.org/dataset/474034
Version: 25 March 2014
Version Date: 2014-03-25

Project
» A Matter of Life or Death? Assessing the physiological roles of PCD-related genes to stress adaptation in diatoms (Diatom PCD genes)

Contributors	Affiliation	Role
Kustka, Adam	Rutgers University	Principal Investigator, Contact
Bidle, Kay D.	Rutgers University (Rutgers IMCS)	Co-Principal Investigator
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Table of Contents

- Dataset Description
  - Methods & Sampling
  - Data Processing Description
- Parameters
- Instruments
- Deployments
- Project Information
- Funding

Dataset Description

These data consist of the raw mass spectrometry files collected as part of an effort to understand the proteomic response of the marine diatom *Thalassiosira pseudonana* to varied CO2 concentration. High and low CO2 acclimated cells were grown with 15N-nitrate or natural abundance nitrate and harvested for proteomic analysis.

Data consist of 37 gigabytes of .raw files produced by the Thermo Scientific mass spectrometer. To obtain the data, please contact BCO-DMO.

Description of the data files:
The data files (37 GB in total size) are in .raw format, as produced by the Thermo Scientific mass spectrometer. The investigators used Proteome Discoverer software to analyze the .raw files. The mass spectrometry files are stored under in folders named MS3027 and MS3184, which were collected for the first and second biological replicates, respectively. In cases where individual LC fractions were subject to mass spectrometry multiple times, file names for the repeated analyses are the VLS number followed by "_<n>", where n represents the replicate analysis. Further details for data acquisition and filtering can be found at Kustka et al. 2014 (in second review; New Phytologist).

Methods & Sampling

Culture conditions and photo-physiology upon CO2 shift
Cells were grown in media prepared from synthetic ocean water according to the Aquil recipe (Sunda et al. 2005) and maintained at 18 degrees C and 200 mmol photon m-2 s-1. Macronutrients were added at 100 mmol per liter NO3-,100 mmol per liter Si(OH)4 and 10 mmol per liter PO4-3. All medium preparation and sample handling was carried out in a class 100 laminar flow hood. All plastic ware was acid-cleaned by soaking

in 10% trace metal grade HCl for 5 to10 days and rinsed with 18.2 M-ohm-cm deionized water. Media and culture flasks were microwave sterilized according to Keller et al. (1988) in polycarbonate bottles. Cell density was determined using a Coulter counter (Beckman-Coulter, Fullerton, CA, USA), and growth rates were computed from linear regressions of ln (cell density) versus time. Cells were acclimated to a constant CO2 (1740 mL per liter) and then shifted to 170 mL per liter CO2 within ~ 15 seconds. The pre-shift conditions were designed to provide a saturating supply of CO2 (1620 mL per liter or 58.6 mM compared to a diatom RuBisCO Km of roughly 30 mM; Badger et al. 1998) and obviate the need for CCM expression. This was done by maintaining cultures in house-built pH stats set at pH 7.61 (NBS scale) for >10 generations. At the shift, 1 N NaOH was added while mixing the culture bottle to achieve a pH of 8.48. CO2 was calculated at these pH values using CO2SYS assuming a total alkalinity of 2.67 mM. The pH stats were assembled as follows. A gel-filled combination pH electrode (Thermo, 9106BNWP) was mounted through the wall of a 1L polycarbonate bottle using a bulkhead mount. The electrode potential was monitored using a pH relay (Eutech Inst., pH200 controller). When the pH increased above a set threshold, the relay switched on a peristaltic pump (Master Flex C/L) and an aquarium air pump to deliver weak acid (0.03 N trace metal grade HCl) while mixing the culture.

Quantitative CO2 responsive proteomics
Cultures were grown under steady state conditions of low and high CO2 (~170 and 1740 mL per liter) in pH stats with Aquil media, as described above except that either low or high CO2 cells were grown with 15N-nitrate (>98%) or nitrate with a natural isotopic composition (i.e., 99.6% 14N). Two sets of independent biological replicates from each condition were processed. For each condition, ~900 mL were harvested at 4 -5 x 105 cells per mL and flash frozen in liquid nitrogen. Protein samples were extracted in 4% SDS, 7.5% glycerol, in 0.1 M NaCO3 and protease inhibitor (Sigma-Aldrich P-2714) and quantified before adding 0.1 mM DTT. Fifty micrograms of protein from each condition were mixed yielding a 1:1 ratio of 15N to 14N labeled protein mixture. Subsequent processing was carried out at the Biological Mass Spectrometry Facility of the UMDNJ-Robert Wood Johnson Medical School. The protein mixture was digested by trypsin. Digested peptides were solubilized in buffer A (20mM ammonium formate, pH10) and subjected to high-pH Reverse phase HPLC (Gilson 306 pumps, 805 manometric module and uv/VIS 155 detector) equipped with a XbridgeTM C18 column (3.5um, 2.1x150mm, Waters, MA). The gradient used for separation of the peptides were 2% buffer B (20mM ammonium formate, 90% acetronitrile, pH10) for 2 min, then 2-45% in 43 min, 45 to 100% B in 5 min. 1 min fractions were collected and vacuum dried before combined or individually analyzed by nano-LC-MSMS.

NanoLC-MS/MS was performed using a RSLC system interfaced with a LTQ Orbitrap Velos (ThermoFisher, CA). Samples were loaded onto a self-packed 100µm x 2cm trap packed with Magic C18AQ, 5µm 200 A (Michrom Bioresources Inc, CA) and washed with Buffer A(0.2% formic acid) for 5 min with flowrate of 10ul/min. The trap was brought in-line with the homemade analytical column (Magic C18AQ, 3µm 200 A, 75 µm x 50cm) and peptides fractionated at 300 nL/min with a multi-stepped gradient (4 to 15% Buffer B (0.16% formic acid 80% acetonitrile) in 25 min and 15-25%B in 65 min and 25-50%B in 55 min). The mass spectrometer acquisition cycled through one MS in Orbitrap (resolution 60,000) followed by 20 MSMS (CID) in LTQ with dynamic exclusion (2 repeat count within 30 sec and exclusion time 60sec). The LC-MSMS data were analyzed using Proteome Discoverer software v1.3 (ThermoFisher). The data was first searched against *T. pseudonana* composed of sequences queried from Uniprot) using Sequest search engine through a "light" search assuming normal nitrogen isotope distribution and a "heavy" search assuming all amino acids were labeled with 15N. For both "heavy" and "light" searches, carbamidoethyl on cysteine was used as a fixed modification. For "light" search, oxidation of methionine was included as a variable modification. For "heavy" search, flexible modification of N-terminal modification of -0.997 Da was included as a variable modification. The identified peptides were quantified with custom built precursor ion quantification method within the software.

Data Processing Description

The peptide quantitation results of each HPLC fraction were combined into one list (MUDPIT). Peptides were filtered to include only top ranked identification with confidence above medium. The heavy/light ratio of each peptide was normalized to the median ratios of all peptides. Heavy/light ratio of protein was calculated as median value of all peptides belonging to this protein. The protein ratio was accepted if three or more peptides were quantified.

[table of contents | back to top]

Parameters

Parameters for this dataset have not yet been identified

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Beckman-Coulter, Fullerton, CA, USA
Generic Instrument Name	Coulter Counter
Dataset-specific Description	Used to count cells
Generic Instrument Description	An apparatus for counting and sizing particles suspended in electrolytes. It is used for cells, bacteria, prokaryotic cells and virus particles. A typical Coulter counter has one or more microchannels that separate two chambers containing electrolyte solutions. from https://en.wikipedia.org/wiki/Coulter_counter

Dataset-specific Instrument Name	Rapid Separation nano LC system (RSLC) interfaced with a LTQ Orbitrap Velos
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	The LTQ-Orbitrap-Velos Pro high resolution mass spectrometer possesses different ion fragmentation capabilities including CID, ETD, and HCD. The instrument is useful for applications that require special fragmentation mechanisms, high resolution (100,000 ppm), high sensitivity, and/or rapid duty cycle. See a description from the Biological Mass Spectrometry Facility of the UMDNJ-Robert Wood Johnson Medical School: http://cabm-ms.cabm.rutgers.edu/instruments.html
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.

Dataset-specific Instrument Name	pH electrode (Thermo 9106BNWP)
Generic Instrument Name	pH Sensor
Dataset-specific Description	A gel-filled combination pH electrode (Thermo, 9106BNWP) was mounted through the wall of a 1L polycarbonate bottle using a bulkhead mount. The electrode potential was monitored using a pH relay (Eutech Inst., pH200 controller).
Generic Instrument Description	An instrument that measures the hydrogen ion activity in solutions. The overall concentration of hydrogen ions is inversely related to its pH. The pH scale ranges from 0 to 14 and indicates whether acidic (more H+) or basic (less H+).

[table of contents | back to top]

Deployments

lab_Kustka

Website	https://www.bco-dmo.org/deployment/474414
Platform	Rutgers_Newark
Start Date	2007-09-01
End Date	2013-08-31
Description	Laboratory-based research for the projects "A Matter of Life or Death? Assessing the physiological roles of PCD-related genes to stress adaptation in diatoms" and "Iron storage in diatoms and N2 fixing cyanobacteria: mechanisms, regulation and biogeochemical significance" were conducted at Dr. Kustka's lab at the Rutgers-Newark campus: 101 Warren Street, Smith Hall Room 140 Newark, New Jersey, 07102

[table of contents | back to top]

Project Information

A Matter of Life or Death? Assessing the physiological roles of PCD-related genes to stress adaptation in diatoms (Diatom PCD genes)

From NSF Award Abstract:
Diatoms are a class of unicellular phytoplankton that account for ~40% of total marine primary productivity in the modern ocean. Since downward fluxes of biogenic silica and organic matter in the modern ocean derive largely from diatom productivity, there is increased interest in the mechanistic processes that regulate their fate. Indeed, the physiological state and life history of diatom assemblages dictate whether diatom Si and its associated organic C are either recycled in the euphotic zone, or exported to depth, placing mechanistic importance on cellular processes mediating bloom to post-bloom transition in response to nutrient stress. Programmed cell death (PCD) triggered by specific environmental stresses (e.g., Fe starvation, viral infection, high light, oxidative stress, and UV exposure) has now been documented in a variety of prokaryotic and eukaryotic unicellular phytoplankton, including diatoms. It provides a mechanistic framework to help explain lysis rates independent of grazing. The expression and activation of metacaspases, putative death proteases, in stressed diatoms, suggest PCD is an integral pathway in these organisms. Currently, the ecological role(s) of PCD-related genes in unicellular phytoplankton and the evolutionary drivers selecting for their retention remain unknown. Their preservation would seem to provide a negative selection pressure, yet their retention and maintenance suggests some sort of ancient, selective advantage. Metacaspases (and other PCD-related genes) may have co-evolved with other metabolic pathways whereby retention and low-level expression served housekeeping or regulatory functions. Elucidation of the ecological role(s) of PCD-related genes, like metacaspases, in diatom field populations requires an understanding of cellular function(s) and induction under relevant stressors. **The premise of this proposal is that a subset of putative, PCD-related genes plays heretofore-unappreciated roles in stress adaptation in marine diatoms. The PIs will investigate whether Fe and N availability differentially regulate their expression and activity in T. pseudonana and in coastal diatoms from the California coast. The researchers will elucidate whether distinct PCD-related genes confer increased fitness under Fe or under N-limitation.** This research follows from the investigators previous results that a subset of PCD-related genes is differentially expressed in T. pseudonana cells in response to Fe stress.

Specific hypotheses are:
(1) Expression and activity of PCD-related genes are controlled by Fe or N availability;
(2) Overexpression of putative PCD-related genes confers increased fitness under nutrient limitation;
(3) Diverse T. psuedonana metacaspases share functional epitope similarities; and
(4) PCD-related genes in coastal diatoms display differential responses to steady-state Fe and N limitation.

This work integrates physiology, biochemistry, genetics, ultra-trace metal clean techniques and field-based sampling in order to elucidate the ecological function of metacaspases in diatoms and to identify their roles and regulation in natural diatom populations under Fe versus N limitation. This novel interdisciplinary approach is particularly suited to address the roles of these fascinating enzymes.

Peer-reviewed Publications:
Kimberlee Thamatrakoln, Benjamin Bailleul, Christopher M. Brown, Maxim Y. Gorbunov, Adam B. Kustka, Miguel Frada, Pierre A. Joliot, Paul G. Falkowski, and Kay D. Bidle. 2013. Death-specific protein in a marine diatom regulates photosynthetic responses to iron and light availability. Proceedings of the National Academy of Sciences; Early Edition, EE. DOI: 10.1073/pnas.1304727110

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0927733

[table of contents | back to top]