Shotgun Proteomics of Pseudonitzschia multiseries Multi stress incubations.

Website: https://www.bco-dmo.org/dataset/719073
Data Type: experimental
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Project
» Collaborative Research: Linking geochemistry and proteomics to reveal the impact of bacteria on protein cycling in the ocean (Bacterial Recyclers)

Contributors	Affiliation	Role
Nunn, Brook L.	University of Washington (UW)	Principal Investigator
Boyd, Philip W.	University of Tasmania (UTAS)	Co-Principal Investigator
Switzer, Megan	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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Dataset Description

Cultures were collected, filtered, and bacterial fractions were lysed, digested and analyzed using proteomic mass spectrometry.

Data are available for download at the EBI PRIDE Archive and at the Chorus Project Archive.
EBI PRIDE
Homepage: http://www.ebi.ac.uk/pride/archive
Project URL: http://www.ebi.ac.uk/pride/archive/projects/PXD006468
Data URL: http://www.ebi.ac.uk/pride/archive/projects/PXD006468/files

Chorus Project
Data URL: https://chorusproject.org/anonymous/download/experiment/-8966972342285284 87

Data are published in Boyd, P.W., Dillingham, P.W., McGraw, C.M., Armstrong, E.A., Cornwall, C.E., Feng, Y.y., Hurd, C.L., Gault-Ringold, M., Roleda, M.Y., Timmins-Schiffman, E., Nunn, B.L. (2016). Physiological responses of a Southern Ocean diatom to complex future ocean conditions. Nature Climate Change 6, 207-213.
DOI: 10.1038/nclimate2811

Methods & Sampling

Bottles were removed from the incubators, and the sampling ports carefully disconnected within the laminar flow hood. The cells were then re-suspended by gently inverting each bottle. Samples for pH were taken after the bottles were returned to the incubator. Sampling for all experimental parameters in each bottle was carried out on day 0, 4 and 9 (all treatments), and then days 11 and 14 for treatments B and D and on day 15 for A and day 17 for A and C. The following protocols were employed at each sampling point. For cell counts 1 ml samples were fixed with 50% glutaraldehyde to a final concentration of 0.5% and stored at 4 °C. Cells were counted with an Olympus CKX 41 inverted microscope using a 0.1 ml nannoplankton chamber (PhycoTech). Protocols for in vivo and in vitro chlorophyll analysis, active fluorescence, and the calculation of chlorophyll-based cell growth rates followed those in refs 56, 57. Cellular particulate C and N were analysed in a Thermo

Flash 2000 CHN Elemental Analyser. Particulate P and biogenic Si were analysed following procedures in ref. 60 and ref. 61, respectively, and converted to cellular elemental composition based on cell counts. The low cell abundances in treatment C resulted in some assays being close to the limits of detection and in some cases being lower than the blanks. No subsamples were taken for dissolved iron analysis during the experiment, but confirmation of no trace-metal contamination of the treatments was obtained indirectly by monitoring several physiological metrics—such as C/chlorophyll, growth rate or cellular silica (see Supplementary Table 4)—that are sensitive to iron supply.

Data-dependent tandem mass spectrometry was carried out on a Thermo Scientific Q-Exactive tandem mass spectrometer following protocols detailed in (Poulson-Ellestad, K. L. *et al*. Metabolomics and proteomics reveal impacts of chemically mediated competition on plankton. *Proc. Natl Acad. Sci. USA* **111**, 9009–9014 (2014).

Cells were pelleted (10,000 × g; 10 min) on ice and lysed using a titanium microtip sonicating probe. Each sample received 10 sonication events (10–15 s each) in 0.2% sodium 3-(4-(1,1-bis(hexyloxy)ethyl)pyridinium-1-yl) propane-1-sulfonate (PPS silent surfactant; Agilent Technologies) in 50 mM ammonium bicarbonate. The details of the digestion were per the manufacturer’s guidelines. Disulfide bonds were reduced with DTT and alkylated with iodoacetamide. Each sample received trypsin at an enzyme-to-protein ratio of 1:50, were vortexed, and were incubated on a Thermomixer for 4 h at 37 °C. Peptide concentrations were measured for each sample using a Thermo Scientific NanoDrop 2000/2000c spectrophotometer. The peptide bond absorbance was monitored at 205 nm, and samples were diluted to yield a final concentration of 100 µg protein·mL−1 . Mass Spectrometry-Based Proteomics. Samples were separated and introduced into the Thermo Scientific Q-Exactive tandem mass spectrometer by reversed-phase chromatography using a 30-cm-long, 75-µm-i.d. fused silica capillary column packed with C18 silica particles (Magic C18AQ, 100 Å, 5 µ; Michrom, Bioresources) fitted with a 2-cm-long, 100-µm-i.d. precolumn (Magic C18AQ, 200 Å, 5 µ; Michrom) . Peptides were eluted using an acidified [formic acid, 0.1% (vol/vol)] water/acetonitrile gradient (2–35% acetonitrile over 90 min). Mass spectrometry was performed on a Thermo Fisher QExactive (QE). Based on peptide concentrations, a total of 1 µg of peptide digest in 10 µL of 2% acetonitrile, 0.1% formic acid was sampled per LC/MS analysis.

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Data Files

File
PsNitz_multiseries_PRIDE_boyd.csv (Comma Separated Values (.csv), 389 bytes) MD5:be88ad6975f3c9ac5bc7df315a2d3a1f Primary data file for dataset ID 719073

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Parameters

Parameter	Description	Units
Repository	Name of database where data are currently served	unitless
Project	Unique project identifier for the database where data are currently served	unitless
URL	Link to the data.	unitless

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Instruments

Dataset-specific Instrument Name	Thermo Flash 2000 CHN Elemental Analyser
Generic Instrument Name	Elemental Analyzer
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	Olympus CKX 41 inverted microscope
Generic Instrument Name	Inverted Microscope
Generic Instrument Description	An inverted microscope is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. It was invented in 1850 by J. Lawrence Smith, a faculty member of Tulane University (then named the Medical College of Louisiana). Inverted microscopes are useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope. Inverted microscopes are also used in micromanipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath using reflecting objectives. The stage on an inverted microscope is usually fixed, and focus is adjusted by moving the objective lens along a vertical axis to bring it closer to or further from the specimen. The focus mechanism typically has a dual concentric knob for coarse and fine adjustment. Depending on the size of the microscope, four to six objective lenses of different magnifications may be fitted to a rotating turret known as a nosepiece. These microscopes may also be fitted with accessories for fitting still and video cameras, fluorescence illumination, confocal scanning and many other applications.

Dataset-specific Instrument Name	Thermo Scientific Q-Exactive tandem 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

Collaborative Research: Linking geochemistry and proteomics to reveal the impact of bacteria on protein cycling in the ocean (Bacterial Recyclers)

Text from NSF award abstract:

Although proteins represent the primary source of new organic nitrogen in the ocean, the identification of individual proteins and mechanisms modulating their preservation has faced analytical and computational challenges in deciphering the vast suite of possible sequences and degradation by-products. Recent efforts to link geochemical cycling, biomedical proteomics and bioinformatics has demonstrated that only a small subset of the suite of proteins produced by marine diatoms appear to survive the degradation process, and those that do are largely protected by physical and enthalpic barriers to microbial attack. Although these discoveries help to explain the survival of individual proteins, they also generate multiple questions regarding bacteria as the dominant recyclers of organic nitrogen and carbon and needs for specific approaches to characterize modified protein products. Bacteria dominate the water column and sedimentary systems in both numbers and diversity, yet their relative contribution to the preserved proteomic pool appears low.

In this project, researchers at Old Dominion Universityand the University of Washington will join forces to decipher the bacterial role in protein recycling and their potential contribution. By integrating high mass accuracy tandem mass spectrometry-based proteomics with stable isotope-based geochemical analysis, they hope to identify those bacterial proteins initially synthesized during organic matter recycling. Three research objectives drive this investigation: (1) to determine the potential contribution of bacteria proteins to marine organic matter; (2) to identify those protein(s) synthesized by heterotrophic marine bacteria during initial stages of organic matter degradation; (3) to determine if glycan (carbohydrate) modifications represent an important component of preserved, yet unidentified, peptides seen in our analysis of oceanic particles and sediments.

Broader Impacts: This project will provide multiple opportunities for interdisciplinary student training in marine chemistry and proteomics as well as address the goal of disseminating results and tools to a broad audience. In the more traditional role, this project will expand the career for a female principal investigator in marine proteomics, support both graduate and undergraduate students at ODU which include opportunities for minority enrichment and provide training for a postdoctoral fellow at UW. On the broader level, the ODU PI participates in high school outreach programs for high achieving students in the local school which provides for summer internships and enrichment programs.

Relevant Links:

Old Dominion University: Marine Organic Geochemistry and Ecology Laboratory (MOGEL) Lab Website
Bering Sea Ecosystem Study: Data Archive
Environmental Proteomics: Bacteria Recyclers in the Ocean
Environmental Proteomics: Proteomics of Colwellia psychretheca at subzero temperatures

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1233589

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