Shotgun Proteomics of Bering Sea algal incubations.

Website: https://www.bco-dmo.org/dataset/718886
Data Type: experimental
Version:
Version Date: 2017-11-08

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
Harvey, Rodger	Old Dominion University (ODU)	Co-Principal Investigator
Noble, William S.	University of Washington (UW)	Co-Principal Investigator
Switzer, Megan	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Table of Contents

Coverage

Spatial Extent: **Lat**:59.9037 **Lon**:-176.1278

Dataset Description

Bering Sea water was collected during Bering Sea Ecosystem Study (BEST; Wiese et al. 2012) cruises on the outer shelf during the spring of 2009 (59.9037°N, 176.1278°W; sampling depth 5 m; water column depth 136 m) and 2010 (56.7272° N, 169.4271°W; sampling depth 36 m; water column depth 104 m) in areas which coincided with the developing spring bloom adjacent to the retreating ice.

Cruise: BEST Cruise 2012 (Bering Sea Ecosystem Study)

Water was collected from ship trap samples, filtered, and algal fractions were lysed, digested and analyzed using proteomic mass spectrometry.

Data are available for download at the EBI PRIDE Archive. Project number: PXD006417

Homepage: https://www.ebi.ac.uk/pride/archive
Project URL: https://www.ebi.ac.uk/pride/archive/projects/PXD006417
Data URL: https://www.ebi.ac.uk/pride/archive/projects/PXD006417/files

Data are published in Moore, E.K., Faux, J., Goodlett, D.R., Harvey, H.R., and Nunn, B.L. 2014. Protein recycling in Bering Sea algal incubations. Marine Ecology Progress Series. 515:45-59. DOI: 10.3354/meps10936

Methods & Sampling

Bering Sea water was collected during Bering Sea Ecosystem Study (BEST; Wiese et al. 2012) cruises on the outer shelf during the spring of 2009 (59.9037°N, 176.1278°W; sampling depth 5 m; water column depth 136 m) and 2010 (56.7272° N, 169.4271°W; sampling depth 36 m; water column depth 104 m) in areas which coincided with the developing spring bloom adjacent to the retreating ice. The phytoplankton community at the time of sampling was diatom dominated (Lomas et al. 2012, Moran et al. 2012). In each year, single 20 l carboys were filled from the CTD rosette taken from the chlorophyll maximum based on chlorophyll fluorescence at the time of sampling. In order to increase the amount of algal material for analysis throughout the incubation, 1 l of concentrated phytoplankton material was obtained by gently passing 10 l of CTD water from an additional bottle through a 10 µm mesh and combined with untreated seawater to make up the 20 l incubation. Macrozooplankton were excluded from the incubation by passing CTD water through a 1 mm plankton net before being added to the 20 l carboys.

Incubations were placed in shipboard −1°C cold rooms for 11 d (2009) and 53 d (2010) for the duration of the experimental period. Carboys were covered to exclude light throughout the incubations and aerated with filtered air. At regular time points, carboys were gently mixed until algal material was homogeneous - ly distributed, and 1 l water samples were collected and filtered onto 25 mm pre-combusted glass fiber filters (GF/F) and 37 mm polycarbonate (0.2 µm) filters for analysis (Table 1). In addition, whole water samples were collected at each time point and filtered onto 0.2 µm filters, DAPI stained, and fixed onto microscope slides for bacterial counts. All incubation particles and bacterial slides were stored at −70°C until analysis or counting. Stained bacterial cells were counted on an Olympus BH2-RFCA fluorescent microscope.

Total hydrolyzable amino acids (THAAs) were identified and quantified by gas chromatography/ mass spectrometry (GC/MS) using the EZFaast method (Phenomonex), which uses derivatization of amino acids with propyl chloroformate and propanol for detection (Waldhier et al. 2010). Briefly, suspended particles collected on GF/Fs were hydrolyzed for 4 h at 110°C (Cowie & Hedges 1992) with 6 M ana lyticalgrade HCl and L-γ-Methylleucine as the recovery standard. Following hydrolyzis and derivatization, amino acids were quantified using an Agilent 6890 capillary GC with samples injected at 250°C and separated on a DB-5MS (0.25 mm ID, 30 m) column with H2 as the carrier gas. The oven was ramped from an initial temperature of 110°C to 280°C at 10°C min−1 followed by a 5 min hold. Amino acid identification was accomplished by an Agilent 5973N mass spectrometer run under the same conditions with helium as the carrier gas and mass spectral acquisition over the 50 to 600 Da range. The protein bovine serum albumin (BSA) was analyzed in parallel to correct for responses among individual amino acids and calculation of molar ratios. The analytical precision (% relative standard deviation) for amino acid analysis was ±5%. Amino acids were normalized to percent carbon and nitrogen present in bulk samples analyzed by standard combustion methods. Total protein content was also estimated by the Bradford assay (Bradford 1976).

Incubation particles collected on polycarbonate filters were extracted for proteins with pulse sonication in 6 M urea with a Branson 250 sonication probe at 20 kHz for 30 s on ice. The extracts were then frozen at −80°C, thawed, and sonicated again for 30 s on ice. This was repeated for a total of 5 sonications and 4 freeze/thaw cycles. Filter extracts of each incubation time point were then digested in 3 replicate groups: (1) standard tryptic digestion with reduction and alkylation (Nunn et al. 2010); (2) digestion with Endoproteinase GluC (Endo GluC), which cleaves peptide bonds C-terminal to glutamic acid (Drapeau et al. 1972) and to a lesser extent aspartic acid (Birktoft & Breddam 1994), to increase the number of proteins identified; and (3), incubation with Peptide N-Glycosidase F (PNGase F), which hydrolyzes nearly all types of N-glycan chains from glycoproteins and glycopeptides (Maley et al. 1989), in order to observe potentially modified proteins prior to tryptic digestion. All digests were concentrated using a speedvac to a volume that gave a final protein concentration of 1 µg per 10 µl based on measured protein concentrations of filter extracts. The uniform 1 µg per 10 µl protein concentration ensured that results would not be biased by sampling, or protein concentration differences at different incubation time points.

Protein identification of sample digests was performed via shotgun proteomic tandem mass spectrometry (MS2; Aebersold & Mann 2003). Digests were analyzed using full scan (specific mass to charge ratio [m/z] 350−2000), followed by gas phase fractionation with repeat analyses over multiple narrow, but overlapping, m/z ranges (Yi et al. 2002, Nunn et al. 2006). Mass spectra were evaluated and database searched with an in-house copy of SEQUEST (Eng et al. 1994, 2008). All searches were performed with no assumption of proteolytic enzyme cleavage (e.g. trypsin, Endo GluC) to allow for identification of protein degradation products due to microbial recycling. A fixed modification was set for 57 Da on cysteine and a variable modification of 16 Da on methionine resulting from alkylation and reduction steps, respectively. A variable 1 Da modification was set for asparagine on PNGase F + trypsin digested samples to account for the conversion of asparagine to aspartic acid after cleavage of glycan chains with the use of PNGase F (Plummer et al. 1984), which takes place specifically at the consensus sequence Asn-XxxSer/Thr where Xxx can be any amino acid except proline (Bause & Hettkamp 1979).

[table of contents | back to top]

Data Files

File
BeringSt_Algal_Incubations_Moore.csv (Comma Separated Values (.csv), 193 bytes) MD5:3cb49d88b08ea15c8fa7ddd4cdc70ad9 Primary data file for dataset ID 718886

[table of contents | back to top]

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
Project_URL	Link to project page at the database where data are currently served	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	CTD - profiler
Generic Instrument Description	The Conductivity, Temperature, Depth (CTD) unit is an integrated instrument package designed to measure the conductivity, temperature, and pressure (depth) of the water column. The instrument is lowered via cable through the water column. It permits scientists to observe the physical properties in real-time via a conducting cable, which is typically connected to a CTD to a deck unit and computer on a ship. The CTD is often configured with additional optional sensors including fluorometers, transmissometers and/or radiometers. It is often combined with a Rosette of water sampling bottles (e.g. Niskin, GO-FLO) for collecting discrete water samples during the cast. This term applies to profiling CTDs. For fixed CTDs, see https://www.bco-dmo.org/instrument/869934.

Dataset-specific Instrument Name	
Generic Instrument Name	Fluorescence Microscope
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

Dataset-specific Instrument Name	Agilent 6890 capillary GC
Generic Instrument Name	Gas Chromatograph
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	Agilent 5973N
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.

[table of contents | back to top]

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

[table of contents | back to top]

Funding

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

[table of contents | back to top]