Metaproteomics of Bering Strait Ship-board 10 day bacterial Incubations.

Website: https://www.bco-dmo.org/dataset/719129
Version:
Version Date: 2017-11-13

Project
» Collaborative Research: Proteins as functional biomarkers: integrating organic characterization with proteomics to track routes for carbon and nitrogen recycling and preservation (Proteins as Biomarkers)

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

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Dataset Description

Data are available for download at the EBI PRIDE Archive.
Homepage: http://www.ebi.ac.uk/pride/archive
Project URL: http://www.ebi.ac.uk/pride/archive/projects/PXD006688
Data URL: http://www.ebi.ac.uk/pride/archive/projects/PXD006688/files

Data are published in Timmins-Schiffman, E., May, D.H., Mikan, M., Riffle, M., Frazar, C., Harvey, H.R., Noble, W.S., Nunn, B.L. (2016) Critical decisions in metaproteomics: achieving high confidence protein annotations in a sea of unknowns. The ISME Journal. DOI: 10.1038/ismej.2016.132

Methods & Sampling

Water samples were collected in August of 2013 using a 24 bottle (10 L General Oceanics Niskin X) rosette equipped with CTD sensors; 160 liters of water were collected from the Bering Strait (BSt) chlorophyll maximum layer (7 m depth; 65° 43.44”N, 168° 57.42”W). The integrated initial *Chl a* measurement was 226.88 mg/m2, temperature was 2.06°C and salinity was 32.15.

Water was prefiltered to remove all eukaryotic grazers and particles by sequential filtration: 10.0 µm followed by a 1.0 µm polycarbonate (PC) filter. For the generation of a site/time-specific metagenome, seven 1-L aliquots of 1.0 µm prefiltered water was filtered onto 0.2 µm PC filters for a total of 7 filters. Metaproteomics samples of free-living ocean microbes were collected on 0.2 µm Nuclepore Track-Etch membrane PC filters (Whatman, Maidstone, UK) from shipboard incubations at 0 ºC in the dark over 10 days (T0 = day 0, T10 = day 10). The 10-day incubation was designed to follow the changes in community composition and metabolism with and without an exogenous source of carbon. Upon collection, all filters were immediately frozen in liquid nitrogen and stored at -80 ºC. In the full experiment, multiple parameters (in addition to proteomics) were measured at several time points over the 10-day incubation (manuscript in prep.). There are, of course, limitations to measuring community function during an incubation in a closed system, but those limitations will

be addressed more fully in the biologically- and ecologically-focused future manuscript.

Filters for the metaproteomics analysis were sliced and submerged in 100 µl of 6 M urea and 600 µl of 50 mM NH4HCO3. Whole cell lysing was accomplished with a Branson 250 probe sonicator (duty cycle 40%; output control 3) for 20 seconds (Branson Ultrasonics, Danbury, CT). Between sonication events, each filter was flash-frozen in liquid nitrogen to reduce protease activity. Protease inhibitors are avoided because they interfere with downstream digestion and mass spectrometry. This sonication-freezing cycle was repeated five times. Filters were rinsed two times in 100 µl of ultrapure water to remove remaining cellular debris from the filter. Protein extraction and desalting followed the protocol outlined in Nunn et al., (2015).

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was done on a nanoAcquity UPLC (Waters Corp, Milford, MA) connected to a Q-Exactive-HF (Thermo Fisher Scientific, Waltham, MA) on technical duplicates for each sample. A total of 11 mixed microbial samples were analyzed.

Analytical and trapping columns were made and packed in-house. Laser-pulled analytical columns were packed with 3 µm C18 beads (Dr. Maisch HPLC GmBH, Germany) to 15 cm (75 µm ID) and 2 cm trapping columns (100 µm ID) were packed with 3 µm C12 beads (Dr. Maisch) with a Kasil-frit. Peptides were eluted from the column with 5%-30% ACN, 0.1% formic acid in 90 minutes using a 300 nl/min flow rate. The mass spectrometer was operated in Top 20 DDA mode with an MS1 scan range of 400-1000 m/z, 15 second dynamic exclusion, and to increase sensitivity of microbial peptides, 5 trypsin peptide ions were added to the exclusion list (421.7598, 842.5121, 532.2871, and 1045.5664). Mass accuracy and chromatographic retention time were monitored using a quality control standard of 15 peptides (PRTC-BSA, Thermo Fisher Scientific).

Instrument(s): QExactive Thermo Finnegan: DDA data

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Data Files

File	
BeringSt_Incubations_Mikan_Timmins.csv	(Comma Separated Values (.csv), 210 bytes) MD5:d65f19d9c1398edfece2bee2efc06fd8
Primary data file for dataset ID 719129	

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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
Experiment_ID	unknown	unitless
Project_URL	Link to project page where data are currently served.	unitless

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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	nanoAcquity UPLC (Waters Corp, Milford MA) connected to a Q-Exactive-HF (Thermo-Fisher Scientific, Waltham MA)
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.

Dataset-specific Instrument Name	10 L General Oceanics Niskin X
Generic Instrument Name	Niskin bottle
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

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Project Information

Collaborative Research: Proteins as functional biomarkers: integrating organic characterization with proteomics to track routes for carbon and nitrogen recycling and preservation (Proteins as Biomarkers)

Coverage: Multiple locations: Canadian and US Beaufort Sea

NSF Award Abstract:
Proteins are a major contributor to organic carbon and nitrogen in the ocean and their amino acid building blocks comprise a major fraction of the total nitrogen identified in coastal and oceanic waters and sediments. Over the last decade, tandem mass spectrometry methods developed for the analysis of peptides and protein

reconstruction together with informatics developments have re-assembled the amino acid signatures and unlocked the information inherent in the proteins they represent. This interdisciplinary research team has the combined expertise in marine organic biogeochemistry, proteomics, and bioinformatics to help determine the fate of individual proteins during degradation and likely mechanisms for their preservation. This research aims to identify and quantify the proteins responsible for chemical transformations of organic matter in the ocean, thereby exposing how microbial communities control and contribute to the carbon and nitrogen cycle and to organic matter preservation. This project has the potential to trigger a fundamental change in how we view, analyze, and model microbial degradation processes.

Recent findings from microbial ecologists show that bacteria with broad responses to various nutrients (i.e. the generalists) can fine-tune the gene expression of various proteins as a reflection of substrate availability; the proponents of this project have observed a suite of bacterial proteins present during organic matter recycling and that this protein expression changes as degradation proceeds. These observations hint that bacterial proteins have potential as organic biomarkers to reflect the taxonomic distribution of the functioning catalysts in degradation processes. The investigators propose to build on these results to investigate the potential for organic matter preservation to be linked to proteomic expression of the microbial community that drive carbon and nitrogen cycling. The project objectives are: 1) to address the relationship between marine organic matter composition and protein expression by the bacterial community (metaproteome) which act as catalysts for degradation; 2) to detail protein expression in the context of detailed organic matter characterization and 3) to distinguish how proteins present in oceanic sediments derived from different kingdoms of life (e.g. eukaryotic or bacterial) reflect the complex process of organic matter preservation. The overall goal is to capture proteins as critical markers of both presence and process in marine systems. The investigators will link the rapid advances in protein identification to track bacterial proteins to act as "functional biomarkers" and indicators of carbon and nitrogen utilization.

The project would provide multiple opportunities for interdisciplinary student training in marine geochemistry and proteomics, and address the goal of disseminating results and tools to a broad audience. In the more traditional role, the project will expand the career for a female research faculty member in marine proteomics and support both graduate and undergraduate student research at each participating institution. On the broader community level, both Harvey and Nunn are also heavily involved in high school outreach programs.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1633939

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