Proteomics Colwellia psychrerythraea strain 34H at subzero temperatures.

Website: https://www.bco-dmo.org/dataset/719009
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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
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Dataset Description

Bacteria was collected from cultures, filtered, and 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/PXD006274
Data URL: http://www.ebi.ac.uk/pride/archive/projects/PXD006274/files

Chorus Project
Data URL: https://chorusproject.org/anonymous/download/experiment/-1211384729456854716

Data were published in Nunn et al (2015).

Methods & Sampling

Colwellia psychrerythraea strain 34H (ATCC No. BAA-681; GenBank Accession No. AF396670) used in this study was originally isolated from Arctic marine sediments (Huston et al., 2000). Cells were cultured from frozen glycerol stocks by incubating at -1∞C with daily inversion in half-strength Marine 2216 broth until early stationary growth phase (2 weeks or less). Prior to the initiation of the experiments, cells were harvested by centrifugation (2800◊g; 20 min; 4∞C). After washing with cold (-1∞C), 0.2µm filtered ASW {0.1M NaCl, 0.01M KCl, 0.06M MgCl2, 8mM MgSO47H20, 5mM TAPSO [3-(N- tris(hydroxymethyl) methylamino]-2-hydroxypro-pane-sulfonic acid], pH 7.5}, cells were resuspended in ASW (-1∞C) with 1.5g C ml-1 extracellular polysacharide substances to an OD600nm of 0.2 corresponding to an average (±standard deviation) cell count of 5.7E7 ± 1.1E7 cells ml-1. Triplicate 100µl of aliquots of cell suspensions were fixed in 2% formaldehyde, and stored at 4∞C for abundance analysis by epifluorescence microscopy as described by Junge and colleagues (2004b). For [3H]-leucine and [3H]-thymidine incubation experiments, 500µl of aliquots of

the cell suspension were placed into 1.5ml Eppendorf tubes for immediate use.

Bacterial metabolic activity was measured using leucine ([3H]-leu) and thymidine ([3H]-thy) incorporation assays, following methodologies based on the analysis of bacterial activities in freshwater ice and saline ice. To determine rates of [3H]-leu incorporation and [3H]-thy incorporation in the prepared cell suspensions, time-course experiments were conducted at temperatures of -1, -5 and -10∞C and sampling occurred at 0, 1, 12 and 24 h, and 1, 2, 4 and 8 week time points. Triplicate negative (killed) controls were completed at each temperature by adding 100µl of 50% trichloroacetic acid (TCA; 4∞C) to control tubes prior to tracer addition. Methods described by Junge and colleagues (2006) were primarily followed, with the exception of modifications including: (i) samples were placed at their intended temperatures immediately after the addition of the radiolabel tracer, not flash frozen with LN2, (ii) three ethanol washes were performed during the processing stage and (iii) in addition to the [3H]-leu incorporation assay to measure metabolic activity based on protein synthesis, [3H]-thy incorporation was also measured to investigate rates of DNA synthesis.

Based on peptide concentrations, a total of 1µg of peptide digest in 10µl of 5% acetonitrile (ACN), 0.1% formic acid was sampled per LC-MS/MS analysis. Samples were separated and introduced into the mass spectrometer (MS) by reverse-phase chromatography using a 25cm long, 75µm i.d., fused silica capillary column packed with C18 particles (Magic C18AQ, 100 ≈, 5) fitted with a 2cm long, 100µm i.d. precolumn (Magic C18AQ, 200 ≈, 5). Peptides were eluted using an acidified (formic acid, 0.1% v/v) water-acetonitrile gradient (5ñ35% acetonitrile in 90 min). Mass spectrometry was performed on a Thermo Fisher QExactive. The top 20 most intense ions were selected for MS2 acquisition from precursor ion scans of 400ñ1600 m z-1. Quality control peptide mixtures were analyzed every sixth injection to monitor chromatography and MS sensitivity. Skyline was used to determine that QC standards did not deviate >10% through all analyses (Maclean et al., 2010).

Instrument(s): Thermo QExactive

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Data Files

File
Colwellia_Junge.csv (Comma Separated Values (.csv), 392 bytes) MD5:7418e0ecee5eeed88559a02a17d31dde Primary data file for dataset ID 719009

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Related Publications

MacLean, B., Tomazela, D. M., Shulman, N., Chambers, M., Finney, G. L., Frewen, B., … MacCoss, M. J. (2010). Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. Bioinformatics, 26(7), 966–968. doi:10.1093/bioinformatics/btq054
Methods

Nunn, B. L., Slattery, K. V., Cameron, K. A., Timmins-Schiffman, E., & Junge, K. (2015). Proteomics of Colwellia psychrerythraeaat subzero temperatures - a life with limited movement, flexible membranes and vital DNA repair. Environmental Microbiology, 17(7), 2319–2335. doi:10.1111/1462-2920.12691
Results

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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 Fisher QExactive
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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