Metalloproteome protein data of the metalloproteome of Pseudoalteromonas sp. strain BB2-AT2 cultures originally collected from Scripps Pier, California coast in 1995

Website: https://www.bco-dmo.org/dataset/808619
Version: 0
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Project
» US GEOTRACES PMT: Cobalt Biogeochemical Cycling and Connections to Metalloenzymes in the Pacific Ocean (PMT Cobalt and Metalloenzymes)
» Collaborative Research: Underexplored Connections between Nitrogen and Trace Metal Cycling in Oxygen Minimum Zones Mediated by Metalloenzyme Inventories (CliOMZ)

Contributors	Affiliation	Role
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Coverage

Spatial Extent: **Lat**:32.86671 **Lon**:-117.25587
Temporal Extent: 1995

Dataset Description

Metalloproteome protein data of the metalloproteome of Pseudoalteromonas sp. strain BB2-AT2. The metalloproteome is the combined data of the metalloproteome proteins and cytosolic metallome (see dataset https://www.bco-dmo.org/dataset/808610). Physiological work was conducted at the Woods Hole Oceanographic Institution, Woods Hole, MA in 2019. These data will be published in Mazzotta, et al. (in press).

Related Datasets:
BB2-AT2 Cell Metal Quota https://www.bco-dmo.org/dataset/808598
BB2-AT2 Cytosolic Metallome https://www.bco-dmo.org/dataset/808610

These data were also supported by a Camille and Henry Dreyfus Foundation Environmental Chemistry Postdoctoral Fellowship.

Methods & Sampling

Location: All physiological work within this dataset was conducted at Woods Hole Oceanographic Institution on a strain of Pseudoalteromonas collected from Scripps Pier, California coast in 1995.

Methodology:

Referenced from Mazzotta, et al. in press:
Marine broth medium was prepared by microwave sterilization49 of 800 mL of 0.2 µm-filtered Vineyard Sound seawater with the addition of 0.2 µm-filter-sterilized solutions of 4 g peptone (Fisher Scientific) and 0.8 g yeast extract (BD Difco). Marine agar plates were prepared with the same composition as that of the marine broth described herein, with the inclusion of 12 g granulated agar (Fisher Scientific) and sterilization achieved by autoclaving for 30 min. Cultures of Pseudoalteromonas sp. BB2-AT2 were revived from a 20% glycerol stock of BB2-AT2 (provided by Kay Bidle, Rutgers University) and used to inoculate approximately 1 mL of marine broth. The inoculated solution was allowed to incubate at 23 °C for 3 hours, then streaked onto a marine agar plate with 50 µL inoculum and incubated at 23 °C overnight. Pellets of BB2-AT2 were grown in volume 500 mL of marine broth medium by inoculation at 23 °C with selection of a single colony from a marine agar plate. Growth curves were obtained with a SpectraMax Me5 unit (Molecular Devices) through absorbance measurements at 600 nm pipetting aliquots into a Corning clear 96 well plate at each time point. Samples were analyzed at 23 °C with a kinetic run over a period of 18 hours with a read interval of 30 minutes, with aliquots added at every timepoint. To validate this approach these plate reader growth rates were successfully intercompared with growth measurements using a Shimadzu UV-1601 spectrophotometer in a quartz cuvette (b = 1 cm) with a 2.5 mL aliquot of inoculated medium (Supplementary Information). Pellets were harvested in mid-exponential phase by centrifugation at 8,000 rpm for 20 minutes using an Eppendorf 5810R centrifuge at 3 °C, the solution was decanted, and the pellet washed with 0.2 µm-filtered seawater (4 mL x 3). Pellets of biomass

For metalloproteomic analysis, all plasticware used was acid-washed unless otherwise mentioned. Buffers were prepared in Milli-Q water with Tris base (Fisher BioReagents) and NaCl (Sigma Aldrich, trace metal basis) with removal of extraneous metal contaminants from solution by Chelex prepared accordingly.50 The pellet of BB2-AT2 was suspended in approximately 6 mL of 50 mM Tris buffer (pH 8.8) under anaerobic conditions (<1 ppm O2) in an N2–filled anaerobic chamber (Coy Laboratory Products Inc.) using a Fisherbrand Model 120 sonic dismembrator, sonicated for 40 s at 2s on/off intervals while on ice. The resulting solution was then diluted to approximately 30 mL with additional 50 mM Tris buffer (pH 8.8) and centrifuged at 4,000 rpm at 3 °C for 15-20 min. The resulting supernatant was decanted and then purified by anion exchange chromatography using an Agilent 1100 series HPLC. A single-use GE HiTrap Q HP anion exchange column was conditioned prior to use by washing the column with 50 mM Tris buffer (pH 8.8) for 5 min at a flow rate of 1 mL/min, then 50 mM Tris buffer containing 1 M sodium chloride (pH 8.8) for 5 min at a flow rate of 1 mL/min and finally with 50 mM Tris buffer (pH 8.8) for 5 min at a flow rate of 1 mL/min. The supernatant was then loaded onto the column via a 60 mL loading syringe onto the column using Carter 12/8 cassette peristaltic pump at a rate of 0.5 mL/min. The sample-loaded column was then connected to the Agilent HPLC system and eluted with a gradient of sodium chloride at a rate of 0.25 mL/min. The method includes the following gradient- 50 mM Tris buffer (pH 8.8) for 5 minutes, followed by a linear increase from 100% 50 mM Tris buffer (pH 8.8) to 100% 50 mM Tris buffer containing 1 M NaCl (pH 8.8) over the course of 40 min and then 50 mM Tris buffer (pH 8.8, 1 M NaCl) for 8 min. Fractions were collected with a Bio-Rad 2110 fraction collector in 2 mL microtubes at a rate of 1 fraction every 4 min, and immediately stored on ice. Anion exchange fractions were concentrated prior to separation by size exclusion chromatography. 5000 Da molecular weight cutoff PES Vivaspin columns were washed with 0.5 mL (x 2) Tris pH 8.8 buffer prior to use. Anion exchange fractions were loaded onto the Vivaspin columns and centrifuged at 5000 rpm for 30 min using an Eppendorf MiniSpin. 0.1 mL of each concentrated anion exchange fraction was injected into a Michrom BioResources, Inc. HPLC system containing a Tosoh Bioscience 5-µm particle size TSKgel G3000SWxl size exclusion column with guard column. Samples were eluted using an isocratic method of 50 mM Tris buffer (pH 7.5, 50 mM sodium chloride) at a flow rate of 0.5 mL/min. Fractions from size exclusion chromatography were collected using a CTC Analytics HTC Pal autosampler system into an acid-washed 1.2 mL 96-well storage plate (Thermo Scientific), covered for storage until analysis with an acid-washed 96 cap sealing mat (Thermo Scientific). For protein digestion, 90 µL of each sample was diluted with 96 µL 100 mM ammonium bicarbonate solution and 10 µL acetonitrile. Then 15 µL of a freshly-prepared 10 mM dithiothreitol solution was added to the samples and incubated at 70 °C for 30 min with shaking at 400 rpm. The samples were then allowed to cool to room temperature, and 30 µL freshly-prepared 20 mM iodoacetamide was added to each sample and allowed to incubate for 30 min in the dark. Following reduction and alkylation, 10 µL of a 0.03 µg/µL trypsin stock (Promega Gold) was added to each sample and then incubated for approximately 18 hours at 37 °C with shaking at 400 rpm. Samples were then analyzed by an LC-MS on a Q-Exactive (Thermo Scientific) after separation on a C18 column following a gradient of 2-95% acetonitrile over a 90 min gradient using a Michrom Paradigm MG4 liquid chromatography system. Samples were ionized by electrospray with 1800 mV potential. Proteomic analyses were used for peptide spectra matching using the SEQUEST algorithm within Proteome Discoverer (v. 2.1) and visualized in Scaffold (v. 4);

protein threshold = 99.9%; minimum # peptides = 2; peptide threshold = 99%.

Instruments:

All work performed in N2–filled anaerobic chamber (Coy Laboratory Products Inc.).
Anion exchange chromatography: Agilent 1100 series HPLC.
Size exclusion chromatography: Michrom BioResources, Inc. HPLC system containing a Tosoh Bioscience 5-µm particle size TSKgel G3000SWxl size exclusion column with guard column.
Samples were then analyzed by an LC-MS on a Q-Exactive (Thermo Scientific) using a Michrom Paradigm MG4 liquid chromatography system.
Metals analyzed on iCAP-Q inductively coupled plasma-mass spectrometer (Thermo) with an SC-4 DX FAST autosampler (Elemental Scientific, Inc.).

Data Processing Description

Anion exchange fraction (AEF) 8, molecular weight fraction (MWF) 1 was subtracted from all cytosolic metallome data reported herein.

Metal Concentration (µM)
55Mn 0.0060839
56Fe 0.0155962
59Co 0.0003049
64Zn 0.0047519
60Ni 0.0541603
63Cu 0.0008257
95Mo 0.0012932

*All metal data used for metalloproteomic assignments with metalloproteome_proteins
**Negative values were converted to nd; nd was replaced with 0 for computational purposes

Proteomic analyses were used for peptide spectra matching using the SEQUEST algorithm within Proteome Discoverer (v. 2.1) and visualized in Scaffold (v. 4).

BCO-DMO Data Manager Processing Notes
* Original data submitted in Excel file 200329_BB2-AT2 Metalloproteome.xlsx sheet "Metalloproteome_Proteins" extracted as csv with the following modifications.
* The first two rows of the sheet containing AEF and MWF values were unpivoted into data columns. Column "protein_abundance" was added to contain the protein abundances which were the rows under AEF and MWF.
* Values "?" were replaced with blank values.
* Columns renamed to meet BCO-DMO naming conventions designed for interoperability. Column names can not start with a number in order to support usage in various frameworks such as matlab and C++. Prefix "Conc_" added to metal columns (e.g. 55Mn -> Conc_55Mn)
* Column "Strain_Gene#_protein name, Genome location" e.g. value "BB2AT2_1028, glycine dehydrogenase, 110554 - 113445" containing multiple parameters was split into 4 columns Strain_Gene, Protein_name, Genome_location_start and Genome_location_end.

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Related Publications

Mazzotta, M. G., McIlvin, M. R., & Saito, M. A. (2020). Characterization of the Fe metalloproteome of a ubiquitous marine heterotroph, Pseudoalteromonas (BB2-AT2): multiple bacterioferritin copies enable significant Fe storage. Metallomics, 12(5), 654–667. https://doi.org/10.1039/d0mt00034e
Results

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Tosoh Bioscience 5-µm particle size TSKgel G3000SWxl size exclusion column
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Michrom BioResources, Inc. HPLC system containing a Tosoh Bioscience 5-µm particle size TSKgel G3000SWxl size exclusion column with a guard column.
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 1100 series HPLC
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	Anion exchange chromatography: Agilent 1100 series HPLC.
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	Michrom BioResources, Inc. HPLC system
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	Michrom BioResources, Inc. HPLC system containing a Tosoh Bioscience 5-µm particle size TSKgel G3000SWxl size exclusion column with a guard column.
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	iCAP-Q inductively coupled plasma-mass spectrometer (Thermo)
Generic Instrument Name	Inductively Coupled Plasma Mass Spectrometer
Dataset-specific Description	Metals analyzed on iCAP-Q inductively coupled plasma-mass spectrometer (Thermo) with an SC-4 DX FAST autosampler (Elemental Scientific, Inc.).
Generic Instrument Description	An ICP Mass Spec is an instrument that passes nebulized samples into an inductively-coupled gas plasma (8-10000 K) where they are atomized and ionized. Ions of specific mass-to-charge ratios are quantified in a quadrupole mass spectrometer.

Dataset-specific Instrument Name	LC-MS on a Q-Exactive (Thermo Scientific)
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Samples were analyzed by an LC-MS on a Q-Exactive (Thermo Scientific) using a Michrom Paradigm MG4 liquid chromatography system.
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

US GEOTRACES PMT: Cobalt Biogeochemical Cycling and Connections to Metalloenzymes in the Pacific Ocean (PMT Cobalt and Metalloenzymes)

Coverage: Laboratory Study and Cultures from Northeast Pacific Line P Transect 48.8167 N 128.667 W

NSF abstract:

Cobalt is important for many forms of marine life, yet it is one of the scarcest nutrients in the sea. Cobalt's oceanic abundance and distribution, along with other scarce nutrients, can influence the growth of microscopic plants (phytoplankton). This in turn can influence carbon cycles in the ocean and atmosphere. Therefore, knowledge of the controls on cobalt's abundance and chemical forms in seawater is a valuable component of our ability to understand the ocean's influence on global carbon cycling. Within phytoplankton and other marine microbes, metals such as cobalt, iron, nickel, and copper are used as critical components of enzymes responsible for key cellular reactions. Since these enzymes require metals to work, they are named metalloenzymes. Participating in a Pacific Ocean cruise from Alaska to Tahiti, this project will study the oceanic distributions of dissolved cobalt and the cellular content of a group of metalloenzymes known to influence biogeochemical cycles. The project will provide scientific impact by creating new knowledge about oceanic micronutrients in regions of economic interest with regard to fisheries and deep-sea mining. Measurement of proteins in the North Pacific will provide data of broad biological and chemical interest and will be made available through a new NSF-funded "EarthCube Ocean Protein Portal" data base. Educational impact will stem from participation of a graduate student and two young technicians, as well as the PI's development of a high school chemistry curriculum for use in two local high schools, thus allowing teachers to include real oceanic and environmental data at their first introduction to chemistry.

Cobalt has a complex biogeochemical cycle. Both its inorganic and organic forms are used by biology in the upper ocean and it is removed from solution by being scavenged in the intermediate and deep ocean. This scavenging removal results in cobalt having the smallest oceanic inventory of any biologically utilized element. Recent studies, however, have found that large dissolved cobalt plumes occur in major oxygen minimum zones due to a combination of less scavenging and additions from sedimentary and remineralization fluxes. The GP15 US GEOTRACES Pacific Meridional Transect (PMT) provides an opportunity to examine the influence of oxygen depletion on cobalt chemistry. Moreover, the study of the protein component of microbial communities using new proteomic techniques will provide evidence of how different major microorganisms respond to the chemical environment (e.g. through transporter production for specific nutrients and micronutrients) as well as the biochemical basis for metal requirements related to the use of specific metalloenzymes. Specifically, the PMT provides an opportunity to confirm that the Pacific oxygen minimum zones contain a large amount of cobalt and to test the hypotheses that simultaneous zinc scarcity could induce wide-scale biochemical substitution of cobalt for zinc in the North Pacific Ocean.

Collaborative Research: Underexplored Connections between Nitrogen and Trace Metal Cycling in Oxygen Minimum Zones Mediated by Metalloenzyme Inventories (CliOMZ)

Coverage: Eastern Tropical Pacific

NSF abstract:
Though scarce and largely insoluble, trace metals are key components of sophisticated enzymes (protein molecules that speed up biochemical reactions) involved in biogeochemical cycles in the dark ocean (below 1000m). For example, metalloenzymes are involved in nearly every reaction in the nitrogen cycle. Yet, despite direct connections between trace metal and nitrogen cycles, the relationship between trace metal distributions and biological nitrogen cycling processes in the dark ocean have rarely been explored, likely due to the technical challenges associated with their study. Availability of the autonomous underwater vehicle (AUV) Clio, a sampling platform capable of collecting high-resolution vertical profile samples for biochemical and microbial measurements by large volume filtration of microbial particulate material, has overcome this challenge. Thus, this research project plans an interdisciplinary chemistry, biology, and engineering effort to test the hypothesis that certain chemical reactions, such as nitrite oxidation, could become limited by metal availability within the upper mesopelagic and that trace metal demands for nitrite-oxidizing bacteria may be increased under low oxygen conditions. Broader impacts of this study include the continued development and application of the Clio Biogeochemical AUV as a community resource by developing and testing its high-resolution and adaptive sampling capabilities. In addition, metaproteomic data will be deposited into the recently launched Ocean Protein Portal to allow oceanographers and the metals in biology community to examine the distribution of proteins and metalloenzymes in the ocean. Undergraduate students will be supported by this project at all three institutions, with an effort to recruit minority students. The proposed research will also be synergistic with the goals of early community-building efforts for a potential global scale microbial biogeochemistry program modeled after the success of the GEOTRACES program, provisionally called "Biogeoscapes: Ocean metabolism and nutrient cycles on a changing planet".

The proposed research project will test the following three hypotheses: (1) the microbial metalloenzyme distribution of the mesopelagic is spatially dynamic in response to environmental gradients in oxygen and trace metals, (2) nitrite oxidation in the Eastern Tropical Pacific Ocean can be limited by iron availability in the upper mesopelagic through an inability to complete biosynthesis of the microbial protein nitrite oxidoreductase, and (3) nitrite-oxidizing bacteria increase their metalloenzyme requirements at low oxygen, impacting the distribution of both dissolved and particulate metals within oxygen minimum zones. One of the challenges to characterizing the biogeochemistry of the mesopelagic ocean is an inability to effectively sample it. As a sampling platform, we will use the novel biogeochemical AUV Clio that enables high-resolution vertical profile samples for biochemical and microbial measurements by large volume filtration of microbial particulate material on a research expedition in the Eastern Tropical Pacific Ocean. Specific research activities will be orchestrated to test the hypotheses. Hypothesis 1 will be explored by comparison of hydrographic, microbial distributions, dissolved and particulate metal data, and metaproteomic results with profile samples collected by Clio. Hypothesis 2 will be tested by incubation experiments using 15NO2- oxidation rates on Clio-collected incubation samples. Hypothesis 3 will be tested by dividing targeted nitrite oxidoreductase protein copies by qPCR (quantitative polymerase chain reaction)-based nitrite oxidizing bacteria abundance (NOB) to determine if cellular copy number varies with oxygen distributions, and by metalloproteomic analyses of NOB cultures. The demonstration of trace metal limitation of remineralization processes, not just primary production, would transform our understanding of the role of metals in biogeochemical cycling and provide new ways with which

to interpret sectional data of dissolved and particulate trace metal distributions in the ocean. The idea that oxygen may play a previously underappreciated role in controlling trace metals due not just to metals' physical chemistry, but also from changing biological demand, will improve our ability to predict trace metal distributions in the face of decreasing ocean oxygen content.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
Gordon and Betty Moore Foundation: Marine Microbiology Initiative (MMI)	GBMF3782
NSF Division of Ocean Sciences (NSF OCE)	OCE-1736599
NSF Division of Ocean Sciences (NSF OCE)	OCE-1924554

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