Whole cellular metal quotas, metal to phosphorous ratios, and metal to carbon ratios of Pseudoalteromonas sp. strain BB2-AT2 cultures originally collected from Scripps Pier, California coast in 1995

Website: https://www.bco-dmo.org/dataset/808598
Data Type: experimental
Version: 1
Version Date: 2020-04-20

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

Whole cellular metal quotas, metal to phosphorous ratios, and metal to carbon ratios of Pseudoalteromonas sp. strain BB2-AT2 cultures originally collected from Scripps Pier, California coast in 1995. 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 Cytosolic Metallome https://www.bco-dmo.org/dataset/808610
BB2-AT2 Metalloproteome Proteins https://www.bco-dmo.org/dataset/808619

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.

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

The measured quotas reflect contributions of intracellular and extracellular metals. Biomass from triplicate 10 mL cultures were centrifuged at 8,000 rpm for 20 minutes at 3 °C at mid-exponential phase of growth. The cell pellet was washed by resuspension in ~1 mL filtered seawater, transferred to an acid-cleaned microfuge tube and centrifuged again for 15 min at 14,000 RPM at 4 °C. The whole cell pellet was digested in 800 µl of 5% trace metal grade HNO3 (Optima) containing 1 ppb Indium (In) then cells were resuspended multiple times. After digesting for 7 days at room temperature solids were removed by centrifugation, and supernatants were collected for quota analysis. Process blank digestions containing acid but no cells were performed in parallel. Digests were diluted by a factor of 10 with additional 5% nitric acid (also containing 1 ppb In), transferred to acid-washed 96 deepwell plates (Thermo Scientific) before being analyzed on an ICAP-Q inductively coupled plasma-mass spectrometer (Thermo) with an SC-4 DX FAST autosampler (Elemental Scientific, Inc.). Metal concentrations were calibrated to a multi-element standard curve (Spex, Certiprep) over a range of 1 – 50 ppb. Phosphorus concentrations were also measured by ICPMS and calibrated to a separate standard curve ranging from 100–1500 ppb using a stock solution of Na3PO4. Samples were analyzed in KED mode using helium as a collision gas after a 45 s sample uptake window. Mass windows were scanned 3- times during measurements. In was used to correct for variation in sample delivery and plasma suppression between samples. Blanks were subtracted from measured concentrations. Technical replicates (n=3, 3, 2) were averaged for each biological triplicates (Supplemental Information). Averages of biological replicates and their standard deviation were then calculated.

Sample metadata:
All culture samples grown in replete marine broth (see Experimental Procedure for details)
BB2-AT2_1_1 BB2-AT2 biological replicate 1, technical replicate 1
BB2-AT2_1_2 BB2-AT2 biological replicate 1, technical replicate 2
BB2-AT2_1_3 BB2-AT2 biological replicate 1, technical replicate 3
BB2-AT2_2_1 BB2-AT2 biological replicate 2, technical replicate 1
BB2-AT2_2_2 BB2-AT2 biological replicate 2, technical replicate 2
BB2-AT2_2_3 BB2-AT2 biological replicate 2, technical replicate 3
BB2-AT2_3_1 BB2-AT2 biological replicate 3, technical replicate 1
BB2-AT2_3_2 BB2-AT2 biological replicate 3, technical replicate 2

Instruments:

All work performed in N2–filled anaerobic chamber (Coy Laboratory Products Inc.).
Metals analyzed on iCAP-Q inductively coupled plasma-mass spectrometer (Thermo) with an SC-4 DX FAST autosampler (Elemental Scientific, Inc.).

Data Processing Description

Process blank metal concentrations subtracted from raw data to obtain concentration values of metals:
Metal Concentration (mM)
55Mn 1.036E-04
56Fe 3.490E-04
59Co 4.854E-06
60Ni 6.598E-05
63Cu 5.211E-05
66Zn 5.226E-04
95Mo 6.518E-06

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Data Files

File
cell_metal_quotas.csv (Comma Separated Values (.csv), 3.32 KB) MD5:4ec302cb1f87a517b90d95b80c491281 Primary data file for dataset ID 808598

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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

Parameter	Description	Units
Sample_Name	Culture name	unitless
Bio_Repl	Biological replicate number	unitless
Tech_Repl	Technical replicate number	unitless
Conc_55Mn	Total concentration of manganese 55Mn	millimolar (mM)
Conc_56Fe	Total concentration of manganese 56Fe	millimolar (mM)
Conc_59Co	Total concentration of manganese 59Co	millimolar (mM)
Conc_60Ni	Total concentration of manganese 60Ni	millimolar (mM)
Conc_63Cu	Total concentration of manganese 63Cu	millimolar (mM)
Conc_66Zn	Total concentration of manganese 66Zn	millimolar (mM)
Conc_95Mo	Total concentration of manganese 95Mo	millimolar (mM)
Conc_31P	Total concentration of phosphorous 31P	molar (M)
Ratio_55Mn_to_P	Ratio of 55Mn ion (mmol) to phosphorous (mol). 55Mn:P	dimensionless
Ratio_56Fe_to_P	Ratio of 56Fe ion (mmol) to phosphorous (mol). 56Fe:P	dimensionless
Ratio_59Co_to_P	Ratio of 59Co ion (mmol) to phosphorous (mol). 59Co:P	dimensionless
Ratio_60Ni_to_P	Ratio of 60Ni ion (mmol) to phosphorous (mol). 60Ni:P	dimensionless
Ratio_63Cu_to_P	Ratio of 63Cu ion (mmol) to phosphorous (mol). 63Cu:P	dimensionless
Ratio_66Zn_to_P	Ratio of 66Zn ion (mmol) to phosphorous (mol). 66Zn:P	dimensionless
Ratio_95Mo_to_P	Ratio of 95Mo ion (mmol) to phosphorous (mol). 95Mo:P	dimensionless
Ratio_55Mn_to_C	Ratio of 55Mn ion (mmol) to carbon (mol). 55Mn:C	dimensionless
Ratio_56Fe_to_C	Ratio of 56Fe ion (mmol) to carbon (mol). 56Fe:C	dimensionless
Ratio_59Co_to_C	Ratio of 59Co ion (mmol) to carbon (mol). 59Co:C	dimensionless
Ratio_60Ni_to_C	Ratio of 60Ni ion (mmol) to carbon (mol). 60Ni:C	dimensionless
Ratio_63Cu_to_C	Ratio of 63Cu ion (mmol) to carbon (mol). 63Cu:C	dimensionless
Ratio_66Zn_to_C	Ratio of 66Zn ion (mmol) to carbon (mol). 66Zn:C	dimensionless
Ratio_95Mo_to_C	Ratio of 95Mo ion (mmol) to carbon (mol). 95Mo:C	dimensionless

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Instruments

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.

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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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