Global proteome analyses of the nitrite-oxidizing bacterium Nitrospira marina grown under atmospheric and low oxygen concentrations

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Project
» 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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Abstract
Global proteome analyses of the nitrite-oxidizing bacterium Nitrospira marina grown under atmospheric and low oxygen concentrations. Accession numbers correspond to the sequenced genome available in the JGI IMG/M repository (ID number: 2596583682).

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Coverage

Temporal Extent: 2016 - 2017

Methods & Sampling

Cultivation conditions and sampling:
Cultures of the marine nitrite-oxidizing bacterium *Nitrospira marina* were grown under atmospheric and low oxygen concentrations (see Bayer et al 2020 for details). Cells were harvested for proteomic analysis during exponential growth. Each culture was mixed with an equal volume of RNALater and filtered by vacuum filtration onto 25 mm, 0.2 µm pore size Supor filters. Filters were frozen at -80°C until extraction.

Protein extraction and purification:
Samples were resuspended with 1800 µL of 1% SDS extraction buffer (1% SDS, 0.1M Tris/HCl pH 7.5, 10mM EDTA). Each sample was incubated at room temperature for 15 min, heated at 95C for 10 min, and shaken at room temperature (RT) at 350 rpm for 1 h. The protein extracts were decanted and centrifuged at 14100 x g for 20 min at RT. The supernatants were removed and concentrated by membrane centrifugation to approximately 300 µL in 5 K MWCO Vivaspin units (Sartorius Stedim, Goettingen, Germany). Each sample was precipitated with cold 50% methanol/50% acetone/0.5 mM HCl for 3 days at –20C, centrifuged at 14100 x g for

30 min at 4C, decanted and dried by vacuum concentration (Thermo Savant Speedvac) for 10 min or until dry. Pellets were resuspended in 1% SDS extraction buffer and left at RT for 1 h to completely dissolve. Total protein was quantified (Bio-Rad DC protein assay, Hercules, CA) with BSA as a standard.

Extracted proteins were purified from SDS detergent, reduced, alkylated and trypsin digested while embedded within a polyacrylamide tube gel, modified from a previously published method (Lu and Zhu et al 2005). A gel premix was made by combining 1 M Tris HCl (pH 7.5) and 40% Bis-acrylimide L 29:1 (Acros Organics) at a ratio of 1:3. The premix (103 µL) was combined with an extracted protein sample (35 µg-50 µg), TE Buffer, 7 µL 1% APS and 3 µL of TEMED (Acros Organics) to a final volume of 200 µL. After 1 h of polymerization at RT, 200 µL of gel fix solution (50% ETOH, 10% acetic acid in LC/MS grade water) was added to the top of the gel and incubated at RT for 20 min. Liquid was then removed and the tube gel was transferred into a new 1.5 mL microtube containing 1.2 mL of gel fix solution then incubated at RT, 350 rpm in a Thermomixer R (Eppendorf) for 1 h. The gel fix solution was then removed and replaced with 1.2 mL destaining solution (50% MeOH, 10% Acetic Acid in water) and incubated at 350rpm for 2h. Liquid was then removed, gel cut up into 1 mm cubes and then added back to tubes containing 1 mL of 50:50 acetonitrile:25 mM ammonium bicarbonate (ambic) incubated for 1 h, 350 rpm at RT. Liquid was removed and replaced with fresh 50:50 acetonitrile:ambic and incubated at 16C at 350 rpm overnight. The above step was repeated for 1 h the following morning. Gel pieces were then dehydrated twice in 800 µL of acetonitrile for 10 min at room temperature and dried for 10 min in a ThermoSavant DNA110 speedvac after removing solvent. 600 µL of 10 mM DTT in 25 mM ambic was added to reduce proteins incubating at 56C, 350 rpm for 1 h. Unabsorbed DTT solution was then removed with volume measured. Gel pieces were washed with 25 mM ambic and 600 µL of 55 mM iodacetamide was added to alkylate proteins at RT, 350 rpm for 1 h. Gel cubes were then washed with 1 mL ambic for 20 min, 350 rpm at RT. Acetonitrile dehydrations and speedvac drying were repeated as above. Trypsin (Promega #V5280) was added in appropriate volume of 25 mM ambic to rehydrate and submerse gel pieces at a concentration of 1:20 µg trypsin:protein. Proteins were digested overnight at 350 rpm and 37C. Unabsorbed solution was removed and transferred to a new tube. 50 µL of peptide extraction buffer (50% acetonitrile, 5% formic acid in water) was added to gels, incubated for 20 min at RT then centrifuged at 14,100 x g for 2 min. Supernatant was collected and combined with unabsorbed solution. The above peptide extraction step was repeated combining all supernatants. Combined protein extracts were centrifuged at 14,100 x g for 20 min, supernatants transferred into a new tube and dehydrated down to approximately 10-20 µL in the speedvac. Concentrated peptides were then diluted in 2% acetonitrile 0.1% formic acid in water for storage until analysis. All water used in the tube gel digestion protocol was LC/MS grade, and all plastic microtubes were ethanol rinsed and dried prior to use.

Instruments:
Global proteomic data was produced using a Dionex Ultimate nanoLC system coupled to a Thermo Fusion mass spectrometer with a Thermo Flex ion source. 1 µg of each sample (measured before trypsin digestion) was concentrated onto a trap column (0.2 x 10 mm ID, 5 µm particle size, 120 Å pore size, C18 Reprosil-Gold, Dr. Maisch GmbH) and rinsed with 100 µL 0.1% formic acid, 2% acetonitrile (ACN), 97.9% water before gradient elution through a reverse phase C18 column (0.1 x 250 mm ID, 3 µm particle size, 120 Å pore size, C18 Reprosil-Gold, Dr. Maisch GmbH) at a flow rate of 500 nL/min. The chromatography consisted of a nonlinear 220 min gradient from 5% to 95% buffer B, where A was 0.1% formic acid in water and B was 0.1% formic acid in ACN (all solvents were Fisher Optima grade). The mass spectrometer was set to perform MS scans on the orbitrap (240000 resolution at 200 m/z) with a scan range of 380 m/z to 1580 m/z. MS/MS was performed on the ion trap using data-dependent settings (top speed, dynamic exclusion 15 seconds, excluding unassigned and singly charged ions, precursor mass tolerance of ±3ppm, with a maximum injection time of 50 ms).

Data Processing Description

Data Processing:
Global proteomic protein identifications were applied to data-dependent acquisition mode raw files using the SEQUEST peptide to spectrum mapping algorithm in Proteome Discoverer version 2.1 using trypsin enzyme digestion allowing 2 missed cleavages, with fragment tolerance of 0.6 Da and parent tolerance of 10.0 ppm, allowing fixed modification of +57 for carbamidomethyl on cysteine and +16 for methionine oxidation. Spectral counts were calculated in Scaffold's Proteome Software Version 4.10 using peptide false discovery rate of 0.2% and protein false discovery rate of 4.6%.

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Data Files

File
Nmarina_global_proteome.csv (Comma Separated Values (.csv), 228.84 KB) MD5:4a7ef7b5b66233847dbbc212d7393029

Global proteome analyses of the nitrite-oxidizing bacterium Nitrospira marina grown under atmospheric and low oxygen concentrations. Accession numbers correspond to the sequenced genome available in the JGI IMG/M repository (ID number: 2596583682).

Column descriptions:

Identified_proteins = Gene annotations of the translated nucleotide sequences in the Nitrospira marina genome that matched the identified peptide spectra

Accession_number = Accession numbers of the respective genes in the Nitrospira marina genome

Spectral_counts_A_atm_O2 = Total number of spectra identified for each protein under growth of Nitrospira marina in atmospheric oxygen conditions

Spectral_counts_B_atm_O2 = Total number of spectra identified for each protein under growth of Nitrospira marina in atmospheric oxygen conditions

Spectral_counts_C_atm_O2 = Total number of spectra identified for each protein under growth of Nitrospira marina in atmospheric oxygen conditions

Spectral_counts_D_low_O2 = Total number of spectra identified for each protein under growth of Nitrospira marina in low oxygen conditions

Spectral_counts_E_low_O2 = Total number of spectra identified for each protein under growth of Nitrospira marina in low oxygen conditions

Spectral_counts_F_low_O2 = Total number of spectra identified for each protein under growth of Nitrospira marina in low oxygen conditions

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Related Publications

Bayer, B., Saito, M. A., McIlvin, M. R., Lücker, S., Moran, D. M., Lankiewicz, T. S., … Santoro, A. E. (2020). Metabolic versatility of the nitrite-oxidizing bacterium Nitrospira marina and its proteomic response to oxygen-limited conditions. The ISME Journal, 15(4), 1025–1039. doi:10.1038/s41396-020-00828-3
Results

Lu, X., & Zhu, H. (2005). Tube-Gel Digestion. Molecular & Cellular Proteomics, 4(12), 1948–1958. doi:10.1074/mcp.m500138-mcp200 https://doi.org/10.1074/mcp.M500138-MCP200
Methods

Saito, M. A., McIlvin, M. R., Moran, D. M., Santoro, A. E., Dupont, C. L., Rafter, P. A., … Waterbury, J. B. (2020). Abundant nitrite-oxidizing metalloenzymes in the mesopelagic zone of the tropical Pacific Ocean. Nature Geoscience, 13(5), 355–362. doi:10.1038/s41561-020-0565-6
General

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Related Datasets

IsRelatedTo

DOE Joint Genome Institute. Nitrospira marina Nb-295. 2014/09. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; Available from:

http://www.ncbi.nlm.nih.gov/bioproject/PRJNA262287. NCBI:BioProject: PRJNA262287.

JGI IMG/M repository ID number: 2596583682. https://img.jgi.doe.gov/cgi-bin/mer/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2596583682

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Parameters

Parameter	Description	Units
Identified_proteins	Gene annotations of the translated nucleotide sequences in the Nitrospira marina genome that matched the identified peptide spectra	unitless
Accession_number	Accession numbers of the respective genes in the Nitrospira marina genome	unitless
Spectral_counts_A_atm_O2	Total number of spectra identified for each protein under growth of Nitrospira marina in atmospheric oxygen conditions	unitless
Spectral_counts_B_atm_O2	Total number of spectra identified for each protein under growth of Nitrospira marina in atmospheric oxygen conditions	unitless
Spectral_counts_C_atm_O2	Total number of spectra identified for each protein under growth of Nitrospira marina in atmospheric oxygen conditions	unitless
Spectral_counts_D_low_O2	Total number of spectra identified for each protein under growth of Nitrospira marina in low oxygen conditions	unitless
Spectral_counts_E_low_O2	Total number of spectra identified for each protein under growth of Nitrospira marina in low oxygen conditions	unitless
Spectral_counts_F_low_O2	Total number of spectra identified for each protein under growth of Nitrospira marina in low oxygen conditions	unitless

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Instruments

Dataset-specific Instrument Name	MWCO Vivaspin units (Sartorius Stedim, Goettingen, Germany)
Generic Instrument Name	Centrifuge
Generic Instrument Description	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

Dataset-specific Instrument Name	Dionex Ultimate nanoLC system
Generic Instrument Name	High-Performance Liquid Chromatograph
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	Thermo Fusion mass spectrometer with a Thermo Flex ion source
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	Thermo Savant Speedvac; ThermoSavant DNA110 speedvac
Generic Instrument Name	Vacuum centrifuge concentrator
Generic Instrument Description	A centrifuge that includes a vacuum chamber within which a centrifuge rotord is rotatably mounted for spinning a plurality of vials containing a solution at high speed while subjecting the solution to a vacuum condition for concentration and evaporation. Alternative names: sample concentrator; speed vacuum; speed vac.

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Project Information

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
NSF Division of Ocean Sciences (NSF OCE)	OCE-1924512

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