Raw LC-MS/MS data, with list of identified peptides, and DNA sequences from a proteomic profile of Mariprofundus ferrooxydans

Website: https://www.bco-dmo.org/dataset/636756
Data Type: Other Field Results, experimental
Version: 28 Jan 2016
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Project
» Proteomic profiling of neutrophilic, iron-oxidizing Mariprofundus ferrooxydans, strain PV-1 (Proteomic profiling of Mariprofundus ferrooxydans)

Program
» Center for Dark Energy Biosphere Investigations (C-DEBI)

Contributors	Affiliation	Role
Barco, Roman A.	Bigelow Laboratory for Ocean Sciences	Principal Investigator
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Dataset Description

Raw LC-MS/MS data, with list of identified peptides in xml format, and DNA sequences.

Related publications:

Barco, RA, Emerson, D, Sylvan, JB, Orcutt, BN, Jacobson Meyers, ME, Ramírez, GA, Zhong, JD, Edwards, KJ. 2015. New insights into microbial iron oxidation as revealed by the proteomic profile of an obligately iron-oxidizing chemolithoautotroph. *Appl. Environ. Microbiol.* 81(17): 5927-37. doi: 10.1128/AEM.01374-15

Methods & Sampling

A proteomic profile of the chemolithoautotrophic, neutrophilic, iron-oxidizing Mariprofundus ferrooxydans was obtained in duplicates and analyzed via LC-MS/MS. Additionally, a proteomic analysis of the membrane fraction is included. Cultures were harvested at late log phase by following the protocol in Barco and Edwards (2014). Briefly, proteins were extracted with 0.1N NaOH/2% SDS solution, clarified and concentrated by nanofiltration. The concentrated samples were run on SDS-PAGE gels in duplicate lanes. All the bands from each lane were excised and analyzed via LC-MS/MS. Membrane fractions were obtained in the following way: the cell-separation protocol was performed per Barco and Edwards (2014) and the cell pellet was resuspended in 0.5M NaCl/40mM Tri-base buffer at pH 8.5 and sonicated for 10 cycles of 30 secs pulses followed by 30 secs of cooling. This sample was clarified and the supernatant was ultracentrifuged at 100,000 x g for 2 hrs. The reddish pellet was solubilized with 0.5% Ultrol Grade, n-Dodecyl-beta-D-maltoside and run on a SDS-PAGE gel. DNA: DNA was extracted by using the FastDNA Spin soil kit according to the manufacturer's instructions (MP

Biomedicals). Extracted DNAwas stored at 20 degrees C until processing. Genomic gaps were amplified by PCR on a Veriti thermal cycler (Life Technologies) as follows: 1 step of denaturation at 95 degrees C for 4 min; 35 cycles of denaturation, melting, and extension (95 degrees C for 30 s, 51 degrees C for 30 s, and 72 degrees C for 3 min, respectively); 1 step of extension at 72 degrees C for 10 min; and 1 final step of cooling at 4 degrees C. Primers used for the gap containing the Cyc1PV-1 gene were 79F (5'-GAAGCGATGGGAAATGTGAAT-3') and 375F (5'-CACACTGGAAGATGTTCTGG-3'). Primers used for the gap containing the Cyc2PV-1 gene were 555F (5'-ACTGATGGGTATCAACAACC-3') and 92R (5'-CCTATCTGTACCGAGCATTC-3'). The amplicons were purified by using the QIAquick PCR purification kit (Qiagen). Amplicon sizes were checked in a 1% agarose gel via electrophoresis. Purified PCR amplicons were submitted for Sanger sequencing and primer walking (Laragen).

Data Processing Description

Excised bands were trypsin-digested and submitted for LC-MS/MS analysis which involved a Thermo LTQ-Orbitrap XL mass spectrometer equipped with an Eksigent Nanoliquid Chromatography 1-D plus system. The resulting MS/MS spectra were searched against the proteomes of Mariprofundus ferrooxydans strains PV-1(Uniprot database) and M34 (IMG database) using Proteome Discoverer SEQUEST Daemon search engine. DNA: sequences were analyzed in Geneious v. R6 (Biomatters Ltd.).

Raw LC-MS/MS files are publicly available through the PRIDE Archive at http://www.ebi.ac.uk/pride/archive/projects/PXD001050 and http://www.ebi.ac.uk/pride/archive/projects/PXD001439.

DNA sequences were deposited in Genbank under accession numbers KR106296, KR106297, KR091570, and BK009249.

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Data Files

File
raw_LCMS_DNA.csv (Comma Separated Values (.csv), 1.95 KB) MD5:4d562d92c94d991b51f126786c968039
Primary data file for dataset ID 636756

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Parameters

Parameter	Description	Units
type	Description of sample type.	dimensionless
taxon	Taxon studied.	dimensionless
repository	Name of the repository where the raw data are stored.	dimensionless
identifier	Unique identifer: Either the PRIDE database project identifier or the NCBI accession number.	dimensionless
URL	Link to the PRIDE database or NCBI.	dimensionless
description	Description of the method and sample.	dimensionless

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Instruments

Dataset-specific Instrument Name	Thermo LTQ-Orbitrap XL
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Excised bands were trypsin-digested and submitted for LC-MS/MS analysis which involved a Thermo LTQ-Orbitrap XL mass spectrometer equipped with an Eksigent Nanoliquid Chromatography 1-D plus 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.

Dataset-specific Instrument Name	
Generic Instrument Name	Thermal Cycler
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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Project Information

Proteomic profiling of neutrophilic, iron-oxidizing Mariprofundus ferrooxydans, strain PV-1 (Proteomic profiling of Mariprofundus ferrooxydans)

Description from C-DEBI website:
The aim of this proposal is to gain a better understanding of what subsets of proteins are actually being expressed during neutrophilic, microbial iron (Fe)-oxidation. The recently isolated *Mariprofundus ferrooxydans*, strain PV-1, will be used as a marine model organism to investigate proteomic differences under different Fe substrates: aqueous Fe2+ and solid Fe0. Two-dimensional gel electrophoresis (2D-GE) and shotgun proteomic methods (LC-MS/MS) will be employed to obtain results from the cultures grown under different conditions. The research being proposed would constitute the foundation for the development of diagnostic tools for the accordance, distribution, and activity level of Fe-oxidation, a globally important biogeochemical process at and below the ocean floor.

This project was funded by a C-DEBI Graduate Student Fellowship.

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Program Information

Center for Dark Energy Biosphere Investigations (C-DEBI)

Website: http://www.darkenergybiosphere.org

Coverage: Global

The mission of the Center for Dark Energy Biosphere Investigations (C-DEBI) is to explore life beneath the seafloor and make transformative discoveries that advance science, benefit society, and inspire people of all ages and origins.

C-DEBI provides a framework for a large, multi-disciplinary group of scientists to pursue fundamental questions about life deep in the sub-surface environment of Earth. The fundamental science questions of C-DEBI involve exploration and discovery, uncovering the processes that constrain the sub-surface biosphere below the oceans, and implications to the Earth system. What type of life exists in this deep biosphere, how much, and how is it distributed and dispersed? What are the physical-chemical conditions that promote or limit life? What are the important oxidation-reduction processes and are they unique or important to humankind? How does this biosphere influence global energy and material cycles, particularly the carbon cycle? Finally, can we discern how such life evolved in geological settings beneath the ocean floor, and how this might relate to ideas about the origin of life on our planet?

C-DEBI's scientific goals are pursued with a combination of approaches:
(1) coordinate, integrate, support, and extend the research associated with four major programs—Juan de Fuca Ridge flank (JdF), South Pacific Gyre (SPG), North Pond (NP), and Dorado Outcrop (DO)—and other field sites;
(2) make substantial investments of resources to support field, laboratory, analytical, and modeling studies of the deep subseafloor ecosystems;
(3) facilitate and encourage synthesis and thematic understanding of submarine microbiological processes, through funding of scientific and technical activities, coordination and hosting of meetings and workshops, and support of (mostly junior) researchers and graduate students; and
(4) entrain, educate, inspire, and mentor an interdisciplinary community of researchers and educators, with an emphasis on undergraduate and graduate students and early-career scientists.

Note: Katrina Edwards was a former PI of C-DEBI; James Cowen is a former co-PI.

Data Management:
C-DEBI is committed to ensuring all the data generated are publically available and deposited in a data repository for long-term storage as stated in their Data Management Plan (PDF) and in compliance with the NSF Ocean Sciences Sample and Data Policy. The data types and products resulting from C-DEBI-supported research include a wide variety of geophysical, geological, geochemical, and biological information, in addition to education and outreach materials, technical documents, and samples. All data and information generated by C-DEBI-supported research projects are required to be made publically available either following publication of research results or within two (2) years of data generation.

To ensure preservation and dissemination of the diverse data-types generated, C-DEBI researchers are working with BCO-DMO Data Managers make data publicly available online. The partnership with BCO-DMO helps ensure that the C-DEBI data are discoverable and available for reuse. Some C-DEBI data is better served by specialized repositories (NCBI's GenBank for sequence data, for example) and, in those cases, BCO-DMO provides dataset documentation (metadata) that includes links to those external repositories.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0939564

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