Synthesis of publicly-available sequence datasets of the 16S rRNA gene in environmental DNA extracted from seafloor and subseafloor samples from the Dorado outcrop, Lō'ihi Seamount, North Pond, and Juan de Fuca Ridge flank

Website: https://www.bco-dmo.org/dataset/789136
Data Type: Cruise Results, Other Field Results, experimental
Version: 1
Version Date: 2020-02-04

Project
» Microbial activity in the crustal deep biosphere (Slow Life in Crust)

Contributors	Affiliation	Role
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Abstract
To summarize crustal bacterial and archaeal taxa for this review, we synthesized publicly-available sequence datasets of the 16S rRNA gene in environmental DNA extracted from seafloor and subseafloor basalts generated using 454, Illumina and Ion Torrent amplicon platforms. These include seafloor basalts from the Dorado Outcrop and the Lō'ihi Seamount in the Pacific Ocean and subseafloor basalts from North Pond on the western flank of the Mid-Atlantic Ridge and the Juan de Fuca Ridge flank in the northeastern Pacific Ocean. Datasets from rock colonization experiments conducted in the subseafloor at the Juan de Fuca Ridge flank site were also included, as well as microbial community surveys of the subseafloor crustal fluids from the anoxic Juan de Fuca site and the oxic North Pond site.

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Coverage

Spatial Extent: **N**:45.62 **E**:-46 **S**:10 **W**:-155.27
Temporal Extent: 2014 - 2019

Dataset Description

Metadata for sequence datasets used in ocean crust microbiome survey.

This metadata table and supporting PDF document describe data analysis performed for a review chapter to be published in an edited book:

Authors: Beth N. Orcutt, Timothy D'Angelo, Sean P. Jungbluth, Julie A. Huber, Jason B. Sylvan
Chapter Title: Microbial Life in Oceanic Crust
Book title: The Microbiology of the Deep-Sea
Editors: Donato Giovannelli, Costantino Vetriani

Publisher: Springer International Publishing AG

Methods & Sampling

Analysis of publicly available 16S rRNA gene sequence datasets for taxonomic profiling

To summarize crustal bacterial and archaeal taxa for this review, we synthesized publicly-available sequence datasets of the 16S rRNA gene in environmental DNA extracted from seafloor and subseafloor basalts generated using 454, Illumina and Ion Torrent amplicon platforms. These include seafloor basalts from the Dorado Outcrop (Lee et al., 2015) and the Lō'ihi Seamount (Jacobsen Meyers et al., 2014) in the Pacific Ocean and subseafloor basalts from North Pond on the western flank of the Mid-Atlantic Ridge (Jørgensen & Zhao, 2016) and the Juan de Fuca Ridge flank in the northeastern Pacific Ocean (LaBonté et al., 2017). Datasets from rock colonization experiments conducted in the subseafloor at the Juan de Fuca Ridge flank site (Smith et al., 2016; Ramírez et al., 2019) were also included, as well as microbial community surveys of the subseafloor crustal fluids from the anoxic Juan de Fuca site (Jungbluth et al., 2016) and the oxic North Pond site (Tully et al., 2017; Meyer et al., 2016). For comparison, we included select reference datasets from oxic (Reese et al., 2018; Zinke et al., 2018) and anoxic sediment (LaBonté et al., 2017) and the overlying bottom seawater (Lee et al., 2015) from these same study sites.

Raw sequence data from the reviewed studies were downloaded from the NCBI Short Read Archive. Sequencing reads generated using Illumina and Ion Torrent platforms were quality filtered and processed to unique Amplicon Sequence Variants (ASVs) using DADA2 (Callahan et al, 2016), with taxonomy determined by the naïve Bayesian classifier in DADA2 using a training set from the SILVA v132 database (Quast et al., 2013; Yilmaz et al., 2014; Glöckner et al., 2017). For the 454 GS-FLX sequence datasets, operational taxonomic units (OTUs) constructed with 97% or greater sequence similarity in the original analyses were reprocessed in mothur V.1.37.6 (Schloss et al., 2009) against the same SILVA database. All short read datasets were merged and summarized to the relative abundance at phylum resolution (or to class level for Proteobacteria phyla) using Phyloseq v1.24.0 (McMurdie & Holmes, 2013). Figures were produced using ggplot2 R package version 2.2.1 (Wickham, 2016) in RStudio (RStudio Team, 2017). Taxonomic grouping in each sample separated taxa into common (>5% abundance in at least one sample) versus rare (never more than 5% in any sample). Supplemental Figure S1 shows the breakdown of Gammaproteobacteria families in the samples presented in Figure 4 of the main text, and Supplemental Figure S2 highlights the abundance of rare taxa (never >5% abundance in any sample). The Bray-Curtis distances between samples was calculated using the same dataset described above, summarized to relative abundance at the Family taxonomic level using Phyloseq and the Vegan package (Oksanen et al., 2018). A Non-Metric Multidimensional Scaling (NMDS) ordination was produced from this distance matrix. It should be noted that common rules for beta diversity comparisons, such as common library preparation/sequencing protocols and library-size normalization, were not performed in this analysis due to the diversity of the datasets being considered and the resulting NMDS ordination having high-stress (>20%). Therefore, the results should be viewed as broadly qualitative and not quantitative.

All data processing steps and markdown files are available via github: https://github.com/orcuttlab/ocean-crust-micro

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Data Files

File	
microbiome_amplicon_metadata.csv	(Comma Separated Values (.csv), 22.19 KB) MD5:c94774915e2b3cd6811678f02be8ae2a
Primary data file for dataset ID 789136	

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Supplemental Files

File
Supplemental material for "Microbial life in Oceanic Crust" book chapter filename: Orcutt_OceanCrustMBIOchapter_Supplemental_v5.pdf (Portable Document Format (.pdf), 428.30 KB) MD5:69374d66fb24425a9550717e7520b420 Supplemental material for "Microbial life in Oceanic Crust" book chapter by Orcutt et al.

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Related Publications

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. doi:10.1038/nmeth.3869
Software

Glöckner, F. O., Yilmaz, P., Quast, C., Gerken, J., Beccati, A., Ciuprina, A., … Ludwig, W. (2017). 25 years of serving the community with ribosomal RNA gene reference databases and tools. Journal of Biotechnology, 261, 169–176. doi:10.1016/j.jbiotec.2017.06.1198
Methods

Jacobson Meyers, M. E., Sylvan, J. B., & Edwards, K. J. (2014). Extracellular Enzyme Activity and Microbial Diversity Measured on Seafloor Exposed Basalts from Loihi Seamount Indicate the Importance of Basalts to Global Biogeochemical Cycling. Applied and Environmental Microbiology, 80(16), 4854–4864. doi:10.1128/aem.01038-14 https://doi.org/10.1128/AEM.01038-14
IsDerivedFrom

Jungbluth, S. P., Bowers, R. M., Lin, H.-T., Cowen, J. P., & Rappé, M. S. (2016). Novel microbial assemblages inhabiting crustal fluids within mid-ocean ridge flank subsurface basalt. The ISME Journal, 10(8), 2033–2047. doi:10.1038/ismej.2015.248
Methods

Jørgensen, S. L., & Zhao, R. (2016). Microbial Inventory of Deeply Buried Oceanic Crust from a Young Ridge Flank. Frontiers in Microbiology, 7. doi:10.3389/fmicb.2016.00820
IsDerivedFrom

Labonté, J. M., Lever, M. A., Edwards, K. J., & Orcutt, B. N. (2017). Influence of Igneous Basement on Deep Sediment Microbial Diversity on the Eastern Juan de Fuca Ridge Flank. Frontiers in Microbiology, 8. doi:10.3389/fmicb.2017.01434
IsDerivedFrom

Lee, M. D., Walworth, N. G., Sylvan, J. B., Edwards, K. J., & Orcutt, B. N. (2015). Microbial Communities on Seafloor Basalts at Dorado Outcrop Reflect Level of Alteration and Highlight Global Lithic Clades. Frontiers in Microbiology, 6. doi:10.3389/fmicb.2015.01470
IsDerivedFrom

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE, 8(4), e61217. doi:10.1371/journal.pone.0061217
Software

Meyer, J. L., Jaekel, U., Tully, B. J., Glazer, B. T., Wheat, C. G., Lin, H.-T., … Huber, J. A. (2016). A distinct and active bacterial community in cold oxygenated fluids circulating beneath the western flank of the Mid-Atlantic ridge. Scientific Reports, 6(1). doi:10.1038/srep22541
IsDerivedFrom

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2018) vegan: Community Ecology Package. R package version 2.5-2. https://CRAN.R-project.org/package=vegan
Software

Orcutt, B., D'Angelo, T., Jungbluth, S. P., Huber, J. A., & Sylvan, J. B. (2020). Microbial life in oceanic crust. https://doi.org/10.31219/osf.io/2wxe6
Results

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research, 41(D1), D590–D596. doi:10.1093/nar/gks1219

Methods

RStudio Team (2017) RStudio: Integrated Development for R. Version 1.0.153. RStudio, Inc., Boston, MA. http://www.rstudio.com/ http://www.rstudio.com/.
Software

Ramírez, G. A., Garber, A. I., Lecoeuvre, A., D'Angelo, T., Wheat, C. G., & Orcutt, B. N. (2019). Ecology of Subseafloor Crustal Biofilms. Frontiers in Microbiology, 10. doi:10.3389/fmicb.2019.01983
IsDerivedFrom

Reese, B. K., Zinke, L. A., Sobol, M. S., LaRowe, D. E., Orcutt, B. N., Zhang, X., … Girguis, P. (2018). Nitrogen Cycling of Active Bacteria within Oligotrophic Sediment of the Mid-Atlantic Ridge Flank. Geomicrobiology Journal, 35(6), 468–483. doi:10.1080/01490451.2017.1392649
IsDerivedFrom

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., … Weber, C. F. (2009). Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Applied and Environmental Microbiology, 75(23), 7537–7541. doi:10.1128/aem.01541-09 https://doi.org/10.1128/AEM.01541-09
Software

Smith, A. R., Fisk, M. R., Thurber, A. R., Flores, G. E., Mason, O. U., Popa, R., & Colwell, F. S. (2016). Deep Crustal Communities of the Juan de Fuca Ridge Are Governed by Mineralogy. Geomicrobiology Journal, 34(2), 147–156. doi:10.1080/01490451.2016.1155001
IsDerivedFrom

Tully, B. J., Wheat, C. G., Glazer, B. T., & Huber, J. A. (2017). A dynamic microbial community with high functional redundancy inhabits the cold, oxic subseafloor aquifer. The ISME Journal, 12(1), 1–16. doi:10.1038/ismej.2017.187
IsDerivedFrom

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org. https://doi.org/10.1007/978-3-319-24277-4
Software

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., … Glöckner, F. O. (2013). The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Research, 42(D1), D643–D648. doi:10.1093/nar/gkt1209
Methods

Zinke, L. A., Reese, B. K., McManus, J., Wheat, C. G., Orcutt, B. N., & Amend, J. P. (2018). Sediment Microbial Communities Influenced by Cool Hydrothermal Fluid Migration. Frontiers in Microbiology, 9. doi:10.3389/fmicb.2018.01249
IsDerivedFrom

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Parameters

Parameter	Description	Units
Plot_Order	Numerical order on the "Sample" Axis of invididual samples in Figure 4 of the main text. Values: integers from 1 to 120 for samples included in plot; none, samples from blank DNA extractions used for comparison; not-in-plot_used-in-NMDS, additional sediment comparison samples not included in plots but used in NMDS analysis	unitless
Sample_Name	Unique name of the sample used in the plot	unitless
SRA_Run	Unique Seqence Read Archive (SRA) Accession Number to download fastq-formatted file of sequence data for the Sample_Name from the NCBI Archive	unitless

SRA_LibraryName	The unique library name given to the Sample_Name by the authors as listed on the NCBI archive	unitless
Study_Nickname	Short hand code referencing the first author and location of a given study	unitless
Sample_Type	Environmental type that the sample was collected from. Values: Basalt, Seafloor or subseafloor basalt core samle; FLOCS, mineral colonization experiment from an in situ sytem; Fluids, subsurface crustal fluids collected from a subseafloor observatory; Sediment, sediment core samples; SW, bottom seawater near field sites; blank, DNA extraction blank	unitless
Temp	Description of the temperature of the sampling environment. Values: cool, <10 degrees C; warm, >10 degrees C; na, not applicable	unitless
Location	Descriptive name of field site where Sample_Name originated. Values: NorthPond; Dorado; Loihi; JuanDeFuca	unitless
Depth	Descriptive category of the relative position of the Sample_Name in the environment. Values: seafloor, collected from the seafloor; subsurface, below the seafloor; none, not applicable	unitless
Sequencer_Type	Sequencing platform used to sequence extracted DNA from the Sample_Name. Values: IonTorrent; Illumina; 454	unitless
region16S	Variable region(s) of the 16S rRNA gene that was sequenced from the extracted DNA from the Sample_Name, as desxcribed in the primary literature. Values: V4; V6; V4-V6; V1-V3	unitless
Primers	Primer set used to amplify the 16S rRNA variable region(s) from the DNA prior to sequencing of the Sample_Name, as described in the primary literature. Values: 519F-805R; 515F-806R; 967F-1046R; 518F-1064R; 28F-388R; 27F-518R	unitless
DNAextraction	Short-hand name for protocol used for extracting DNA from the sample, as described in the primary literature. Values: MPBiomedicalsFastDNA; CTABPhenolChloroform; TCEPPhenolChloroform; MoBioPowerSoil; EnzymePhenolChloroform; SDSPhenolChloroform	unitless
DOI	Digital Object Identitfyer information for publications that describe the original study for the data used here	unitless
SRA_Study	Sequence Read Archive Identifier number for finding original datafiles on the NCBI Archive	unitless

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Project Information

Microbial activity in the crustal deep biosphere (Slow Life in Crust)

Coverage: Juan de Fuca Ridge flank CORKs, 47N/127W

NSF Award Abstract:
The marine deep biosphere is the habitat for life existing under the sea floor. The zone has remarkably low energy sources creating a paradox of how life can persist there. Resolving this energy paradox is a grand challenge in deep biosphere research. The Juan de Fuca Ridge flank off the coast of Washington, USA, is an accessible, low energy environment making it an attractive location for addressing this challenge. A series of experiments will be conducted on the seafloor at the Juan de Fuca Ridge flank, using established subseafloor observatories that access the crustal deep biosphere, to provide the first direct in situ measurement of microbial activity in the crustal subsurface. This project will provide essential information about the ability of life to survive under conditions that we are not able to replicate in the laboratory, but that are increasingly important for understanding microbial community interaction in the environment. This information can then be used in models of global microbial activity for estimating the impact of this biosphere on elemental cycling, transforming our understanding of microbial processes within this vast subseafloor habitat. To communicate these discoveries to the public, the project will include a ship-to-shore outreach program during the cruise. In addition public lectures will be presented, and an interactive display of deep-sea video footage will be set up for the annual public Open House at the Bigelow Laboratory for Ocean Sciences in Maine. Diverse undergraduate students and a postdoctoral researcher will be recruited to participate in the research and public outreach activities.

This project proposes to leverage existing subsurface infrastructure on the eastern flank of the Juan de Fuca Ridge with advances in single-cell based molecular and geochemical approaches to make fundamental new discoveries about the activity of life in the deep crustal biosphere. During a two-week research cruise, the research team will incubate crustal fluids in situ and in the laboratory with labeled substrates for tracking single-cell activity, coupled with radioisotope tracer activity and potentiostat measurements, with the objective of determining in situ and potential rates of activity and cellular physiology. The research will also identify which metabolisms active microorganisms utilize under in situ and laboratory conditions, the rates of these processes, and the microorganisms involved. The results are expected to provide explicit hypothesis testing of microbial activity and in situ microbial growth rates from the crustal deep biosphere to transform understanding of microbial activity in the crustal deep biosphere and generate critical information about the ability of life to survive under low energy conditions.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1737017

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