# Fungal iTAG analyses on Peru Margin sediment core samples from R/V JOIDES Resolution cruise JRES-201 in the Peru Margin from January to March 2002

Website: https://www.bco-dmo.org/dataset/654116 Data Type: Cruise Results Version: 15 Aug 2016 Version Date: 2016-08-15

#### Project

» <u>Exploring of the Ecological Role(s) of Marine Fungi in the Deep Subseafloor</u> (Fungal and prokaryotic activity in subseafloor)

#### Program

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

Contributors	Affiliation	Role
Edgcomb, Virginia P.	Woods Hole Oceanographic Institution (WHOI)	Principal Investigator
Burgaud, Gaetan	Universite de Brest	International Collaborator
<u>Pachiadaki, Maria G.</u>	Woods Hole Oceanographic Institution (WHOI)	Co-Principal Investigator
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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### Methods & Sampling

Peru Margin subsurface sediments were sampled on IODP leg 201 site 1229A (10°58.5721' S 77°57.4590'W) in 150.5 m water depth. Core depth at 1229A was 187 mbsf.

DNA was extracted from either 2 or 20 grams of exterior and interior core sediment from frozen (stored at - 80 degree C) Peru Margin samples collected at 6 mbsf (2H2) and 95 mbsf (11H5), respectively, using the PowerSoil DNA Isolation Kit (MoBio Laboratories, USA). Extractions were also performed for replicate samples collected from the exterior of both cores. The manufacturer's protocol was modified to include five repetitions of homogenization for 1-minute intervals, with 1 minute rest in between, using a FastPrep benchtop homogenizer (MP Biomedicals, Santa Ana, CA) set to 4.0 m/s. A final purification step using isopropanol precipitation was also added. Duplicate extractions were performed for both interior and exterior regions of each core. Partial small-subunit ribosomal DNA (SSU rDNA) fragments were PCR amplified from DNA extracts using the key-tagged fungal-targeting primer set nu-SSU-0817-5' and nu-SSU-1196-3' (Borneman and Hartin, 2000). Replicate PCR amplifications (3-6) were run for each sample using Phusion High-Fidelity DNA Polymerase (ThermoFisher Scientific, USA) and 5X Phusion HF Buffer. PCR conditions were: 98 degrees C for 30 seconds followed by 40 cycles of 98 degrees C for 10 seconds, 56 degrees C for 30 seconds, and 72 degrees C for 30 seconds, and a final incubation for 7 minutes at 72 degrees C. PCR products were visualized by agarose gel electrophoresis and positive results were excised and purified from the gel using the ZymoClean Gel DNA recovery Kit (Zymo Research, USA). Purified replicate PCR amplifications from each

sample were combined prior to iTAG sequencing using Illumina MiSeq PE300 at Georgia Genomics Center.

#### **Data Processing Description**

Raw read files were deposited in GenBankSRA under accession number SRP072127.

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### **Data Files**

### File

Peru\_Margin\_iTAGs.csv(Comma Separated Values (.csv), 245 bytes) MD5:d95c0a9e868ac38da6f7cb39f624606b

Primary data file for dataset ID 654116

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### **Parameters**

Parameter	Description	Units
cruise_id	Cruise identifier	unitless
location	Sampling location	unitless
lat	Latitude of sampling location	decimal degrees
lon	Longitude of sampling location	decimal degrees
core_depth	Depth of the sediment core	meters below seafloor (mbsf)
sample_depth	Depth from which the samples were taken	meters below seafloor (mbsf)
accession_num	GenBank SRA accession number	unitless
accession_link	Hyperlink to GenBank SRA for the accession number	unitless

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### Instruments

Dataset- specific Instrument Name	
Generic Instrument Name	Advanced Piston Corer
Generic Instrument Description	The JOIDES Resolution's Advanced Piston Corer (APC) is used in soft ooze and sediments. The APC is a hydraulically actuated piston corer designed to recover relatively undisturbed samples from very soft to firm sediments. More information is available from IODP (PDF).

Dataset- specific Instrument Name	
Generic Instrument Name	Automated DNA Sequencer
Dataset- specific Description	Purified replicate PCR amplifications from each sample were combined prior to iTAG sequencing using Illumina MiSeq PE300 at Georgia Genomics Center.
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

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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# Deployments

### JRES-201

Website	https://www.bco-dmo.org/deployment/626163	
Platform	R/V JOIDES Resolution	
Report	http://dmoserv3.whoi.edu/data_docs/C-DEBI/cruise_reports/201PREL-1.pdf	
Start Date	2002-01-27	
End Date	2002-03-29	
Description	Leg 201 Controls on Microbial Communities in Deeply Buried Sediments, Eastern Equatorial Pacific and Peru Margin Sites 1225-1231 27 January-29 March 2002 Cruise report obtained from <a href="http://www-odp.tamu.edu/publications/pubs.htm">http://www-odp.tamu.edu/publications/pubs.htm</a>	

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# **Project Information**

Exploring of the Ecological Role(s) of Marine Fungi in the Deep Subseafloor (Fungal and

### prokaryotic activity in subseafloor)

Coverage: Peru Margin and Canterbury Basin New Zealand

The deep sedimentary biosphere, extending hundreds of meters below the seafloor harbors unexpected diversity of Bacteria, Archaea and microbial eukaryotes. Far less is known about microbial eukaryotes in subsurface habitats, albeit several studies have indicated that fungi dominate microbial eukaryotic communities and fungal molecular signatures (of both yeasts and filamentous forms) have been detected in samples as deep as 1740mbsf. Here we compare and contrast fungal ribosomal RNA gene signatures and whole community metatranscriptomes present in sediment core samples from 6 and 95mbsf from Peru Margin site 1229A and from samples from 12 and 345 mbsf from Canterbury Basin site U1352. The metatranscriptome analyses reveal higher relative expression of amino acid and peptide transporters in the less nutrient rich Canterbury Basin sediments compared to the nutrient rich Peru Margin, and higher expression of motility genes in the Peru Margin samples. Higher expression of genes associated with metals transporters and antibiotic resistance and production was detected in Canterbury Basin sediments. A poly-A focused metatranscriptome produced for the Canterbury Basin sample from 345 mbsf provides further evidence for active fungal communities in the subsurface in the form of fungal-associated transcripts for metabolic and cellular processes, cell and membrane functions, and catalytic activities. Fungal communities at comparable depths at the two geographically separated locations appear dominated by distinct taxa. Differences in taxonomic composition and expression of genes associated with particular metabolic activities may be a function of sediment organic content as well as oceanic province. Microscopic analysis of Canterbury Basin sediment samples from 4 and 403 mbsf produced visualizations of septate fungal filaments, branching fungi, conidiogenesis and spores. These images provide another important line of evidence supporting the occurrence and activity of fungi in the deep subseafloor biosphere.

This project was funded by C-DEBI sub-award #49538097.

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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 <u>Data Management Plan (PDF)</u> and in compliance with the <u>NSF Ocean Sciences Sample and Data Policy</u>. 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)	<u>OCE-0939564</u>

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