Microbial metatranscriptomics: upper and lower halocline water column data from the R/V Urania cruise in the Mediterranean Sea during 2012 (Pickled Protists project)

Website: https://www.bco-dmo.org/dataset/554030
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Project
» Pickled Protists or Community Uniquely Adapted to Hypersalinity? (Pickled Protists)

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Dataset Description

Metatranscriptome of whole microbial community from upper and lower halocline water column of Thetis DHAB

Related Reference:

Pachiadaki, M.G., Yakimov, M.M., Leadbetter, E., Edgcomb, V.P. 2014. Unveiling microbial activities along the halocline of Thetis, a deep hypersaline anoxic basin in the Eastern Mediterranean Sea. The ISME J. doi:10.1038/ismej.2014.100.

Methods & Sampling

Water column samples were collected with a 12 L Niskin bottles housed on a rosette (General Oceanics) equipped with a SBE- 911plus conductivity-temperature-depth (CTD) sensor (Sea- Bird Electronics). Determination of oxygen concentration at selected depths was carried out using the Winkler method with an automatic endpoint detection burette (716 DNS Titrino, Metrohm AG). Water from two distinct horizons (each representing a distinct range of salinity and oxygen between the top and bottom of each Niskin bottle) was collected from the Thetis interface; the upper interface (UI) layer corresponding to 7.0 – 16.3% salinity, and the lower (LI) layer with 21.4 – 27.6% salinity. Oxygen in the UI sample ranged from 9.5 µmol L-1 to undetectable, and remained undetectable in the lower sample. From each horizon, ca. 12 L of water were collected on Durapore membranes (47 mm, 0.65 µm, Millipore) under gentle pressure (~100 ml min−1), using a peristaltic pump (Ecoline ISM 1079). The filters were stored in RNA Shield at -80oC until analysis. The filters were transferred into Lysing Matrix E tubes (MP Biomedicals). Four ml of RNeasy Midi Kit Buffer RTL (Qiagen) were added, homogenized for 60 seconds at 4.0 m/s using a FastPrep®-24 (MP Biomedicals), and centrifuged for 10 minutes at 4,000 x g. Liquids were transferred to clean tubes, 1 volume of 70% ethanol was added to each tube, and extracts were processed following the RNeasy kit instructions. Extractions were treated with DNase and purified with the MEGA Clear kit as described above. Fluid and filter extracts were combined for each sample and concentrated by ethanol precipitation. Absence of DNA was confirmed by 40 cycles of PCR using

the general bacterial SSU rRNA gene primer 8F and the universal primer 1492R. For each extraction of total RNA (two replicates per depth horizon), cDNA was synthesized using the Ovation RNA-Seq System V2 Kit (NuGEN) following the manufacturer's instructions. Finally, cDNAs were purified with the MinElute Reaction Cleanup Kit (Qiagen) and sent for paired-end sequencing. One lane of Illumina HiSeq 2x100bp was requested for each sample (two replicates per lane).

Data Processing Description

Forward and reverse reads were paired and filtered for quality control using CLC Genomics Workbench 5.0 (CLCBio) and a minimum quality score of 28, a minimum read length of 94 bp, allowing no sequences with ambigious nucleotides. The same platform was used to perform assembly of contigs and mapping of reads to contigs. The Rapid Analysis of Multiple Metagenomes with a Clustering and Annotation Pipeline was used through the CAMERA platform to assign contigs to clusters of orthologous gene (COG) families, gene ontologies (GO), and protein families (Pfam) using four translation tables.

BCO-DMO Processing:
- original file: EdgcombThetisMetatranscriptomeData_final.xlsx
- added conventional header with dataset name, PI name, version date
- renamed parameters to BCO-DMO standard
- added cruise id, lat, lon
- added html links to GenBank BioProject

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Data Files

File
Thetis_metatranscriptomics.csv (Comma Separated Values (.csv), 1.21 KB) MD5:51fcf469f88ba70aca55687280e9dff2 Primary data file for dataset ID 554030

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Parameters

Parameter	Description	Units
cruise_id	cruise identification	unitless
site	sampling location	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
sampling_method	sampling method	unitless
sample_descrip	description of sample	unitless
project_id	GenBank Bioproject number	unitless
accession_number	GenBank accession number	unitless
sample	sample identification	unitless
tax_id	GenBank Taxonomy ID number	unitless
filename	sequence result file name	unitless

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Instruments

Dataset-specific Instrument Name	Automated Sequencer
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Roche 454 FLX Titanium platform
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	Niskin bottle
Generic Instrument Name	Niskin bottle
Dataset-specific Description	12L Niskin bottles mounted on a General Oceanics rosette sampler equipped with a SBE-911plus conductivity-temperature-depth (CTD) sensor (Sea- Bird Electronics)
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

Dataset-specific Instrument Name	MS_SID
Generic Instrument Name	Submersible Incubation Device-In Situ Microbial Sampler
Dataset-specific Description	Microbial Sampler - In Situ Incubation Device (MS-SID)
Generic Instrument Description	The Submersible Incubation Device-In Situ Microbial Sampler (SID-ISMS) system was developed for the 2011 NSF funded DHAB Metazoans Mediterranean Brine research project and first used on cruise AT18-14. The system includes several integrated components including: a 2 liter incubation chamber; fixation filters and water sample bottles; a High Range CTD (Neil Brown Ocean Sensors, Inc., USA) equipped with two turbidity sensors (Wet Labs ECOView); an Aanderra 2808F oxygen optode; an SDSL-data link; and a sonardyne beacon, a pinger and a 24 volt deep-sea battery. The sensors and sampling devices are mounted on a frame that is attached to the hydro-wire. Lowering rate and recovery speed are controlled by a winch mounted on the surface vessel.

Dataset-specific Instrument Name	Thermal Cycler
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

Urania-2012-09

Website	https://www.bco-dmo.org/deployment/554001
Platform	R/V Urania
Start Date	2012-09-14
End Date	2012-10-01
Description	Microbial sample collection

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Project Information

Pickled Protists or Community Uniquely Adapted to Hypersalinity? (Pickled Protists)

Coverage: Mediterranean Sea

Protists are an essential component of microbial food webs and play a central role in global biogeochemical cycles, and thus are key players in sustaining the healthy functioning of any ecosystem. Over the past few years a rich diversity of protists has been revealed in a range of extreme environments, indicating that the frontiers of eukaryotic life are still being explored. Only recently, one of the most extreme marine environments known to science was discovered in the eastern Mediterranean Sea at a depth of ~3500m, namely deep hypersaline anoxic basins (DHABs). These basins are characterized by extremely high salt concentrations (up to saturation) that have been considered anathema to life. Instead, highly diverse communities of bacteria exist in the waters of these basins. With the exception of a preliminary study to this proposal that indicated a diverse and active assemblage of protists in the water column along the halocline and below the halocline, these DHABs remain largely unexplored regarding eukaryotic life forms. The sediments of the DHABs have not been explored for protists at all.

The investigators will collect water column and sediment samples on a short cruise to two basins with different brine chemistries. An exciting combination of molecular, cultivation-independent and culture-based approaches will be used to study the microbial communities of two basins. Investigators will use those approaches to determine adaptive strategies of marine protist communities to hypersaline, anoxic environments and the degree of their potential impact on biogeochemical cycling as a result of their predation activities, the degree to which the dominant protists maintain bacterial or archaeal symbionts, and the identity of those symbionts. The original research proposal identified Bannock and Discovery Basins as the field study areas, however the 2009 cruise collected samples at Discovery and Urania Basin. Methods to be employed include RNA-based sequence analysis of diversity based on 18S rDNA genes, statistical analyses of community composition and phylotype richness, geochemical documentation of the water column and sediments using classical and microelectrode approaches, expression profiling using 3'-UTR fragments of mRNAs, sequencing of complete gene transcripts for proteins appearing to confer adaptation to hypersalinity, analysis of the proteome signatures, FISH-SEM to characterize novel extremophiles, CARD-FISH to identify eukaryote prey and putative symbionts, and TEM to assess morphology and endobiont presence in common benthic morphotypes.

Hypersaline environments rank highly in the list of extreme systems that have attracted increasing notice in science as well as by the lay public. For example, considering predictions of increasing temperatures and drought in certain regions of our planet, the number of hypersaline habitats may increase dramatically causing this ecosystem to gain importance on a global scale. Thus, an understanding of the ecosystem in these habitats will help predict future ecosystem functioning due to global change. From a different perspective, revealing the mechanisms of adaptation to high salinity has become a major objective, both for biological science and for potential commercial exploitation of natural products associated with those adaptations.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0849578

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