454 pyrotags for Bacterial and Archaeal taxa in deep hypersaline anoxic basin (DHAB) halocline sediments from the R/V Atlantis AT18-14 cruise in the Eastern Mediterranean; 35.3 N 21.7 E in 2011 (Pickled Protists project)

Website: https://www.bco-dmo.org/dataset/553979
Version: 2015-03-19

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

Contributors	Affiliation	Role
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Dataset Description

Pyrotags of bacteria and archaea obtained from Urania, Discovery, and L'Atalante DHAB halocline sediments.

Sequences have been submitted to GenBank under BioProject PRJNA270764. They will be publicly available once the manuscript is accepted (Kormas et al. submitted to Extremophiles)

Methods & Sampling

ROV Jason was used to collect push cores 6.35cm diameter obtained from the Deep Submergence Lab at Woods Hole Oceanographic Institution (www.whoi.edu/groups/DSL/) and configured with a seal to prevent contamination during ascent from the halocline sediments of L' Atalante, Urania and Discovery basins in the eastern Mediterranean Sea at a depth of about 3500-4000m. Sediments were profiled for oxygen and immediately frozen upon return to surface.

Data Processing Description

RNA was purified using the MEGAclear kit (Ambion, USA). Reverse transcription of the purified RNA samples was performed using the QuantiTect kit (Qiagen, USA). Tag-pyrosequencing of the 16S rRNA gene was performed using PCR amplification of the V4-V6 region of the 16S rRNA gene and the primer pair S-DBact-0341-b-S-17 (5'-CCTACGGGNGGCWGCAG-3') and S-D-Bact-0785-a-A-21 (5'-GACTACHVGGGTATCTAATCC-3') for Bacteria (Klindworth et al. 2012), and archaea349F (5'-GYGCASCAGKCGMGAAW-3') and archaea806R (5'-GGACTACVSGGGTATCTAAT-3') for Archaea (Takai & Horikoshi 2000), as described in Dowd et al., (2008). A one-step, 30-cycle PCR reaction was performed using HotStarTaq Plus Master Mix Kit (Qiagen, USA). Following

PCR, all amplicon products (ca. 450 bp) from different samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, USA). Samples were sequenced utilizing the Roche 454 FLX Titanium platform and reagents according to the guidelines at the MRDNA Ltd. (Shallowater, TX, USA) sequencing facility. Processing of the resulting sequences, i.e. trimming and quality control, was performed with the MOTHUR software (v 1.30) including denoising of the flowgrams using PyroNoise and data normalization to the smallest number of sequences in the resulting libraries. Sequences ≥250 bp with no ambiguous base assignments and no homopolymers ≥8 bp were included in downstream analyses. Single singletons, i.e. sequences that appeared only with once in the whole dataset, were excluded from further analysis. These sequences were aligned using the SILVA SSU database (release 108). All sequences were binned into Operational Taxonomic Units (OTUs) and were clustered (average neighbor algorithm) at 97% sequence similarity. Coverage values were calculated with MOTHUR (v 1.30).

BCO-DMO Processing:
- original file: EdgcombDHABprokaryoteTags.xlsx
- replaced positions for Discovery and Urania with those in event log for pushcores
- added html links to GenBank BioProject
- added cruise_id, lat, lon

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Data Files

File
sediment_pyrotags_prok.csv (Comma Separated Values (.csv), 1.02 KB) MD5:3326a169dbf6eefadc5ff1241c58d997 Primary data file for dataset ID 553979

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Parameters

Parameter	Description	Units
project_id	GenBank Project identification	unitless
seq_target	sequence target	unitless
sample	GenBank Sample identification	unitless
SRA	GenBank Sequence Read Archive identification	unitless
sample_location	sampling location	unitless
cruise_id	cruise identification	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees

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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	ROV Jason
Generic Instrument Name	ROV Jason
Dataset-specific Description	Sampled with push cores of 6.35cm diameter and configured with a seal to prevent contamination during ascent.
Generic Instrument Description	The Remotely Operated Vehicle (ROV) Jason is operated by the Deep Submergence Laboratory (DSL) at Woods Hole Oceanographic Institution (WHOI). WHOI engineers and scientists designed and built the ROV Jason to give scientists access to the seafloor that didn't require them leaving the deck of the ship. Jason is a two-body ROV system. A 10-kilometer (6-mile) fiber-optic cable delivers electrical power and commands from the ship through Medea and down to Jason, which then returns data and live video imagery. Medea serves as a shock absorber, buffering Jason from the movements of the ship, while providing lighting and a bird’s eye view of the ROV during seafloor operations. During each dive (deployment of the ROV), Jason pilots and scientists work from a control room on the ship to monitor Jason’s instruments and video while maneuvering the vehicle and optionally performing a variety of sampling activities. Jason is equipped with sonar imagers, water samplers, video and still cameras, and lighting gear. Jason’s manipulator arms collect samples of rock, sediment, or marine life and place them in the vehicle’s basket or on "elevator" platforms that float heavier loads to the surface. More information is available from the operator site at URL.

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

AT18-14

Website	https://www.bco-dmo.org/deployment/58732
Platform	R/V Atlantis
Start Date	2011-11-25
End Date	2011-12-08
Description	According to the pre-cruise plan, the two main science objectives are: (1) water column sampling at two basins: Discovery and Urania Basins, at 3 depths: brine (approx 3500-4000m depth), halocline (~3500m), and reference (~2000m) using a new sampler, the SID-ISMS (under construction), with the vessel CTD/Niskin rosette as backup and (2) sediment coring at both basins, using ROV Jason. Cores will be collected in 3 locations for each basin, the "bathtub ring" where the halocline impinges on the seafloor, the brine, and a reference core sample from above the halocline. Station "Discovery" (35° 19.213’ N 21° 41.351’ E) will be occupied for 6 days as will "Station 2" (35° 13.674’ N 21° 28.58’ E). The proposed science activities include: (1) water column sampling using the SID-ISMS to collect in situ filtered water (ship must hold position during deployment while instrument is working) and preserved in situ for molecular work; (2) water column sampling using the SID-ISMS to collect in situ filtered and preserved samples for FISH/microscopy experiments; (3) grazing experiment using SID-ISMS to collect water from halocline of each basin and measure the grazing rates of protozoa over a 6 hour period. The instrument must remain at depth during the 6 hour SID-ISMS grazing experiments. The sampler can be lifted to ~3000 m depth to get it away from the bottom, but the ship must maintain position to avoid dragging the sampler; (4) coring of "bathtub ring" at each basin using the ROV Jason that will be used to locate the bathtub ring and then collect cores at that location; (5) coring of brine at each basin (ROV Jason will reach into the brine from the bathtub ring area and will collect cores). Corers will be a combination of large Jason pushcores (property of co-PI Bernhard) and also some RNAlater samplers (similar to those used by Tim Shank (WHOI). The RNAlater samplers must be fabricated (and perhaps some borrowed from the Shank lab group); and (6) coring of a reference sample from outside the halocline (above) at each basin (normal seawater sediments). The research team aboard the R/V Atlantis, headed south on 25 November 2011 from Piraeus (port of Athens, Greece), to the study areas about 100 miles west of the island of Crete. No cruise report will be submitted for this cruise, but the science party did maintain a blog at the Dive and Discover site for Dive and Discover Expedition 14 - The Mediterranean Deep Brines (URL: http://www.divediscover.whoi.edu/expedition14/index.html). During the cruise, 5 ROV Jason dives, 10 SID-ISMS deployments, 1 multicorer (no samples recovered), and 3 Niskin rosette casts were completed. Image data from the ROV Jason dives for the AT18-14 cruise are available from the WHOI ROV Jason Virtual Van by clicking the year 2011 on the page's left side panel, and then clicking on the link for AT18-14. The cruise was supported by NSF-BIOLOGY awards:http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=0849578 (Edgcomb)http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=1061391 (Bernhard) Original cruise data are available from the NSF R2R data catalog

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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

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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