NCBI and iMicrobe accessions for transcriptomes of T. oceanica and T. weissflogii grown in varied Fe and light (GeTFeHvCOdia project)

Website: https://www.bco-dmo.org/dataset/636775
Data Type: experimental
Version: 2016-01-28

Project
» Genomic and Transcriptomic Comparison of Iron and Light Physiology in Coastal and Oceanic Diatoms (GeTFeHvCOdia)

Contributors	Affiliation	Role
Jenkins, Bethany D.	University of Rhode Island (URI)	Principal Investigator
Chappell, Phoebe Dreux	Old Dominion University (ODU)	Co-Principal Investigator
Copley, Nancy	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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Dataset Description

Marine MicroEukaryote Transcriptome Sequencing Project (MMETSP) Sample Links:
iMicrobe accession: http://data.imicrobe.us/project/view/104
GenBank SRA accession: http://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA231566

Related Datasets:

Thalassiosira Fe and light limitation - culture conditions
Thalassiosira Fe and light limitation - ENA accessions

References:

Media:
Guillard RRL, Hargraves PE (1993). *Stichochrysis immobilis* is a diatom, not a chyrsophyte. *Phycologia* **32:** 234-236.

Culturing, RNA Extraction:
Chappell, P.D.*, L.P. Whitney*, J.R. Wallace, A.I. Darer, S. Jean-Charles, and B.D. Jenkins. 2015. Genetic indicators of iron limitation in wild populations of *Thalassiosira oceanica* from the northeast Pacific Ocean – *ISME J.* 9(3): 592-602. doi: 10.1038/ismej.2014.171 (* indicates co-first author)

Sequencing:
Keeling, P.J. *et al.* (2014) The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP): illuminating the functional diversity of eukaryotic life in the oceans through transcriptome sequencing. *PLoS Biol, 12*(6), e1001889. doi: 10.1371/journal.pbio.1001889

Methods & Sampling

Thalassiosira oceanica (CCMP 1005) and *T. weissflogii* (CCMP 1010) isolates were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (West Boothbay Harbor, ME, USA). *T. oceanica was* isolated in 1958 at 33.1833°N, 65.25°W North Atlantic; *T. weissflogii was* isolated in 1969, at approximately 37°N 65°W Gulf Stream, between Bermuda and New York. Culture experiments were performed using a modified version of f/2 made in 0.2 µm filtered and microwave-sterilized Sargasso seawater (Guillard and Hargraves, 1993) see culture conditions dataset for modifications to f/2 for each different treatment. The inoculum for the low iron treatment came from replete cultures that had undergone two successive dilutions (1:10) into media without added iron, resulting in f/2 media with < 4 nM Fe. All macronutrient stocks were processed through a Chelex® 100 ion-exchange column (Bio-Rad Laboratories Inc., Hercules, CA, USA) containing resin prepared according to Price et al. (1989) and 0.2 µm Acrodisc® filter-sterilized (Pall Corporation, Port Washington, NY, USA). All media preparation and culture transferring was performed in a Class-100 HEPA filtered hood.

For all experiments, triplicate cultures were grown at a light level of 140 E/m^2/s or 45 E/m^2/s at 25 ^{o} C (click on Get Data, above) in a Percival incubator (Percival Scientific, Perry, IA, USA) and incubated gently shaking on a MaxQ® 2000 orbital shaker (Thermo Fisher Scientific, Waltham, MA, USA). Growth of the cultures was monitored daily with fluorescence measurements and cell counts (data not shown). Cultures were harvested when growth of the nutrient limited cultures began to decrease when compared to the replete cultures. Biomass was collected by gentle filtration onto 2 µm filters. Filters were placed in screw cap tubes containing 500 µL Qiagen Buffer RLT (Qiagen, Venlo, Netherlands), flash frozen in liquid nitrogen, and stored at -80°C until RNA extractions were conducted.

Total RNA was extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol, with the following exceptions: cells were lysed using 0.5 mm and 0.1 mm zirconia/silica beads (BioSpec, Bartlesville, OK, USA) mixed with the lysis buffer and bead beaten until the solution looked homogenous (approximately 1 minute). The lysis solution was then put over Qiashredder columns (Qiagen) to remove any large plant material that could clog the spin columns. To aid in the removal of DNA, two DNase digestions were performed. First, Qiagen's RNase-free DNase Set (an on-column treatment) was used according to the manufacturer's instructions. The second DNA removal step was conducted using the Turbo DNA-free kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. The RNA was then quantified in duplicate using the Qubit Fluorometer (Life Technologies). Following quantification, RNA samples were pooled and precipitated with a standard ammonium acetate/ethanol precipitation and sent for sequencing. An aliquot of the RNA extracted from individual replicates of the low iron and replete treatments of *T. weissflogii* (CCMP 1010) were also sent for sequencing.

Data Processing Description

Data in SRA and ENA databases is linked as raw sequence data in fastq format. Details of processing of sample files associated with MMETSP sample assemblies on iMicrobe site is given in Keeling *et al.* 2014

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Data Files

File
accessions.csv (Comma Separated Values (.csv), 6.39 KB) MD5:6669d33db04aa5a5b33f2314bf3ae8ea Primary data file for dataset ID 636775

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Parameters

Parameter	Description	Units
species	sample species	unitless
MMETSP_sample_id	iMicrobe sample name and link	unitless
iMicrobe_sample_accession	iMicrobe sample accession number and link	unitless
NCBI_BioSample_id	GenBank BioSample identification and link	unitless
NCBI_study_accession	GenBank Study accession number and link	unitless
NCBI_BioProject_id	GenBank BioProject identification and link	unitless
NCBI_SRA_id	GenBank Sequence Read Archive accession number and link	unitless

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Automated DNA Sequencer
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.

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Deployments

Jenkins_2015

Website	https://www.bco-dmo.org/deployment/636824
Platform	URI
Start Date	2015-01-01
End Date	2015-12-31
Description	genomics study

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Project Information

Genomic and Transcriptomic Comparison of Iron and Light Physiology in Coastal and Oceanic Diatoms (GeTFeHvCOdia)

Coverage: laboratory experiments with temperate diatoms

Project description from NSF award abstract:
Diatoms are among the most important primary producers in the ocean. Coastal species can respond to dynamic inputs of nutrients into the surface ocean and form large biomass blooms. Phytoplankton growth in much of the ocean is limited by one nutrient or another, and species that persist in these environments must be able to function under these low nutrient conditions. Oceanic diatom species have lower Fe requirements for growth and may have evolved to subsist in low Fe environments by changing the composition of Fe-demanding metabolic pathways. Iron and light responsive pathways are intimately linked because of the large Fe requirement of photosynthesis and the potential for both to limit growth and the efficiency of the biological carbon pump. Physiological and field studies have shown that many diatoms are susceptible to Fe/light co-limitation, but we lack information on the biochemical basis for co-limitation and how this differs between diatom species. This project will use a combination of genomics tools to investigate how coastal and oceanic diatoms in the ecologically important *Thalassiosira* genus respond to differing conditions of Fe and light. The investigators will compare the genome sequence of the oceanic diatom T. oceanica, which has recently been sequenced by the PI in collaboration with Illumina, Inc., to published diatom genomes to identify potential differences and similarities in the Fe and light metabolism in oceanic and coastal diatoms. They will use normalized libraries of Expressed Sequence Tags (EST) to characterize the transcriptome of *T. oceanica*, an oceanic strain of *T. weissflogii*, and the coastal diatom *T. rotula* grown in a matrix of Fe-limiting and replete conditions and at low and growth-saturating light levels. And they will quantify gene expression levels for the transcriptome-wide response in these experiments using digital gene expression (DGE), and use the EST data to map the DGE tags. Data from the DGE and EST experiments will be used to compare how diatom metabolism responds to variable light and Fe concentrations and to identify target genes for following limitation in natural diatom populations. Expression of these genes will be monitored in time-course experiments with additional manipulations of Fe and light levels to identify gene markers indicative of different physiological states using quantitative PCR (qPCR). The investigators will also design antibodies to a select number of proteins to monitor protein expression, and they will use qPCR and antibodies to follow responses in diatom communities in field samples collected from a cruise transitioning between Fe-replete and Fe-limiting environments as part of other funded research efforts. This work will further our understanding of how diatoms are adapted to different environments and what the genetic basis for their ecological success may be. Results from this work will help us predict how diatoms may respond to changing light regimes as a result of increased stratification due to climate change and help predict if species from different habitats will have similar or varied responses.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0962208

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