Links to published dmdA, cDNA, and RNA sequences from the Dauphin Island Cubitainer Experiment (DICE) from samples collected on R/V E.O. Wilson in the Gulf of Mexico, Alabama (En-Gen DMSP Cycling project)

Website: https://www.bco-dmo.org/dataset/3792
Version: 20 June 2014
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Project
» En-Gen: A Functional Genomics Approach to Organic Sulfur Cycling in the Ocean (En-Gen DMSP Cycling)

Contributors	Affiliation	Role
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Dataset Description

Links are provided to published dmdA amplicon, cDNA transcript, and mircoarray sequences resulting from Dauphin Island Cubitainer Experiments (DICE) as part of the project "En-Gen: A Functional Genomics Approach to Organic Sulfur Cycling in the Ocean".

Experimental design, methods, and results are further described in:
E. C. Howard, S. Sun, C. R. Reisch, D. A. del Valle, R. P. Kiene, and M. A. Moran (2010). Changes in DMSP Demethylase Gene Assemblages in Response to an Induced Phytoplankton Bloom. Applied and Environmental Microbiology, vol. 77, p. 524. DOI: 10.1128/AEM.01457-10
Rinta-Kanto, H. Burgmann, S. M. Gifford, S. Sun, S. Sharma, R. P. Kiene, and M. A. Moran (2011). Analysis of Sulfur-Related Gene Expression by Roseobacter Communities Using a Taxon-Specific Functional Gene Microarray. Environmental Microbiology, vol. 13, p. 453. DOI: 10.1111/j.1462-2920.2010.02350.x
J. M. Rinta-Kanto, S. Sun, S. Sharma, R. P. Kiene, and M. A. Moran (2012). Bacterial Community Transcription Patterns During a Marine Phytoplankton Bloom", Environmental Microbiology, vol. 14, p. 228. DOI: 10.1111/j.1462-2920.2011.02602.x

Methods & Sampling

See Howard et al. (2010), Rinta-Kanto et al. (2011), and Rinta-Kanto et al. (2012) for detailed methods, which are paraphrased below:

"In October 2006, seawater was collected from near-surface waters in the Gulf of Mexico off the coast of Dauphin Island (temp = 27 degrees C, salinity = 34) by lowering a clean bucket ~10 cm below the surface.

Water was filtered through a 200 micrometer mesh into six 20-liter polyethylene Cubitainers (Fold-A-Carrier; Reliance Products, Ltd.). Three experimental Cubitainer microcosms were amended with sodium nitrate (10 uM) and potassium phosphate (0.6 uM) to induce bloom conditions; three control microcosms received no nutrient amendments. The microcosms were incubated at 27 degrees C with a 12-h-on/12-h-off light cycle (200 umol quanta per square meter per second) for the duration of the experiment. At the initial time point (T = 0), the full contents of one control and one experimental microcosm (20 liters each) were sacrificed for detailed chemical and biological measurements."

Howard et al. (2010):
"For DNA samples, water from each time point was sequentially filtered through 8-um and 3-um prefilters, and particles were collected on 0.2-um pore-size polycarbonate filters. DNA was extracted using the PowerMax soil DNA isolation kit (MoBio Laboratories, Inc.). Concentrations were estimated by absorbance on a NanoDrop spectrophotometer (Thermo Scientific). DNA extracted from duplicate control and experimental samples was used as template for PCRs, followed by pyrosequencing of amplicons. Universal dmdA primers were modified from the work of Varaljay et al (2010). PCR products were run on 1% (dmdA amplicons) or 2% (bacterial and archaeal 16S rRNA amplicons) agarose gels, followed by purification using the Qiagen QIAquick gel extraction kit. Products were cleaned using the Ampure system (Agencourt Bioscience Corporation). Two separate pools were assembled, and these were sequenced on separate halves of the PicoTiterPlate (454 Life Sciences). 1,300 ng DNA was used in Roche GS FLX LR70 pyrosequencing at the University of South Carolina Environmental Genomics Facility (Columbia, SC).

For reads of high quality, adaptor and key sequences were removed and sequences were clustered using the CD-HIT program. For analysis of dmdA amplicons, reference sequences (the longest read in the cluster) were analyzed via BLASTX against an in-house DmdA database. rRNA sequences were deposited in the Community Cyberinfrastructure for Advanced Marine Microbial Ecology Research and Analysis (CAMERA) database with project ID CAM-PROJ_DICE (sample CAM_S_A001= experimental microcosms; sample CAM_S_A002 = control microcosms). dmdA amplicon sequences were deposited in the NCBI Short Read Archive with project ID 49967."

Rinta-Kanto et al. (2011):
"The microarray was designed based on *R. pomeroyi* DSS-3 genes and their orthologs in 12 other Roseobacter genomes (http://www.roseobase.org). The gene encoding DMSP demethylase (dmdA) was included on the array. Genes involved in DMSP cleavage to DMS (discovered in Roseobacter genomes subsequent to microarray final design) were not included on the array. Once a list of *R. pomeroyi* DSS-3 genes was compiled, orthologs for these genes in the other 12 Roseobacter genomes were identified using reciprocal BLASTs. Probes were designed from the orthologs using the Hierarchical Probe Design algorithm. The best probes selected by the program were queried against NCBI's nr database using BLASTN, and only those probes with a best hit to a Roseobacter gene were retained. The final array consisted of 1578 probes targeting 431 genes. The complete list of probes is deposited in NCBI's Gene Expression Omnibus database under accession number GPL10323. (The identifier codes for each probe begin with the *R. pomeroyi* DSS-3 locus tag followed by a number generated by the probe design algorithm describing the placement of the probe in the tree generated during design.)"

Rinta-Kanto et al. (2012):
"RNA samples were collected from the microcosms on Day 5 by prefiltering ~1 L subsamples through 8.0 and 3.0 um poresize polycarbonate filters and collecting bacterioplankton on 0.2-um polycarbonate filters. Filters were placed in sterile 15 ml polypropylene tubes with lysis/binding buffer from the Ambion RNAqueous-kit. Cells were lysed using the Ambion RNAqueous Midi-kit (Applied Biosystems, Austin, TX). RNA was extracted from the lysate using Qiagen RNeasy kit. RNA was quantified after the extraction using a Nanodrop spectrometer.

Samples were processed as described in Poretsky et al. (2009) with some modifications (see Rinta-Kanto et al. 2012). Resulting amplified antisense RNAs were converted to doublestranded cDNA using Universal RiboClone cDNA Synthesis System (Promega). Concentrations of mRNA, amplified RNA and cDNA were measured using a Nanodrop spectrometer, and quality of the mRNA and cDNA was assessed using an Experion Automated Electrophoresis Station (Bio-Rad). Five ug of cDNA from each sample was used for pyrosequencing with the GS FLX sequencing system (Roche). Transcript libraries were deposited in the CAMERA database with a project ID CAM_PROJ_DICE."

References:
Poretsky, R.S., Hewson, I., Sun, S.L., Allen, A.E., Zehr, J.P., and Moran, M.A. (2009) Comparative day/night metatranscriptomic analysis of microbial communities in the North Pacific subtropical gyre. Environ Microbiol 11: 1358–1375. DOI: 10.1002/9781118010518.ch63
Varaljay, V. A., E. C. Howard, S. Sun, M. A. Moran (2009). Deep Sequencing of a DMSP-Degrading Gene (dmdA) Using PCR Primer Pairs Designed from Marine Metagenomic Data. Applied and Environmental Microbiology, vol.

76, p. 609. DOI: 10.1128/AEM.01258-09

Data Processing Description

BCO-DMO added the site coordinates obtained from the CAMERA repository.

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Data Files

File
DICE_sequences.csv (Comma Separated Values (.csv), 2.08 KB) MD5:2bb64a272656a415ff2b6542644ca2ed Primary data file for dataset ID 3792

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Parameters

Parameter	Description	Units
taxon	Description of the taxon of study.	text
site_desc	Description of the general location where samples were collected.	text
lat	Latitude of the sampling site. North = positive.	decimal degrees
lon	Longitude of the sampling site. East = positive.	decimal degrees
month	2-digit month when sampling occurred.	mm (01 to 12)
year	4-digit year when sampling occurred.	YYYY
descrip	Sample description.	text
repository	Name of the repository where sequences can be accessed.	text
project_id	The identifier assigned to the project in the repository.	text
sample_accession	Sample accession number and link to repostiory.	unitless

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Instruments

Dataset-specific Instrument Name	bucket
Generic Instrument Name	bucket
Dataset-specific Description	Water was collected in the field using a clean bucket.
Generic Instrument Description	A bucket used to collect surface sea water samples.

Dataset-specific Instrument Name	Spectrometer
Generic Instrument Name	Spectrometer
Dataset-specific Description	RNA (post-extraction), mRNA, amplified RNA (aRNA), and cDNA were quantified using a Nanodrop spectrometer.
Generic Instrument Description	A spectrometer is an optical instrument used to measure properties of light over a specific portion of the electromagnetic spectrum.

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Deployments

DMSP_Dauphin_Island

Website	https://www.bco-dmo.org/deployment/58888
Platform	R/V E.O. Wilson
Description	October 2006 deployment in the Gulf of Mexico approximately 20 km off the coast of Dauphin Island, AL to collect surface water for the project "En-Gen: A Functional Genomics Approach to Organic Sulfur Cycling in the Ocean". (Latitude: 30°03.041′N, Longitude: 87°59.708′W)

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Project Information

En-Gen: A Functional Genomics Approach to Organic Sulfur Cycling in the Ocean (En-Gen DMSP Cycling)

Coverage: Sapelo Island, GA, USA, 31.4° N Lat, 81.3° W Lon / Dauphin Island, AL, USA, 30.3 ° N Lat, 88.1° W Lon

The recent discovery of key genes that mediate competing pathways at a critical juncture in the marine sulfur cycle has allowed biogeochemists to make rapid advances in understanding where and when sulfur transformations occur in the ocean, and most importantly, what factors regulate them. This project describes an environmental functional genomics project that will rapidly increase our knowledge of the role that bacterioplankton play in dimethylsulfoniopropionate (DMSP) cycling in ocean surface waters, focusing particularly on biological controls of volatile sulfur exchange across the ocean/atmosphere boundary.

The investigators have asked three critical hypotheses to explain the regulation of bacterial DMSP degradation: that involve investigations on the energy constraints of DMSP cycling, the role that DMSP concentration in the oceans plays, and the sulfur requirements for bacterial growth. These research areas serve as the focus for hypothesis-driven laboratory and field studies using functional genomics approaches that will track patterns in gene expression in relation to sulfur metabolism. The hypotheses will be tested with:
1) chemostat systems with a model marine bacterium Silicibacter pomeroyi;
2) microcosm experiments with Gulf of Mexico seawater; and
3) field studies at various sites in the Gulf of Mexico. Marine bacterioplankton play a key role in regulating the

flux of DMSP-derived sulfur to the atmosphere, a process of great importance for global climate regulation and marine productivity.

The investigators will also be involved in graduate and undergraduate student education, and two post-doctoral associates will be trained to address multidisciplinary challenges in environmental microbiology. High school biology students in Athens, GA will participate in marine microbial biology research that includes bacterial diversity and discovery studies in coastal Georgia, follow-up training in molecular tools and bioinformatics in their own classroom, and summer internships at the University of Georgia and Dauphin Island Sea Laboratory.

(The description above is from the NSF Award Abstract).

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0724017

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