

Links to published dmdA sequences from marine bacterioplankton from Sapelo Island, GA (En-Gen DMSP Cycling project)

Website: <https://www.bco-dmo.org/dataset/3791>

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Project

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

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Dataset Description

Links are provided to published dimethylsulfoniopropionate (DMSP) demethylase protein, DmdA, sequence data from marine bacterioplankton that has been deposited in the NCBI GenBank Short Read Archive (SRA).

Experimental design, methods, and results are further described in:

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](https://doi.org/10.1128/AEM.01258-09)

Methods & Sampling

See Varaljay et al. 2010 for detailed methods, which are paraphrased below.

"Surface water was collected between October 2000 and April 2005 at two sampling sites at the [Sapelo Island Microbial Observatory](#) (SIMO) in coastal Georgia: the Dean Creek site (a salt marsh tidal creek) and the Dobby Sound (a coastal ocean inlet). Approximately 20 liters of water was filtered sequentially through 8.0-um, 1.0-um, and 0.2-um pore size polycarbonate membrane filters, with two replicate samples obtained at each site. Cells captured on the 1.0-um (particle associated) and 0.2-um filter (free-living) were stored at -20 degrees C until DNA extraction using a PowerMax Soil DNA Isolation kit (MO BIO Laboratories, Inc.). 76 DNA extracts were used in the study, representing 38 samples of each size fraction. Samples were separately pooled by size fraction in equal amounts to produce composite free-living and particle-associated DNA samples.

Primer pairs giving single amplicons of the correct size from the composite SIMO DNA were chosen for analysis by sequencing. Amplicons suitable for 454 sequencing were prepared by modifying each primer pair with an adaptor sequence at the 5' end of the forward primer according to the method of Huber et al. Additional four-base key sequences in between the adaptor and primer sequence were used to distinguish

inosine and degenerate primer sequences. PCRs were carried out in duplicate using 24 ng template DNA and then pooled before sequencing. Amplicons were cleaned using the AMPure purification method (Agencourt Bioscience Corp., Beverly, MA) according to the 454 Life Sciences protocol (Roche Diagnostics Corp., Branford, CT), with modifications to the volume of purified PCR products (30.0 l) and AMPure beads (50.4 l). Products were quantified spectrophotometrically and combined in equal concentrations in four separate pools based on primer and size fraction. Four-region 454 FLX LR70 sequencing was carried out at the University of South Carolina EnGenCore facility. Amplicon sequences were annotated by BLASTx analysis. This analysis was used to distinguish correct target sequences from closely related paralogous sequences and to classify amplicons by clade."

Data Processing Description

"To account for differences in the number of amplicons sequenced for each primer pair (ranging from 2,000 to 12,000 sequences), a resampling approach was used in which 1,000 sample populations of the same size were randomly drawn from the amplicon pools being compared. This approach was used to normalize the number of 90% dmdA clusters in comparisons between primer pairs and size fractions." (Varaljay et al. 2010)

BCO-DMO added the site coordinates, which were obtained from the [Sapelo Island Microbial Observatory](#) (SIMO) website.

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Data Files

File
bacterioplankton_dmdA_sequences.csv (Comma Separated Values (.csv), 389 bytes) MD5:bae3deaeddbb27f02186c71bc7f4dd81
Primary data file for dataset ID 3791

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Parameters

Parameter	Description	Units
site_desc	Description of the general location where samples were collected.	text
taxon	Description of the taxon of study.	text
accession_number	Accession number and link to NCBI's Short Read Archive (SRA).	unitless
site	Name of the sampling site.	text
lat	Latitude of the sampling site. North = positive.	decimal degrees
lon	Longitude of the sampling site. East = positive.	decimal degrees

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Deployments

DMSP_Sapelo_Island

Website	https://www.bco-dmo.org/deployment/58889
Platform	shoreside Sapelo Island Microbial Observatory
Description	Surface water was collected between October 2000 and April 2005 at two sampling sites at the Sapelo Island Microbial Observatory (SIMO) in coastal Georgia: the Dean Creek site (a salt marsh tidal creek) and the Dobby Sound (a coastal ocean inlet). Samples were collected for the project "En-Gen: A Functional Genomics Approach to Organic Sulfur Cycling in the Ocean". Dean Creek site: 81.2699°W, 31.3929°N Dobby Sound: 81.2915°W, 31.3862°N Both of these sites are located within the Georgia Coastal Ecosystems Long Term Ecological Research (LTER) study area, more specifically the UGAMI site (UGA Marine Institute).

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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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