NCBI accession numbers and related metadata from a study of transcriptomic response of Emiliania huxleyi to 2-heptyl-4-quinolone (HHQ)

Website: https://www.bco-dmo.org/dataset/773272
Data Type: experimental
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Project
» Collaborative Research: Building a framework for the role of bacterial-derived chemical signals in mediating phytoplankton population dynamics (HHQSignals)

Contributors	Affiliation	Role
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Abstract
NCBI accession numbers and related metadata from a study of transcriptomic response of Emiliania huxleyi to 2-heptyl-4-quinolone (HHQ). Sequences from this study are available at the NCBI GEO under accession series GSE131846 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?&acc=GSE131846

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Coverage

Temporal Extent: 2018-06-20 - 2018-06-23

Dataset Description

Sequences from this study are available at the NCBI GEO under accession series GSE131846 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?&acc=GSE131846

Acquisition Description

Batch 2 L cultures of axenic Emiliania huxleyi strain CCMP2090 were grown in natural seawater-based f/2-Si medium (Guillard 1975) in sterile acid-washed polycarbonate bottles. Cultures were maintained on a 14:10 light (80 +/- 5 µmol photons m^{-2} s^{-1}):dark cycle at 17.5 - 17.8 °C. After 48 hr of growth, quadruplicate 2 L cultures were exposed to either 1 ng/ml, 10 ng/ml, or 100 ng/ml concentrations of 2-heptyl-4-quinolone (HHQ). Quadruplicate bottles were also exposed to dimethyl sulfoxide (DMSO) to serve

as a vehicle control (final concentration 0.002% DMSO in all bottles). Cell biomass was collected 24 hr and 72 hr after treatment via centrifugation (9,000 RPM for 8 min at 4 °C) of 400 ml of culture and total RNA extracted using the RNeasy Plus Mini Kit (Qiagen) following the manufacturer's recommendations using 350 µl RLT plus buffer per sample and the optional centrifugation (14,000 RPM for 1 min) step to ensure membranes were dry prior to elution with 30 µl RNase free water. Eluent was reapplied to the membrane, and incubated for 8 min at room temperature before repeating the elution step to increase yield. Strand-specific RNAseq library construction was performed using the KAPA Stranded mRNA-Seq library preparation kit with KAPA mRNA capture beads (Kapa Biosystems) and sequenced on the NextSeq platform (Illumina) to generate 75 bp paired-end reads.

Processing Description

Sequenced reads were conservatively trimmed to remove adaptors, low-complexity and low-quality sequence, and rRNA reads (including chloroplast and mitochondria rRNA) using Trimmomatic (V0.38; Bolger et al. 2014) with a custom adapter file containing Emiliania huxleyi CCMP2090 rRNA sequences and the following settings: ILLUMINACLIP:2:30:10 LEADING:3 TRAILING:3 MAXINFO:40:0.5 MINLEN:50. Read quality was examined before and after trimming using FastQC (V0.11.8; https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and MultiQC (V1.6; Ewels et al. 2016). Paired files produced by Trimmomatic were then concatenated prior to determination of transcript abundances using Salmon (V0.12.0; Patro et al. 2017) and the Ensembl (Kersey et al. 2017) gene predictions for Emiliania huxleyi CCMP1516 (the non-axenic form of CCMP2090; ftp://ftp.ensemblgenomes.org/pub/protists/release-41/fasta/emiliania_huxleyi/cdna/) as a transcript target index (k-mer size = 23). Salmon was run in the quasi-mapping mode with default settings and the following flags: --validateMappings and –gcBias. Quantification results from Salmon were examined using MultiQC (V1.6; Ewels et al. 2016) and then processed using the tximport R package with default settings (V1.10.0; Soneson et al. 2015) to prepare for gene-level analyses. Transcript and gene IDs were linked using the general feature format file for Emiliania huxleyi CCMP1516 available from Ensembl Genomes (ftp://ftp.ensemblgenomes.org/pub/protists/release-41/gff3/emiliania_huxleyi). Normalization and determination of significantly differentially abundant transcripts was preformed using the DESeq2 R package (V1.22.1; Love et al. 2014) using standard functions and workflows recommended by the authors. After estimation of size factors to normalize for differences in library sequencing depth and gene dispersion estimation using the biological replicates, tests for differential expression were carried out for each pairwise comparison of interest with the Wald test using a negative binomial generalized linear model. Logarithmic fold change (LFC) estimates were shrunken by calling the apeglm package (V1.6.0; Zhu et al. 2018) within DESeq2. The resulting p values were adjusted for multiple testing using the Benjamini-Hochberg (BH) procedure and transcripts with a BH-adjusted p value < 0.1 were deemed to be differentially abundant.

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Related Publications

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics, 30(15), 2114–2120. doi:10.1093/bioinformatics/btu170
Methods

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics, 32(19), 3047–3048. doi:10.1093/bioinformatics/btw354
Methods

Guillard, R. R. L. (1975). Culture of Phytoplankton for Feeding Marine Invertebrates. Culture of Marine Invertebrate Animals, 29–60. doi:10.1007/978-1-4615-8714-9_3
Methods

Kersey, P. J., Allen, J. E., Allot, A., Barba, M., Boddu, S., Bolt, B. J., … Yates, A. (2017). Ensembl Genomes 2018: an integrated omics infrastructure for non-vertebrate species. Nucleic Acids Research, 46(D1), D802–D808. doi:10.1093/nar/gkx1011
Methods

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology, 15(12). doi:10.1186/s13059-014-0550-8
Methods

Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. Nature Methods, 14(4), 417–419. doi:10.1038/nmeth.4197
Methods

Soneson, C., Love, M. I., & Robinson, M. D. (2016). Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. F1000Research, 4, 1521. doi:10.12688/f1000research.7563.2
Methods

Zhu, A., Ibrahim, J. G., & Love, M. I. (2018). Heavy-tailed prior distributions for sequence count data: removing the noise and preserving large differences. Bioinformatics, 35(12), 2084–2092. doi:10.1093/bioinformatics/bty895
Methods
Methods

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Parameters

Parameter	Description	Units
series_accession	NCBI GEO identifier	unitless
biosample_accession	NCBI BioSample identifier	unitless
biosample_link	URL for SRA BioSample Page at NCBI	unitless
sample_name	Sample name	unitless
organism	NCBI taxonomy name	unitless
tax_ID	NCBI taxonomy ID	unitless
strain	Organism strain	unitless
sample_type	Sample type	unitless
biomaterial_provider	Name of the lab or PI or a culture collection identifier	unitless
env_biome	Descriptor of the broad ecological contxt of a sample	unitless
samp_size	Amount of size of sample that was collected	unitless
temp	Temperature of the sample at time of sampling	degrees Celsius
light_level_umol_m2_s	Light level	microml photons m-2 s-1
light_dark_hr	Duration of light and dark cycles	hours
media	Type of growth medium used	unitless
collection_date	Date sample was collected; format: DD-Mmm-YYYY	unitless
geo_loc_name	Geographical origin of the sample	unitless
treatment	Treatment	unitless
time_elapsed	Time elapsed since treatment	hours
bio_replicate	Biological replicate number	unitless

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Instruments

Dataset-specific Instrument Name	llumina NextSeq500
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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Project Information

Collaborative Research: Building a framework for the role of bacterial-derived chemical signals in mediating phytoplankton population dynamics (HHQSignals)

Coverage: Bergen, Norway

NSF Abstract: Bacteria and phytoplankton play a central role in the modification and flow of materials and nutrients through the marine environment. While it has been established that interactions between these two domains are complex, the mechanisms that underpin these interactions remain largely unknown. There is increasing recognition, however, that dissolved chemical cues govern these microbial interactions. This project focuses on establishing a mechanistic framework for how bacterially derived signaling molecules influence interactions between phytoplankton and bacteria. The quorum-sensing (QS) molecule, 2-heptyl-4-quinolone (HHQ) will be used as a model compound for these investigations. Previously published work suggests that exposure to very low levels of HHQ results in phytoplankton mortality. Gaining a mechanistic understanding of these ecologically important interactions will help to inform mathematical models for the accurate prediction of the cycling of material through the marine microbial loop. This work initiates a new, hybrid workshop-internship undergraduate research program in chemical ecology, with a focus Bacteria and phytoplankton play a central role in the modification and flow of materials and nutrients through the marine environment. While it has been established that interactions between these two domains are complex, the mechanisms that underpin these interactions remain largely unknown. There is increasing recognition, however, that dissolved chemical cues govern these microbial interactions. This project focuses on establishing a mechanistic framework for how bacterially derived signaling molecules influence interactions between phytoplankton and bacteria. The quorum-sensing (QS) molecule, 2-heptyl-4-quinolone (HHQ) will be used as a model compound for these investigations. Previously published work suggests that exposure to very low levels of HHQ results in phytoplankton mortality. Gaining a mechanistic understanding of these ecologically important interactions will help to inform mathematical models for the accurate prediction of the cycling of material through the marine microbial loop. This work initiates a new, hybrid workshop-internship undergraduate research program in chemical ecology, with a focus into bacteria-phytoplankton interactions. Undergraduate students

participate in an intense summer learning experience where research and field-based exercises are supplemented with short-lecture based modules. Students return to their home institutions and work closely with the PIs to conduct interdisciplinary research relating to the aims and scope of the summer research. This research also provides training and career development to two graduate students and a postdoctoral scientist. Interactions between phytoplankton and bacteria play a central role in mediating biogeochemical cycling and microbial trophic structure in the ocean. The intricate relationships between these two domains of life are mediated via excreted molecules that facilitate communication and determine competitive outcomes. Despite their predicted importance, identifying these released compounds has remained a challenge. The PIs recently identified a bacterial QS molecule, HHQ, produced by globally distributed marine gamma-proteobacteria, which induces phytoplankton mortality. The PIs therefore hypothesize that bacteria QS signals are critical drivers of phytoplankton population dynamics and, ultimately, biogeochemical fluxes. This project investigates the timing and magnitude of HHQ production, and the physiological and transcriptomic responses of susceptible phytoplankton species to HHQ exposure, and quantifies the influence of HHQ on natural algal and bacterial assemblages. The work connects laboratory and field-based experiments to understand the governance of chemical signaling on marine microbial interactions, and has the potential to yield broadly applicable insights into how microbial interactions influence biogeochemical fluxes in the marine environment.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1657808
NSF Division of Ocean Sciences (NSF OCE)	OCE-1657818

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