Pipeline for phylogenetic analysis of the GlcDEF, GOX/LOX, and tsar genes conducted as part of "Community context and pCO2 impact the transcriptome of the "helper" bacterium Alteromonas in co-culture with picocyanobacteria"

Website: https://www.bco-dmo.org/dataset/882970
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Project
» Collaborative Research: Ecology and Evolution of Microbial Interactions in a Changing Ocean (LTPE)

Contributors	Affiliation	Role
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Abstract
Pipeline for phylogenetic analysis of the GlcDEF, GOX/LOX, and tsar genes conducted as part of "Community context and pCO2 impact the transcriptome of the "helper" bacterium Alteromonas in co-culture with picocyanobacteria" (Barreto Filho et al., 2022). The provided code, documentation, input and output files include all the information needed to replicate our findings. The following results abstract describes these data along with related datasets which can be accessed from the "Related Datasets" section of this page. Many microbial photoautotrophs depend on heterotrophic bacteria for accomplishing essential functions. Environmental changes, however, could alter or eliminate such interactions. We investigated the effects of changing pCO2 on gene expression in co-cultures of 3 strains of picocyanobacteria (Synechococcus strains CC9311 and WH8102 and Prochlorococcus strain MIT9312) paired with the ‘helper’ bacterium Alteromonas macleodii EZ55. Co-culture with cyanobacteria resulted in a much higher number of up- and down-regulated genes in EZ55 than pCO2 by itself. Pathway analysis revealed significantly different expression of genes involved in carbohydrate metabolism, stress response, and chemotaxis, with different patterns of up- or down-regulation in co-culture with different cyanobacterial strains. Gene expression patterns of organic and inorganic nutrient transporter and catabolism genes in EZ55 suggested resources available in the culture media were altered under elevated (800 ppm) pCO2 conditions. Altogether, changing expression patterns were consistent with the possibility that the composition of cyanobacterial excretions changed under the two pCO2 regimes, causing extensive ecophysiological changes in both members of the co-cultures. Additionally, significant downregulation of oxidative stress genes in MIT9312/EZ55 cocultures at 800 ppm pCO2 were consistent with a link between the predicted reduced availability of photorespiratory byproducts (i.e., glycolate/2PG) under this condition and observed reductions in internal oxidative stress loads for EZ55, providing a possible explanation for the previously observed lack of “help” provided by EZ55 to MIT9312 under elevated pCO2. The data and code stored in this archive will allow the reconstruction of our analysis pipelines. Additionally, we provide annotation mapping files and other resources for conducting transcriptomic analyses with Alteromonas sp. EZ55.

Table of Contents

Methods & Sampling

See "Related Datasets" section for other results and pipelines from this study.

Strains

Six clones each of the open ocean *Synechococcus* strain WH8102 and the coastal *Synechococcus* strain CC9311 were obtained by dilution to extinction in SN media [1]. The parent cultures of each organism were obtained from the National Center for Marine Algae (Boothbay Harbor, Maine) and were axenic upon receipt. Six clones of *Alteromonas* sp. strain EZ55 and *Prochlorococcus* MIT9312 were also previously obtained and cryopreserved at -80 °C [2]. The EZ55 clones used in our *Synechococcus* co-cultures were the same 6 clones used in our previous transcriptomic study of MIT9312 [2] in order to maximize the comparability of results between that study and the present study. Co-cultures were initiated by mixing each of the six clones of CC9311 and WH8102 with one of the EZ55 clones.

Culture conditions

Synechococcus cultures were grown under similar conditions to those described in our previous experiment with *Prochlorococcus* [2]. Briefly, all cultures were prepared in acid-washed conical-bottom glass centrifuge tubes containing 13 mL of artificial seawater (ASW) amended with nutrient stocks [1] and with acid and/or base to control pCO_2. ASW (per L: 28.41 g NaCl, 0.79 g KCl, 1.58 g CaCl2*2H2O, 7.21 g MgSO4*7H2O, 5.18 g MgCl2*6H2O) was sterilized in acid-washed glass bottles, amended with 2.325 mM (final concentration) of filter-sterilized sodium bicarbonate, then bubbled with sterile air overnight. *Synechococcus* cultures were grown in SEv (per L: 32 µM $NaNO_3$, 2 µM NaH_2PO_4, 20 µL SN trace metal stock, and 20 µL F/2 vitamin stock). The primary differences between this medium and the PEv medium used in our earlier *Prochlorococcus* study are the nitrogen source (NO_3^- vs. NH_4^+, with molar concentration of N and N:P ratios identical to PEv) and the addition of F/2 vitamins [1]. Carbonate chemistry of each media batch was determined prior to pCO_2 manipulations by measuring alkalinity and pH by titration and colorimetry, respectively [2, 3] and then using the *oa* function in *seacarb* package in R to determine how much hydrochloric acid and bicarbonate (for 800 ppm pCO_2) or sodium hydroxide (for 400 ppm pCO_2) was needed to achieve desired experimental conditions [4]. Acid and base amendments were introduced immediately prior to inoculation. Cultures were grown in a Percival growth chamber at 21º C under 150 µmol photons m^{-2} s^{-1} on a 14:10 light:dark cycle. *Synechococcus* cultures were grown on a rotating tissue culture wheel at approximately 60 rpm.

For "EZ55 growth experiments with photorespiration metabolites" and "RNA library preparation and sequencing" details see the related dataset "Synechococcus growth and genetic sequence accessions from pCO2 experiments" https://www.bco-dmo.org/dataset/882390

Detection of glycolate utilization genes

Several genes involved in the bacterial glycolate utilization pathway (glycolate/lactate oxidase, the 3 subunits of glycolate dehydrogenase, and tartronate semialdehyde reductase) were not annotated in the reference genomes for our organisms so we specifically sought to detect them using a reciprocal BLAST analysis. We retrieved any sequences from each of the four reference genomes with high similarity (E-value < 0.001) to the relevant genes from *Escherichia coli* and/or *Synechococcus elongatus* using blastp [7] and then back-matched each retrieved sequence to the *E. coli* or *S. elongatus* reference genome. If the reciprocal match was the same gene used in the original BLAST search, we considered the match significant.

Data Processing Description

This .zip package Phylogenetic_analysis.zip contains files and code necessary to replicate our phylogenetic

analysis of the GlcDEF, GOX/LOX, and tsar genes.

The "Phylogenetic_analysis" folder contains the files necessary to replicate our phylogenetic analysis of the GlcDEF, GOX/LOX, and tsar genes. Only alignments are provided for glcE and tsar genes, in fasta format, as GlcE.align.faa and tsar.align.faa. For GOX/LOX and glcDF, the following file types are provided:

.align.faa -- fasta format alignments

.mdsx -- MEGA format files used for sequence alignment

.modelselect.txt -- MEGA output used to determine which model to use for tree formation

.mtsx -- MEGA format tree session files

.nwk -- final trees in Newick format

Note that glcD, glcD2, glcF, and marine glcDF fusion proteins were analyzed with a single alignment. For organisms with glcD and glcF as separate ORFs, the coding sequences were concatenated with glcD first followed by glcF.

BCO-DMO Data Manager Processing notes:
* Pipeline attached as a zip file bundle to "Data Files" section.
* SRA accessions and related collection and treatment information extracted from NCBI's SRA Run Selector and attached as a supplemental file (SraRunTable_PRJNA377729.csv)

[table of contents | back to top]

Data Files

File
Phylogenetic analysis pipeline filename: Phylogenetic_analysis.zip (ZIP Archive (ZIP), 192.94 KB) MD5:d8de80595ddf7eb84c97c03889160092 This .zip package contains files and code necessary to replicate our phylogenetic analysis of the GlcDEF, GOX/LOX, and tsar genes. The "Phylogenetic_analysis" folder contains the files necessary to replicate our phylogenetic analysis of the GlcDEF, GOX/LOX, and tsar genes. Only alignments are provided for glcE and tsar genes, in fasta format, as GlcE.align.faa and tsar.align.faa. For GOX/LOX and glcDF, the following file types are provided: .align.faa -- fasta format alignments .mdsx -- MEGA format files used for sequence alignment .modelselect.txt -- MEGA output used to determine which model to use for tree formation .mtsx -- MEGA format tree session files .nwk -- final trees in Newick format Note that glcD, glcD2, glcF, and marine glcDF fusion proteins were analyzed with a single alignment. For organisms with glcD and glcF as separate ORFs, the coding sequences were concatenated with glcD first followed by glcF.

[table of contents | back to top]

Supplemental Files

File
BioProject PRJNA377729 SRA Run Table filename: SraRunTable_PRJNA377729.csv (Comma Separated Values (.csv), 45.60 KB) MD5:84d6df19caa3cd3e095c0161d624c5d3 SRA accessions and related collection and treatment information extracted from NCBI's SRA Run Selector. This includes all SRA runs and related BioSamples for BioProject PRJNA377729 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA377729).

[table of contents | back to top]

Related Publications

Barreto Filho, M. M., Lu, Z., Walker, M., & Morris, J. J. (2022). Community context and pCO2 impact the transcriptome of the “helper” bacterium Alteromonas in co-culture with picocyanobacteria. ISME Communications, 2(1). https://doi.org/10.1038/s43705-022-00197-2
Results

[table of contents | back to top]

Related Datasets

IsRelatedTo

Lamont-Doherty Earth Observatory, Columbia University (2017). Phytoplankton, Impacts of Evolution on the Response of Phytoplankton Populations to Rising CO2. 2017/03. NCBI:BioProject: PRJNA377729.[Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA377729.

Morris, J. (2022) **Synechococcus (WH8102 and CC9311) growth and genetic sequence accessions from experiments with variable pCO2 treatments from 2016 to 2018.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-10-13 doi:10.26008/1912/bco-dmo.882390.1 [view at BCO-DMO]
Relationship Description: Related data from the same experiment.

Morris, J. J., Barreto Filho, M. M., Zhiying, L., Walker, M. (2022) **Pipelines for transcriptome analyses conducted as part of "Community context and pCO2 impact the transcriptome of the "helper" bacterium Alteromonas in co-culture with picocyanobacteria".** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-10-04 doi:10.26008/1912/bco-dmo.881942.1 [view at BCO-DMO]
Relationship Description: Related analysos from the same experiment.

Morris, J., Zhiying, L. (2023) **Carbonate chemistry data collected as part of a study of the "Community context and pCO2 impact the transcriptome of the "helper" bacterium Alteromonas in co-culture with picocyanobacteria".** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-12-27 doi:10.26008/1912/bco-dmo.883120.1 [view at BCO-DMO]
Relationship Description: Data from the same experiment.

[table of contents | back to top]

Parameters

Parameters for this dataset have not yet been identified

[table of contents | back to top]

Project Information

Collaborative Research: Ecology and Evolution of Microbial Interactions in a Changing Ocean (LTPE)

Coverage: Lab work: Birmingham, Alabama and New York, New York. Field Work: Bermuda Atlantic Time Series.

NSF Award Abstract:
Carbon dioxide released from fossil fuels is causing the ocean to become more acidic. Much attention has been given to how this will affect shelled animals like corals, but acidification also affects the algae that form the base of the ocean food chain. It is possible that future algal communities will look very different than they do today, with potentially negative consequences for fisheries, recreation, and climate. Alternatively, it is possible that these algae will be able to adapt rapidly enough to avoid the worst of it. This study looks at algae adapting to acidification in real time in the lab, focusing on "marketplace" interactions between the algae and the bacteria they live alongside. The researchers also go to sea to learn whether adaptations from the lab experiments are beneficial under real-world conditions. Ultimately, this project is helping scientists better understand how the ocean's most important and most overlooked organisms will respond to the changes humans are causing in their habitat. The researchers also use their scientific work to create fun educational opportunities from grade school to college, including agar art classes where students learn about microbial ecology by "painting" with freshly-isolated ocean bacteria.

The effect of ocean acidification on calcifying organisms has been well-studied, but less is known about how changing pH will affect phytoplankton. Previous work showed that the mutualistic interaction between the globally abundant cyanobacterium Prochlorococcus and its "helper" bacterium Alteromonas broke down under projected future CO2 conditions, leading to a strong decrease in the fitness of Prochlorococcus. It is possible that such interspecies interactions between microbes are important for many ecological processes, but a lack of understanding of how these interactions evolve makes it difficult to predict how important they are. This project is using laboratory evolution experiments to discover how evolution shapes the interactions between bacteria and algae like Prochlorococcus, and how these co-evolutionary dynamics might influence the biogeochemical processes that shape Earth's climate. Four research cruises to the Bermuda Atlantic Time Series are also planned to study how natural algal/bacterial communities respond to acidification, and whether evolved microbes from laboratory experiments have a competitive advantage in complex, natural communities exposed to elevated CO2. The ultimate goal of this project is to gain a mechanistic understanding of microbial interactions that can be used to inform models of Earth's oceans and biological feedbacks on global climate.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1851085

[table of contents | back to top]