Synechococcus (WH8102 and CC9311) growth and genetic sequence accessions from experiments with variable pCO2 treatments from 2016 to 2018

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

Contributors	Affiliation	Role
Morris, James	University of Alabama at Birmingham (UA/Birmingham)	Principal Investigator
Lu, Zhiying	University of Alabama at Birmingham (UA/Birmingham)	Scientist
Barreto Filho, Marcelo Malisano	University of Alabama at Birmingham (UA/Birmingham)	Student
Walker, Melissa	University of Alabama at Birmingham (UA/Birmingham)	Student
York, Amber D.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Synechococcus (WH8102 and CC9311) growth and genetic sequence accessions from experiments with variable pCO2 treatments. These data were produced as part of a study of the "Community context and pCO2 impact the transcriptome of the "helper" bacterium Alteromonas in co-culture with picocyanobacteria" (Barreto Filho et al., 2022). Sequences files are accessible from the National Center for Biotechnology Information (BioProject PRJNA377729). 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 inMIT9312/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.

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Coverage

Temporal Extent: 2016-10-24 - 2018-01-28

Methods & Sampling

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 pCO2. 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 NaNO3, 2 µM NaH2PO4, 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 (NO3- vs. NH4+, 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 pCO2 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 pCO2) or sodium hydroxide (for 400 ppm pCO2) 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.

Growth experiments

The transcriptomes of all six clonal replicates of each *Synechococcus* strain along with their EZ55 partners were assessed under approximately 400 (based on atmospheric pCO2 measured at Mauna Loa in 2015, when the experiment was planned) or 800 ppm (i.e., approximate predicted year 2100 pCO2 under IPCC scenario A2) pCO2. Prior to RNA extraction, each culture was acclimated to experimental conditions for three transfer cycles (approximately 14 generations). Growth was tracked by flow cytometry using a Guava HT1 Flow Cytometer (Luminex Corporation, Austin, TX). EZ55 cell concentrations were determined by dilution onto YTSS agar (per L, 4 g tryptone, 2.5 g yeast extract, 15 g sea salts, 15 g agar).
Whenever *Synechococcus* cell densities reached 2.6 x 105 cells mL-1, cultures were diluted 26-fold into fresh media. Preliminary experiments revealed that this cell concentration was low enough that growth was not limited by nutrients and pH and pCO2 were not significantly impacted by cyanobacterial carbon concentrating mechanisms. In the final transfer cycle, each culture was split into 5 identical subcultures to increase the biomass available for RNA extraction; all 5 subcultures of each clone were then pooled and collected on a single 0.2 mm polycarbonate filter by gentle syringe filtration, then flash-frozen in liquid nitrogen and stored at -80o C prior to RNA extraction. For WH8102 cultures, an average of 4.04 x 107 WH8102 cells and 3.91 x 108 EZ55 cells were collected per filter, and for CC9311 cultures, an average of 5.47 x 107 CC9311 and 7.33 x 108 EZ55

cells were collected per filter.

RNA library preparation and sequencing

RNA extraction was performed separately for each replicate culture with the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) with a small modification of the lysis step [2]. rRNA was removed with the Ribo-Zero rRNA Removal Kit for Bacteria (Illumina, San Diego, CA, USA) [7]. Following rRNA removal, samples were purified and concentrated with a RNeasy MiniElute cleanup kit (Qiagen). Quantity and quality of post-digestion RNA were assessed with an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). mRNA library preparation for Illumina Hi-seq 2500 paired-end sequencing (PE100) used TruSeq RNA sample prep kit v2 (Illumina, San Diego, CA, USA). DNA fragment length was 100 bp, paired ends were non-overlapping, and the insert size was approximately 300 bp. Individual barcode sequences were added to sequence reads for multiplex sequencing which were run in a single lane at the Sulzberger Columbia University Genome Center (CUGC) (New York, NY, USA).

EZ55 growth experiments with photorespiration metabolites

We investigated the ability of EZ55 to grow on metabolic intermediates in the photorespiration pathway as their sole carbon source. Glycine, glycolate, glucose, and glyoxylate stock solutions (concentrations of 20%, 4%, 4% and 10% W/V, respectively) were filter sterilized using a 0.2 µM filter. The pH of glycolate and glyoxylate stocks was adjusted to approximately 7 using 10 M NaOH. EZ55 clones were inoculated into ASW supplemented with Pro99 nutrients [1] and 0.1% (W/V) glucose [5] and acclimated for 24 hours at 28 ℃ with orbital shaking at 120 rpm. The detection of intracellular H2O2 was performed according to Lu et al. [6], with slight modification. Briefly, 1.5 ml of culture was centrifuged at 8000 rpm for 5 min, the supernatant was removed, and the pellet was resuspended in 1 ml phosphate buffered saline (pH=7.4, Fisher). 5 µl of 1 mM 2′,7′-dichlorodihydrofluorescein diacetate was added to the resuspension and vortexed for 5 seconds and then incubated for 1 h on a shaker (120 rpm) in the dark. The suspension was centrifuged at 8000 rpm for 5 min, the pellet was washed twice with PBS, and finally resuspended in 200 µl PBS. Fluorescence was measured by flow cytometry at excitation/emission wavelengths of 485/535 nm.

Detection of glycolate utilization genes (see related dataset "Pipelines for transcriptome analyses" https://www.bco-dmo.org/dataset/881942)

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.

Problems/Issues
In some of our earliest cultures, too few daily flow cytometry measurements were collected to calculate robust exponential growth rates, because it was impossible to confirm at least three data points corresponding to the logarithmic growth phase of the culture. For these cultures, only malthusian growth rates are reported.

Data Processing Description

Statistical analyses and related code described below can be downloaded from the pipeline package GrowthCurve_analysis.zip in the "Data Files" section of this page. The following refers to the file names within that .zip package.

The supplemental file SynProGrowthCurves.rcode.txt contains the R code necessary to replicate our statistical analysis of the growth curves of both the above Synechococcus cultures as well as the Prochlorococcus cultures reported in our earlier manuscript (Hennon et al. 2018). The packages plyr (Wickham, 2011), lme4 (Bates et al., 2017), and lsmeans (or emmeans (Lenth et al., 2022)) will need to be installed, but after that the code can be copied and pasted into an R window with the working directory set to contain SynGR.csv and our results should be reproducible.

The file SynGR.csv contains the compiled growth rate data used for the statistical analysis. Column headings are Species (CC9311, WH8102, or MIT9312), Replicate (biological replicate clone), Treatment (CO2- for 400 ppm pCO2 or CO2+ for 800 ppm), Transfer (see above), T0 (date of initial culture inoculation), Tend (date of

culture transfer or RNA harvesting), InitDens and FinalDens (cell density in cells per milliliter on T0 and Tend, respectively), t (elapsed time in days between T0 and Tend), m (malthusian growth rate, inverse days), r (exponential growth rate, inverse days), Lag (lag phase duration in days), DieOff (initial loss of cells after transfer as a proportion). Note that r, lag, and DieOff were only calculable for cultures that were monitored frequently enough to allow robust estimates of exponential growth rate; some CC9311 cultures were sampled less frequently.

BCO-DMO Data Manager Processing Notes:
* Entire analysis pipeline including exact format of data the pipeline requires added as a zip package to Data Files section.
* The results data file SynCurves.csv was imported into the BCO-DMO data system and published as the main results table for this dataset.
* SRA accessions and related collection and treatment information extracted from NCBI's SRA Run Selector and attached as a supplemental file (SraRunTable_PRJNA377729.csv)

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Data Files

File
syn_growth.csv (Comma Separated Values (.csv), 18.43 KB) MD5:5edeed6422f8d5951e542a9780250a62 Primary data file for dataset ID 882390

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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).
Growth curve analysis pipeline filename: GrowthCurve_analysis.zip (ZIP Archive (ZIP), 8.66 KB) MD5:1ed63e4f0d70942c0603f069a4f8d2b4 This .zip package contains the files necessary to replicate our growth curve analyses described in Barreto Filho et al. (2022). The file SynCurves.csv contains all of the raw data from the Synechococcus WH8102 and CC9311 growth experiments leading up to our RNA collection. Column headings are Strain (CC9311 or WH8102), Replicate (representing the clonal biological replicate of each culture), Treatment (400 ppm or 800 ppm pCO2), Transfer (each culture was passaged twice before RNA harvesting at the end of the third "transfer cycle"; during the third cycle, each replicate was split into 5 replicate tubes to increase harvested biomass, designated as e.g. replicate 1.1, 1.2, 1.3, etc.), Time (days since the first post-acclimation transfer), and Cell Density (in cells per milliliter, measured by flow cytometry). The file SynProGrowthCurves.rcode.txt contains the R code necessary to replicate our statistical analysis of the growth curves of both the above Synechococcus cultures as well as the Prochlorococcus cultures reported in our earlier manuscript (Hennon et al. 2018). The packages plyr, lme4, and lsmeans (or emmeans) will need to be installed, but after that the code can be copied and pasted into an R window with the working directory set to contain SynGR.csv and our results should be reproducible. The file SynGR.csv contains the compiled growth rate data used for the statistical analysis. Column headings are Species (CC9311, WH8102, or MIT9312), Replicate (biological replicate clone), Treatment (CO2- for 400 ppm pCO2 or CO2+ for 800 ppm), Transfer (see above), T0 (date of initial culture inoculation), Tend (date of culture transfer or RNA harvesting), InitDens and FinalDens (cell density in cells per milliliter on T0 and Tend, respectively), t (elapsed time in days between T0 and Tend), m (malthusian growth rate, inverse days), r (exponential growth rate, inverse days), Lag (lag phase duration in days), DieOff (initial loss of cells after transfer as a proportion). Note that r, lag, and DieOff were only calculable for cultures that were monitored frequently enough to allow robust estimates of exponential growth rate; some CC9311 cultures were sampled less frequently.

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Related Publications

Andersen RA (2005). Algal culturing techniques. Elsevier/Academic Press, Burlington, Mass. ISBN: 0120884267
Methods

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

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Usinglme4. Journal of Statistical Software, 67(1). doi:10.18637/jss.v067.i01
Methods

Gattuso, J.-P., & Lavigne, H. (2009). Technical Note: Approaches and software tools to investigate the impact of ocean acidification. Biogeosciences, 6(10), 2121–2133. https://doi.org/10.5194/bg-6-2121-2009
Methods

Hennon, G. M., Morris, J. J., Haley, S. T., Zinser, E. R., Durrant, A. R., Entwistle, E., … Dyhrman, S. T. (2017). The impact of elevated CO2 on Prochlorococcus and microbial interactions with “helper” bacterium Alteromonas. The ISME Journal, 12(2), 520–531. doi:10.1038/ismej.2017.189.
Methods

Knight, M. A., & Morris, J. J. (2020). Co-culture with Synechococcus facilitates growth of Prochlorococcus under ocean acidification conditions. Environmental Microbiology, 22(11), 4876–4889. doi:10.1111/1462-2920.15277
Methods

Lenth, R., Buerkner, P., Herve, M., Jung, M., Love, J., Miguez, F., Riebl, H., Singmann, H. (2022). emmeans: Estimated Marginal Means, aka Least-Squares Means. Estimated Marginal Means, aka Least-Squares Means. cran.r-project.org. Retrieved from https://cran.r-project.org/web/packages/emmeans/emmeans.pdf
Software

Wickham, H. (2011). The Split-Apply-Combine Strategy for Data Analysis. Journal of Statistical Software, 40(1). https://doi.org/10.18637/jss.v040.i01
Software

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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. 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 analysis from the same experiment.

Morris, J. J., Zhiying, L. (2022) **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".** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-10-25 doi:10.26008/1912/bco-dmo.882970.1 [view at BCO-DMO]
Relationship Description: Related analyses 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.

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Parameters

Parameter	Description	Units
Strain	Strain (CC9311 or WH8102)	unitless
Replicate	Replicate (representing the clonal biological replicate of each culture)	unitless
Treatment	Treatment (400 ppm or 800 ppm pCO2)	unitless
Transfer	Transfer (each culture was passaged twice before RNA harvesting at the end of the third 'transfer cycle'; during the third cycle, each replicate was split into 5 replicate tubes to increase harvested biomass, designated as e.g. replicate 1.1, 1.2, 1.3, etc.)	unitless
Time	Time (days since the first post-acclimation transfer)	unitless
Cell_Density	Cell Density (measured by flow cytometry)	cells per milliliter

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Instruments

Dataset-specific Instrument Name	Illumina Hi-seq 2500 paired-end sequencing (PE100)
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA)
Generic Instrument Name	Bioanalyzer
Generic Instrument Description	A Bioanalyzer is a laboratory instrument that provides the sizing and quantification of DNA, RNA, and proteins. One example is the Agilent Bioanalyzer 2100.

Dataset-specific Instrument Name	Millipore Guava HT1 Flow Cytometer
Generic Instrument Name	Flow Cytometer
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

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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.

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Funding

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

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