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"

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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
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" (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 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).

Data Processing Description

Analyses pipelines:

See "Data Files" section to download zip files containing the folders and files described below.

Each .zip package contains the files and code necessary to replicate our transcriptome analyses. Each organism's folder contains scripts for performing the aligment and counting (fastq_to_counts), RNA-seq pre processing and differential expression analysis (dge_analysis), and gene set testing analysis (Over-representation,ORA, and gene set enrichment analysis, GSEA) in R using the package "Rsubread".

All scripts are provided as .txt files. Additionally, we also have created and made available annotation packages (.db files) for each organism, which are necessary for annotating genes to proteins.

Also, in the Alteromonas_analysis folder we provided a file containing a list of KEGG ORTHOLOGY (KO) identifiers for Altermonas EZ55 genes, which is necessary for the gene set testing analysis for this organism because it does not have a KEGG code. This file was obtained from the KAAS - KEGG Automatic Annotation Server website (https://www.genome.jp/kegg/kaas/, Moriya et al., 2017). For the picocyanobacteria organisms KEGG codes are available and can be found in the scripts.

All of our sequences files are accessible from the National Center for Biotechnology Information (BioProject PRJNA377729, Sequence Read Archive accession numbers SRX2619948-SRX2619957, SRX3033334-SRX3033345, and SRX14411251-SRX14411274). For the picocyanobaceria, reference genomes (.fasta) and annotation (.GFF3) files can be obtained from the Ensemble bacteria website https://bacteria.ensembl.org/index.html. For Alteromonas we provided updated .fasta and annotation (.GTF) files in the Altermonas_analysis folder.

However, since the aligment steps require larger computer power usually being performed on servers inside a linux environment, we also provide our table of counts and metadata (targets) to allow the replication our differential gene expression analysis and gene set testing analysis using average laptops and Rstudio:

Alteromonas: ALL_EZ55_2.Rdata (count table) and targets.xlsx (metadata)

MIT9312: Pro_9312._y_targets_2.Rdata (contains the targets file)

CC9311: Syn_9311.Rdata (contains the targets file)

WH8102: Syn_8102_2.Rdata (contains the targets file)

BCO-DMO Data Manager Processing notes:
* Pipelines attached as a zip file bundles 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)

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Data Files

File
Transcriptome analysis pipeline: bacterium Alteromonas filename: Alteromonas_analysis.zip (ZIP Archive (ZIP), 3.21 MB) MD5:de512511c92a4ec1df264a982026ec7e This .zip package contains files and code necessary to replicate our transcriptome analysis for the “helper” bacterium Alteromonas. This includes scripts for performing the alignment and counting (fastq_to_counts), RNA-seq pre processing and differential expression analysis (dge_analysis), and gene set testing analysis (Over-representation,ORA, and gene set enrichment analysis, GSEA) in R using the package "Rsubread". All scripts are provided as .txt files. Additionally, we also have created and made available annotation packages (.db files) for each organism, which are necessary for annotating genes to proteins. Also, in the Alteromonas_analysis folder we provided a file containing a list of KEGG ORTHOLOGY (KO) identifiers for Altermonas EZ55 genes, which is necessary for the gene set testing analysis for this organism because it does not have a KEGG code. This file was obtained from the KAAS - KEGG Automatic Annotation Server website (https://www.genome.jp/kegg/kaas/). For the picocyanobacteria organisms KEGG codes are available and can be found in the scripts. All of our sequences files are accessible from the National Center for Biotechnology Information (BioProject PRJNA377729, Sequence Read Archive accession numbers SRX2619948-SRX2619957, SRX3033334-SRX3033345, and SRX14411251-SRX14411274). For the picocyanobaceria, reference genomes (.fasta) and annotation (.GFF3) files can be obtained from the Ensemble bacteria website https://bacteria.ensembl.org/index.html. For Alteromonas we provided updated .fasta and annotation (.GTF) files in the Altermonas_analysis folder. However, since the aligment steps require larger computer power usually being performed on servers inside a linux environment, we also provide our table of counts and metadata (targets) to allow the replication our differential gene expression analysis and gene set testing analysis using average laptops and Rstudio: Alteromonas: ALL_EZ55_2.Rdata (count table) and targets.xlsx (metadata) See the "Acquisition Description" section for further details about all the transcriptome analyses files and packages.

File

Transcriptome analysis pipeline: Prochlorococcus strain MIT9312

filename: MIT9312_analysis.zip

(ZIP Archive (ZIP), 359.05 KB)
MD5:077db0c74739b51e25d6a7f8f994acc9

This .zip package contains files and code necessary to replicate our transcriptome analysis for Prochlorococcus strain MIT9312. This includes scripts for performing the alignment and counting (fastq_to_counts), RNA-seq pre processing and differential expression analysis (dge_analysis), and gene set testing analysis (Over-representation,ORA, and gene set enrichment analysis, GSEA) in R using the package "Rsubread".

All scripts are provided as .txt files. Additionally, we also have created and made available annotation packages (.db files) for each organism, which are necessary for annotating genes to proteins.

All of our sequences files are accessible from the National Center for Biotechnology Information (BioProject PRJNA377729, Sequence Read Archive accession numbers SRX2619948-SRX2619957, SRX3033334-SRX3033345, and SRX14411251-SRX14411274). For the picocyanobaceria, reference genomes (.fasta) and annotation (.GFF3) files can be obtained from the Ensemble bacteria website https://bacteria.ensembl.org/index.html.

However, since the aligment steps require larger computer power usually being performed on servers inside a linux environment, we also provide our table of counts and metadata (targets) to allow the replication our differential gene expression analysis and gene set testing analysis using average laptops and Rstudio:

MIT9312: Pro_9312._y_targets_2.Rdata (contains the targets file)

See the "Acquisition Description" section for further details about all the transcriptome analyses files and packages.

Transcriptome analysis pipeline: Synechococcus strain CC9311

filename: CC9311_analysis.zip

(ZIP Archive (ZIP), 661.37 KB)
MD5:b92405aae202f17b0919f7f1673acaac

This .zip package contains files and code necessary to replicate our transcriptome analysis for coastal Synechococcus strain CC9311. This includes scripts for performing the alignment and counting (fastq_to_counts), RNA-seq pre processing and differential expression analysis (dge_analysis), and gene set testing analysis (Over-representation,ORA, and gene set enrichment analysis, GSEA) in R using the package "Rsubread".

All scripts are provided as .txt files. Additionally, we also have created and made available annotation packages (.db files) for each organism, which are necessary for annotating genes to proteins.

All of our sequences files are accessible from the National Center for Biotechnology Information (BioProject PRJNA377729, Sequence Read Archive accession numbers SRX2619948-SRX2619957, SRX3033334-SRX3033345, and SRX14411251-SRX14411274). For the picocyanobaceria, reference genomes (.fasta) and annotation (.GFF3) files can be obtained from the Ensemble bacteria website https://bacteria.ensembl.org/index.html.

However, since the alignment steps require larger computer power usually being performed on servers inside a linux environment, we also provide our table of counts and metadata (targets) to allow the replication our differential gene expression analysis and gene set testing analysis using average laptops and Rstudio:

CC9311: Syn_9311.Rdata (contains the targets file)

See the "Acquisition Description" section for further details about all the transcriptome analyses files and packages.

File

Transcriptome analysis pipeline: Synechococcus strain WH8102

filename: WH8102_analysis.zip

(ZIP Archive (ZIP), 471.22 KB)
MD5:2728a8af7c60fdf8c50b376c80c36270

This .zip package contains files and code necessary to replicate our transcriptome analysis for open ocean Synechococcus strain WH8102. This includes scripts for performing the alignment and counting (fastq_to_counts), RNA-seq pre processing and differential expression analysis (dge_analysis), and gene set testing analysis (Over-representation,ORA, and gene set enrichment analysis, GSEA) in R using the package "Rsubread".

All scripts are provided as .txt files. Additionally, we also have created and made available annotation packages (.db files) for each organism, which are necessary for annotating genes to proteins.

All of our sequences files are accessible from the National Center for Biotechnology Information (BioProject PRJNA377729, Sequence Read Archive accession numbers SRX2619948-SRX2619957, SRX3033334-SRX3033345, and SRX14411251-SRX14411274). For the picocyanobaceria, reference genomes (.fasta) and annotation (.GFF3) files can be obtained from the Ensemble bacteria website https://bacteria.ensembl.org/index.html.

However, since the aligment steps require larger computer power usually being performed on servers inside a linux environment, we also provide our table of counts and metadata (targets) to allow the replication our differential gene expression analysis and gene set testing analysis using average laptops and Rstudio:

WH8102: Syn_8102_2.Rdata (contains the targets file)

See the "Acquisition Description" section for further details about all the transcriptome analyses files and packages.

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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).

File

Transcriptome analysis output tables

filename: transcriptome_supplemental_tables.zip

(ZIP Archive (ZIP), 117.05 KB)
MD5:d503d607e29f5176635a5b761913775b

The transcriptome analysis output containing the supplemental tables S1 through S14 described in the main text and the supplemental information (Barreto Filho et al., 2022). Each is a flat-format spreadsheet.

Column headings are:

Table S1-S4:

species: which species each gene came from

symbol: locus tag of the indicated gene

product: annotation of the indicated gene

logFC: log fold change of the gene between tested conditions

PValue: statistical test of the significance of the difference between treatment groups

Table S5-S8:

partner: which organism EZ55 was partnered with, or "general" for the average co-culture response

treatment: pCO2 treatment level

symbol: locus tag of the indicated gene

product: annotation of the indicated gene

logFC: log fold change of the gene between tested conditions

logFC_rel_axenic: log fold change of the gene between the indicated condition and axenic EZ55

PValue: statistical test of the significance of the difference between treatment groups

Table S9-S14:

symbol: locus tag of the indicated gene

product: annotation of the indicated gene

logFC: log fold change of the gene between tested conditions

PValue: statistical test of the significance of the difference between treatment groups

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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

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.
Results

Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A. C., & Kanehisa, M. (2007). KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Research, 35(Web Server), W182–W185. https://doi.org/10.1093/nar/gkm321
References

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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. (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., 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

Parameters for this dataset have not yet been identified

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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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