Enzyme activity of Alteromonas macleodii exudates

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Project
» Collaborative Research: Extracellular vesicles as vehicles for microbial interactions in marine Black Queen communities (Vesicle Interactions)

Contributors	Affiliation	Role
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Abstract
The cyanobacterium Prochlorococcus has a conspicuously reduced genome causing it to require help from co-existing organisms for survival under a variety of stressful conditions. In this work we used conditioned media experiments to demonstrate that exudates of the heterotrophic bacterium Alteromonas macleodii EZ55 facilitated the survival of Prochlorococcus MIT9312 batch co-cultures as they entered stationary phase. Based on mass spectrometry analysis, Alteromonas exudates contained a wide variety of proteins; here we assessed whether or not these proteins exhibited common enzymatic activities including glycosidase, protease, phosphatase, and hydroperoxidase reactions. We also tested the impact of disrupting membranes by sonication on membrane activity as a test of the possibility that some proteins were packaged within extracellular membrane vesicles.

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Coverage

Location: Laboratories at the University of Alabama at birmingham

Methods & Sampling

Strains and culture conditions: All strains used in this study were taken from those used for a Long-Term Phytoplankton Evolution (LTPE) experiment (1). *Prochlorococcus* strains were streptomycin-resistant derivates of the high light-adapted strain MIT9312 obtained as described previously (2, 3), either before (Ancestor) or after 500 generations of evolution at either 400 ppm or 800 ppm pCO2 conditions (i.e., modern day or projected year 2100 conditions (4)). *Alteromonas* strains were derivatives of strain EZ55, originally isolated

from a *Prochlorococcus* MIT9215 culture (3). As with our *Prochlorococcus* strains, we used both ancestral and evolved varieties of EZ55 co-evolved with *Prochlorococcus* at the two pCO2 treatments and subsequently isolated. *Prochlorococcus* cultures were revived from cultures cryopreserved with 7.5% DMSO in liquid nitrogen vapor, and *Alteromonas* cultures were revived from cultures preserved with 20% glycerol stored at -80o C. Prior to use in experiments, all *Prochlorococcus* cultures were grown in co-culture with *Alteromonas* EZ55 helpers (3) and were acclimated to culture conditions for at least 4 generations prior to data collection.

Alteromonas cultures were grown in YTSS medium (5) and *Prochlorococcus* cultures were grown in Pro99 medium (6) or PEv medium (1), both made in an artificial seawater base (ASW) (1). Prior to addition to co-cultures *Alteromonas* strains were pelleted at 2000 g for 2 minutes and washed twice in sterile ASW, then added to cultures at approximately 106 cells ml-1. *Alteromonas* was grown at 30o C with 120 rpm shaking. Unless otherwise noted, *Prochlorococcus* and co-cultures were grown in static 13 mL conical bottom acid-washed glass tubes under approximately 75 mmol photons m-2 s-1 cool white light in a Percival incubator set to 23o C. When medium additions were employed, all solutions were filter sterilized with a 0.2 mm filter. Cell densities of *Prochlorococcus* cultures to standardize inoculations between experiments were determined using a Guava HT1 flow cytometer (Luminex Corporation, Austin, TX) by the distinctive signature of these cells on plots of forward light scatter vs. red fluorescence (Fig. S1A). Day-to-day culture growth was tracked using the *in vivo* chlorophyll *a* module for the Trilogy fluorometer (Turner Designs, San Jose, CA) with a custom 3D-printed adapter designed for conical bottom tubes. Fluorometer measurements and cell counts were linearly related across the range of cells examined in this study (Pearson correlation coefficient 0.835, $p = 1.38 \times 10-6$, Fig. S1B).

Concentration of* Alteromonas *exudates: EZ55 was grown in Pro99 media supplemented with 0.1% glucose to sustain growth in the absence of *Prochlorococcus* exudates. We scaled cultures up progressively from 12 mL to 2 L. The 2L culture was grown in a vented bottle with an outlet connected to a filter with 0.22 µm pore size. After removing most of the cells by centrifugation, we produced size-fractionated, concentrated exudates using tangential flow filtration using Sartorius Vivaflow 200 cassettes. The 2L culture supernatant was passed first through a 0.22 µm cassette using a Masterflex L/S peristaltic pump (Cole-Parmer) to remove bacterial cells, then through a 50 kDa module and a 5 kDa module in succession to produce >50 kDa and <50 kDa fractions that were each concentrated approximately 100-fold. A portion of the >50 kDa fraction was placed in boiling water for 5 minutes to denature proteins. When these concentrated extracellular products were added to culture media for growth experiments they were diluted 100-fold, returning them to approximately their original concentration prior to filtration.

***Enzyme activities of exudates*:** We used a variety of enzymatic tests to evaluate the activity of *Alteromonas* exudates. We used the fluorescent conjugates 4-nitrophenyl-α-D-glucopyranoside, 4-nitrophenyl-β-D-glucopyranoside, and 4-nitrophenyl-N-acetyl-β-D-glucopyranoside to measure the activities of the glycolytic enzymes α-glucosidase, β-glucosidase and N-acetyl-glucosaminidase, respectively (7). Phosphatase activity was measured by following the hydrolysis of fluorescent substrate 4-methylumbelliferyl phosphate (8). Enzyme activity for these experiments was expressed as the rate of fluorescent product accumulation over the first 2-5 hours, minus the rate of accumulation in an exudate-free media control. Protease activity was measured after a 30 minute incubation based on Sigma's non-specific protease activity assay using casein as a substrate (9). Siderophore activity was determined after a 30 minute incubation using chrome azurol S (10). Both fluorescence and optical density traces for these assays were measured using a Biotek Synergy H1 plate reader (Agilent, Santa Clara, CA). Hydroperoxidase activity was assessed by measuring the elimination of a hydrogen peroxide spike after 2 hours, tracking the hydrogen peroxide concentration using acridinium ester chemiluminescence via the injection protocol described previously (2) but modified for use with the Synergy H1. We also attempted to determine the role, if any, of intact EVs in enzyme function by sonicating a subsample of the >50 kDa fraction prior to assays. To accomplish this, the concentrates were treated with a Fisher Scientific sonic dismembrator for 3 cycles of 10 s with 10 s pause between (100W, 30% output efficiency). Both methylumbelliferyl and 4-nitrophenyl conjugate substrates are routinely used to measure membrane permeability due to their inability to cross membranes (11, 12), so an increase in apparent enzymatic activity post-sonication can be interpreted as an indication of membrane disruption.

Data Processing Description

ll statistical analyses were performed in R v. 4.4.1. Most analyses used linear models followed by post hoc extended marginal means testing of pairwise differences between treatment groups using the *emmeans* package (1). Assumptions of linear regression were checked for models by Shapiro-Wilk tests of the normality of residuals and plots of residuals vs. fitted values for homoscedasticity; where these assumptions were violated we used the Box-Cox procedure to find an optimal power transformation (2). Statistical differences

between lysate and exudate protein localization counts were determined using Fisher's exact test implemented in R.

BCO-DMO Curation Notes

* Processed Enzymes.csv as primary file.
* Added descriptions from readme to files directly.

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Data Files

File	
997078_v1_enzym.csv	(Comma Separated Values (.csv), 4.57 KB) MD5:919feb3880d40cba64984d47ee437edb
Primary data file for dataset ID 997078, version 1	

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Supplemental Files

File	
Enzymes.csv	(Comma Separated Values (.csv), 4.82 KB) MD5:a761c1d54879eea27bca898559df8d1a
Enzymes.R	(R Script, 8.94 KB) MD5:48912d217076f02163a1e993c58b5272
Enzymes.R contains the R code for analyzing the "Enzymes.csv".	

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Related Publications

Cupp-Enyard, C. (2008). Sigma's Non-specific Protease Activity Assay - Casein as a Substrate. Journal of Visualized Experiments, (19). https://doi.org/10.3791/899
Methods

De Spiegeleer, P., Sermon, J., Vanoirbeek, K., Aertsen, A., & Michiels, C. W. (2005). Role of Porins in Sensitivity of Escherichia coli to Antibacterial Activity of the Lactoperoxidase Enzyme System. Applied and Environmental Microbiology, 71(7), 3512–3518. https://doi.org/10.1128/aem.71.7.3512-3518.2005 https://doi.org/10.1128/AEM.71.7.3512-3518.2005
Methods

Hoppe, HG. (1993). Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurement of bacteria, p. 423-431. In P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole (ed.), Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, FL 978-0873715645
Methods

Li, Y., Sun, L.-L., Sun, Y.-Y., Cha, Q.-Q., Li, C.-Y., Zhao, D.-L., Song, X.-Y., Wang, M., McMinn, A., Chen, X.-L., Zhang, Y.-Z., & Qin, Q.-L. (2019). Extracellular Enzyme Activity and Its Implications for Organic Matter Cycling in Northern Chinese Marginal Seas. Frontiers in Microbiology, 10. https://doi.org/10.3389/fmicb.2019.02137
Methods

Livermore, D. M. (2004). Management of Multiple Drug-Resistant Infections. Journal of Antimicrobial Chemotherapy, 54(6), 1162–1162. https://doi.org/10.1093/jac/dkh466
Methods

Lu, Z, S Plummer, J Kizziah, SJ Biller, and J Jeffrey Morris. 2026. Vesicle-associated exudates from Alteromonas enhance growth and survival of Prochlorococcus in batch culture. Applied and Environmental Microbiology X:XXX.
Results

Lu, Z., Entwistle, E., Kuhl, M. D., Durrant, A. R., Barreto Filho, M. M., Goswami, A., & Morris, J. J. (2025). Coevolution of marine phytoplankton and Alteromonas bacteria in response to pCO2 and coculture. The ISME Journal, 19(1). https://doi.org/10.1093/ismejo/wrae259
Methods

Lu, Z., Plummer, S., Kizziah, J., Biller, S. J., & Jeffrey Morris, J. (2025). Enzymatically active exudates from Alteromonas facilitate Prochlorococcus survival in stationary phase. https://doi.org/10.1101/2025.05.28.656624
Methods

Morris, J. J., Johnson, Z. I., Szul, M. J., Keller, M., & Zinser, E. R. (2011). Dependence of the Cyanobacterium Prochlorococcus on Hydrogen Peroxide Scavenging Microbes for Growth at the Ocean's Surface. PLoS ONE, 6(2), e16805. https://doi.org/10.1371/journal.pone.0016805
Methods

Morris, J. J., Kirkegaard, R., Szul, M. J., Johnson, Z. I., & Zinser, E. R. (2008). Facilitation of Robust Growth of Prochlorococcus Colonies and Dilute Liquid Cultures by "Helper" Heterotrophic Bacteria. Applied and Environmental Microbiology, 74(14), 4530–4534. https://doi.org/10.1128/aem.02479-07 https://doi.org/10.1128/AEM.02479-07
Methods

O'Neill, A. J., Miller, K., Oliva, B., & Chopra, I. (2004). Comparison of assays for detection of agents causing membrane damage in Staphylococcus aureus. Journal of Antimicrobial Chemotherapy, 54(6), 1127–1129. https://doi.org/10.1093/jac/dkh476
Methods

R. Andersen, Ed., Algal Culturing Techniques, (Academic Press, Burlington, MA, 2005), pp. 596. https://isbnsearch.org/isbn/9780120884261
Methods

Schwyn, B., & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry, 160(1), 47–56. https://doi.org/10.1016/0003-2697(87)90612-9
Methods

Searle, S. R., Speed, F. M., & Milliken, G. A. (1980). Population Marginal Means in the Linear Model: An Alternative to Least Squares Means. The American Statistician, 34(4), 216–221. https://doi.org/10.1080/00031305.1980.10483031
Methods

Sobecky, P. A., Mincer, T. J., Chang, M. C., & Helinski, D. R. (1997). Plasmids isolated from marine sediment microbial communities contain replication and incompatibility regions unrelated to those of known plasmid groups. Applied and Environmental Microbiology, 63(3), 888–895. https://doi.org/10.1128/aem.63.3.888-895.1997
Methods

Sokal, R. R., and F. J. Rohlf. 2012. Biometry Fourth Edition. Freeman, New York.
Methods

Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., & Miller, H. L. (Eds.). (2007). Climate Change 2007: The Physical Science Basis. Cambridge University Press. https://www.ipcc.ch/report/ar4/wg1/
Methods

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Parameters

Parameter	Description	Units
Strain	The Prochlorococcus/Alteromonas co-culture from which the Alteromonas strain was isolated. LTPE26 was an ancestral culture; LTPE397 was evolved for 500 generations at 400 ppm pCO2 before culture isolation; and LTPE403 was evolved for 500 generations at 800 ppm pCO2 before isolation. "Blank" indicates the value of the negative controls for each assay.	unitless
Enzyme	Indicates which of the enzymatic assay each data point corresponds to. AP, alkaline phosphatase; H2O2, hydroperoxidase/catalase; Protease, Sigma protease assay; Siderophore, siderophore chrome azurol S assay; AG, alpha-glucosidase; NAG, N-acetylglucosaminidase; BG, beta-glucosidase.	unitless
Treatment	E, >50 KDa fraction containing Enzymes; F, 5-50 kDa filtered fraction; S, Sonicated >50 KDa fraction; M, Media control	unitless
Value	The measured value for each assay. The exact type of data measured differs for the different assays: AP: Fluorescence at 449 nm (excitation at 359 nm), H2O2: Proportion of initial H2O2 remaining, Protease: Optical density at 660 nm, Siderophore: Optical density at 630 nm, AG/NAG/BG: Optical density at 405 nm	nm

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Instruments

Dataset-specific Instrument Name	Percival
Generic Instrument Name	Algal Growth Chamber
Generic Instrument Description	A chamber specifically designed for the growth of algae in flasks. The chamber typically provides controlled temperature, humidity, and light conditions.

Dataset-specific Instrument Name	Guava HT1
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. Description from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm

Dataset-specific Instrument Name	Biotek Synergy H1
Generic Instrument Name	plate reader
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

Dataset-specific Instrument Name	Masterflex peristaltic pump (Cole-Parmer)
Generic Instrument Name	Pump
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

Dataset-specific Instrument Name	
Generic Instrument Name	Turner Designs Trilogy fluorometer
Generic Instrument Description	The Trilogy Laboratory Fluorometer is a compact laboratory instrument for making fluorescence, absorbance, and turbidity measurements using the appropriate snap-in application module. Fluorescence modules are available for discrete sample measurements of various fluorescent materials including chlorophyll (in vivo and extracted), rhodamine, fluorescein, cyanobacteria pigments, ammonium, CDOM, optical brighteners, and other fluorescent compounds.

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Project Information

Collaborative Research: Extracellular vesicles as vehicles for microbial interactions in marine Black Queen communities (Vesicle Interactions)

Coverage: Laboratory cultures

NSF Award Abstract:

NSF Award Abstract:
The function and stability of microbial communities in the ocean depends on exchanges of biological products and services between individual cells. Marine microbes are typically far apart from one another, so some of these exchanges occur through the release of products or services into the surrounding water, where they travel to other cells via simple diffusion. Understanding the degree to which such valuable products made by one organism are targeted to a specific partner, and how, has important implications for our understanding of the ecology and evolution of the marine microbiome. This project examines the role played by a poorly understood type of very small particle - extracellular membrane vesicles - in mediating functional interactions within the oceans. Extracellular vesicles are released by most marine microbes and are abundant in ocean waters, but our understanding of their functions remains in its infancy. As vesicles can contain diverse molecules, including active enzymes, and transport them between cells, they may work as a packaging and delivery system for goods and services traded between ecologically important microorganisms. Broader impacts of the project include providing hands-on research experiences for undergraduate and graduate students - including those from groups historically underrepresented in STEM fields - and the development of new active learning exercises to help increase knowledge about the roles microbes play in students' lives.

This project explores vesicle functions across multiple scales, combining -omics analyses, field experiments, and functional studies in cultures of diverse and ecologically important microbes to arrive at new understandings of vesicle contributions to cellular exchanges. These experiments incorporate an evolutionary perspective for exploring the range of vesicle functions and genetic mechanisms affecting their production, examining how their contents have changed in co-cultures of phytoplankton and heterotrophic bacteria following hundreds of generations of experimental laboratory evolution. Fundamental ecological questions are addressed concerning whether vesicles, and their associated functions, act as truly 'public goods' in the oceans or can instead be targeted to a subset of cells, possibly yielding 'club goods' that define interacting, cooperative networks. Collectively, this effort will generate new insights into the mechanisms marine microbes use to interact with one another, and experimentally define the functional potential and ecological impact of EV-mediated trafficking networks in the oceans.

This project is jointly funded by the Biological Oceanography Program and the Established Program to Stimulate Competitive Research (EPSCoR). This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2304066
NSF Division of Ocean Sciences (NSF OCE)	OCE-2304067

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