Chemotaxis of Vibrio alginolyticus to control/phage-infected Synechococcus exudates from 2020-2021 (VIC project)

Website: https://www.bco-dmo.org/dataset/885574
Data Type: model results, experimental
Version: 1
Version Date: 2022-12-19

Project
» Collaborative Research: Viral induced chemotaxis mediating cross-trophic microbial interactions and carbon flux (VIC)

Contributors	Affiliation	Role
Floge, Sheri	Wake Forest University (WFU)	Co-Principal Investigator
Guasto, Jeffrey	Tufts University	Co-Principal Investigator
Henshaw, Richard J.	ETH-Zurich (ETH)	Scientist, Contact
Newman, Sawyer	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
This data set summarises the chemotactic response of a model marine bacteria (Vibrio alginolyticus YM4 wild-type) to filtered exudates of the cyanobacteria Synechococcus sp WH8102. Two filtrate sets were collected, each spanning 6 time points (named T1 -> T6), with the initial assays split into 4 biological replicates (named A,B,C,D). The two treatments were: 1) A control treatment (named "Control", or shortened to "C") 2) A phage-infected treatment (named "Phage", or shortened to "P"), where host Synechococcus were infected with the T-4 like Myovirus S-SSM5, with data collected over the pre-lysis cycle. These treatments are fully described in: https://doi.org/10.1038/s43705-022-00169-6. The filtrates were kept frozen at -80C in 1ml aliquots, and only thawed to room temperature immediately prior to experimental use. For each time point (T1-6), and replicate (A-D), three sets of chemotaxis assays were collected: Phage vs ASW, Control vs ASW, Phage vs Control. (Each with three biological replicates with fresh cell suspensions, with three physical replicates per sample, totaling nine samples at each time point/sample replicate). The chemotactic preference of Vibrio alginolyticus was measured by analyzing the cell distribution over time, and comparing the cell populations on opposite sides of the channel. Upon completion of the assays, the data shows that the bacteria will preferentially chemotax towards the phage-infected cells, with the strongest response occurring immediately in the infection cycle (T1), contrary to the assumed belief that it is post-lysis behavior that drives microbial chemotaxis in the oceans.

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Coverage

Temporal Extent: 2020-12-03 - 2021-10-08

Dataset Description

The data in this data set is organized as follows:

Accumulation Curves: The final processed data for each time point (T1-6), each replicate (A-D) with the average and SEM values tabled.

Filtrates_T#R: Where # is the time point number (1-6) and R the replicate (A-D). Each folder contains:

1) Three Excel sheets corresponding to the raw cell counts for each biological replicate (each with 3 repeats)

2) A supplemental folder containing a cell array with the full bacteria positions for each time point for each replicate (.mat format)

The chemotaxis data was collected by Dr. Richard J. Henshaw of Tufts University (Boston, MA, USA) from 3rd December 2020 to 8th October 2021. Queries about this dataset should be directed to rhenshaw@ethz.ch.

Methods & Sampling

Vibrio alginolyticus (YM4; wild-type) from -80°C stock were grown overnight in Marine 2216 media (Difco) by incubating at 30°C and shaking at 600 revolutions per minute (RPM). The overnight culture was diluted 100-fold into fresh pre-warmed 2216 media and grown for three hours (30°C, shaking at 600 RPM) to O.D. ≈ 0.2. 2 ml of culture was then washed and resuspended (1,500 RCF for 5 min) in 0.5ml of artificial seawater (ASW).

Artificial seawater (ASW) was prepared following the NCMA ESAW Medium recipe, which was adapted from Harrison et al.and modified by Berges, and filtered through a 0.2um filter immediately prior to use.

A simple three-inlet gradient generation microfluidic device was used to produce the chemical gradients. Microfluidic devices were made using standard soft lithography techniques. Polydimethylsiloxane (Dow Corning SYLGARD 184) channels were cast on photoresist (Microchem) molds fabricated via photolithography and plasma bonded to standard glass slides. Gradient generation channels were designed with three inlets (width 0.5mm) carrying the chemostimulus solution, cell suspension and ASW media, respectively. Prior to use, the chambers were pretreated with a 0.5% BSA solution to mitigate cell adhesion. The three solutions were flow stratified for a minimum of 2min using a syringe pump(Harvard Apparatus), whereby flow rates were adjusted to maintain a 4:1:4 ratio of the stream widths. Upon halting the flow, a monotonic chemotaxis profile was established through diffusion. A chemostimulus gradient develops in the channel via diffusion, and the chemotactic response of the cell population was observed over time. Imaging was performed with phasecontrast microscopy (4×, 0.13 NA objective; Nikon Ti-E) at 1 fps over the course of ~10 min using a CMOS camera (Blackfly S, Teledyne FLIR). An example of the gradient generator used can be found in http://dx.doi.org/10.1038/s41567-021-01247-7

This data set summarises the chemotactic response of a model marine bacteria (*Vibrio alginolyticus* YM4 wild-type) to filtered exudates of the cyanobacteria *Synechococcus* sp WH8102. Two filtrate sets were collected, each spanning 6 time points (named T1 -> T6), with the inital assays split into 4 biological replicates (named A, B, C, D). The two treatments were:

1). A control treatment (named "Control", or shortened to "C")

2). A phage-infected treatment (named "Phage", or shortened to "P"), where host Synechococcus were infected with the T-4 like Myovirus S-SSM5, with data collected over the pre-lysis cycle.

These treatments are fully described in: https://doi.org/10.1038/s43705-022-00169-6. The filtrates were kept frozen at -80C in 1ml aliquots, and only thawed to room temperature immediately prior to experimental use.

For each time point (T1-6), and replicate (A-D), three sets of chemotaxis assays were collected: Phage vs ASW, Control vs ASW, and Phage vs Control. (Each with three biological replicates with fresh cell suspensions, with three physical replicates per sample, totaling nine samples at each time point/sample replicate). The chemotactic preference of *Vibrio alginolyticus* was measured by analyzing the cell distribution over time, and comparing the cell populations on opposite sides of the channel.

Data Processing Description

All data were processed using custom MATLAB scripts (version 2021a). The processing summary is:

1) Locate particles using a bandpass filter/peak finding algorithm
(available: https://site.physics.georgetown.edu/matlab/)

2) Bin particles according to x-position to examine distribution across the field of view

An example of the analysis script is included in the dataset.

Data Files

File
Filtrate_Chemotaxis_Data filename: Filtrate_Chemotaxis.zip   (ZIP Archive (ZIP), 19.29 GB) MD5:ebeed51cfcb9854018786961e26262ea

Supplemental Files

File
Filtrate Chemotaxis Analysis Scripts filename: Filtrate_Chemotaxis_Analysis.zip   (ZIP Archive (ZIP), 8.95 KB) MD5:c95a81fdfa46f53b94038aeb7c2f57cd This file package contains MatLab functions within .m files. Each file contains more description of usage, input, and output in the header of each m-file. Below is a brief description of the purpose of each function. --- "Chemotaxis_Analysis.m" contains setup and parameters, you should only need to change numbers/details here. --- "cntrd.m" contains function: cntrd(im,mx,sz,interactive) PURPOSE: calculates the centroid of bright spots to sub-pixel accuracy. Inspired by Grier & Crocker's feature for IDL, but greatly simplified and optimized for matlab. --- pkfnd.m contains function: pkfnd(im,th,sz) PURPOSE: finds local maxima in an image to pixel level accuracy.   This provides a rough guess of particle centers to be used by cntrd.m. Inspired by the lmx subroutine of Grier and Crocker's feature.pro --- "bpass.m" contains function: bpass(image_array,lnoise,lobject,threshold) PURPOSE: Implements a real-space bandpass filter that suppresses pixel noise and long-wavelength image variations while retaining information of a characteristic size. ---

Related Publications

Dehkharghani, A., Waisbord, N., Dunkel, J., & Guasto, J. S. (2019). Bacterial scattering in microfluidic crystal flows reveals giant active Taylor–Aris dispersion. Proceedings of the National Academy of Sciences, 116(23), 11119–11124. https://doi.org/10.1073/pnas.1819613116
Methods

Howard-Varona, C., Roux, S., Bowen, B. P., Silva, L. P., Lau, R., Schwenck, S. M., Schwartz, S., Woyke, T., Northen, T., Sullivan, M. B., & Floge, S. A. (2022). Protist impacts on marine cyanovirocell metabolism. ISME Communications, 2(1). https://doi.org/10.1038/s43705-022-00169-6

Methods

Stehnach, M. R., Waisbord, N., Walkama, D. M., & Guasto, J. S. (2021). Viscophobic turning dictates microalgae transport in viscosity gradients. Nature Physics, 17(8), 926–930. https://doi.org/10.1038/s41567-021-01247-7 https://doi.org/http://dx.doi.org/10.1038/s41567-021-01247-7
Methods

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Related Datasets

IsRelatedTo

Floge, S., Guasto, J., Henshaw, R. J. (2023) **Chemotaxis of Vibrio alginolyticus towards live phage-infected/control Synechococcus cells (VIC project).** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-10-17 http://lod.bco-dmo.org/id/dataset/913619 [view at BCO-DMO]

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	CMOS camera (Blackfly S, Teledyne FLIR)
Generic Instrument Name	Camera
Dataset-specific Description	Imaging was performed with phasecontrast microscopy (4×, 0.13 NA objective; Nikon Ti-E) at 1 fps over the course of ~10 min using a CMOS camera (Blackfly S, Teledyne FLIR). An example of the gradient generator used can be found in http://dx.doi.org/10.1038/s41567-021-01247-7.
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	Nikon Ti-E Inverted Microscope
Generic Instrument Name	Inverted Microscope
Dataset-specific Description	Imaging was performed with phasecontrast microscopy (4×, 0.13 NA objective; Nikon Ti-E) at 1 fps over the course of ~10 min using a CMOS camera (Blackfly S, Teledyne FLIR). An example of the gradient generator used can be found in http://dx.doi.org/10.1038/s41567-021-01247-7.
Generic Instrument Description	An inverted microscope is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. It was invented in 1850 by J. Lawrence Smith, a faculty member of Tulane University (then named the Medical College of Louisiana). Inverted microscopes are useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope. Inverted microscopes are also used in micromanipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath using reflecting objectives. The stage on an inverted microscope is usually fixed, and focus is adjusted by moving the objective lens along a vertical axis to bring it closer to or further from the specimen. The focus mechanism typically has a dual concentric knob for coarse and fine adjustment. Depending on the size of the microscope, four to six objective lenses of different magnifications may be fitted to a rotating turret known as a nosepiece. These microscopes may also be fitted with accessories for fitting still and video cameras, fluorescence illumination, confocal scanning and many other applications.

Dataset-specific Instrument Name	Harvard Apparatus syringe pump
Generic Instrument Name	Pump
Dataset-specific Description	Gradient generation channels were designed with three inlets (width 0.5mm) carrying the chemostimulus solution, cell suspension and ASW media, respectively. Prior to use, the chambers were pretreated with a 0.5% BSA solution to mitigate cell adhesion. The three solutions were flow stratified for a minimum of 2min using a syringe pump(Harvard Apparatus), whereby flow rates were adjusted to maintain a 4:1:4 ratio of the stream widths.
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

Project Information

Collaborative Research: Viral induced chemotaxis mediating cross-trophic microbial interactions and carbon flux (VIC)

Coverage: Culture-based work

NSF Award Abstract:
Drifting photosynthetic microbes in surface ocean waters carry out nearly half of global carbon (C) fixation, both supporting the marine food web and reducing atmospheric carbon dioxide (CO_2) levels. The fate of C in ocean ecosystems is controlled by myriad individual interactions within a highly interconnected planktonic food

web, the sheer complexity of which has hindered predictive understanding of global C cycling. Chemical cues govern microbial interactions, and during infection, marine viruses manipulate the metabolism of phytoplankton and bacteria, facilitating the release of dissolved organic matter from infected cells. This research aims to determine how viral metabolic reprogramming of and organic matter release from intact, infected phytoplankton influences microbial interactions and C cycling. The interdisciplinary, collaborative nature of the project will enable direct training of two postdoctoral researchers, one graduate student, and undergraduate students in viral ecology, microfluidics, and metabolomics. An educational outreach program that engages middle school students in hands-on, high speed imaging of microbes will be expanded, and the project will culminate in a three-day workshop to advance the application of microfluidic devices and mass spectrometry analyses in microbial ecology.

The overarching hypothesis behind this research is that viral infection alters the chemical landscape of intact, infected picophytoplankton cells, attracting neighboring chemotactic bacteria and protistan zooplankton, and altering C flux pathways. To test this idea, a series of linked multi-scale laboratory-based experiments will be run to 1) Characterize the response of diverse model marine microbes to dissolved organic matter (DOM) released from intact, virus-infected picophytoplankton using microfluidics-based chemotaxis assays, 2) Identify key viral-derived DOM compounds eliciting chemotactic responses using stable isotope labeling, metabolomics analyses, and chemotaxis assays, and 3) Quantify micron-scale cross-trophic encounter dynamics and evaluate their impact on bulk-scale C cycling using liter-scale measurements of C dynamics linked to high spatiotemporal resolution live imaging of microbial food webs. The ultimate goal of the project is to develop a mechanistic understanding of the role of intact, virus-infected cells in oceanic C cycling.

Funding