Analysis of temporal dynamics of three virus types persistently co-infecting Pleurochrysis carterae CCMP645 from laboratory experiments at the Bigelow Laboratory for Ocean Sciences, Maine from 2015-2016

Website: https://www.bco-dmo.org/dataset/670442
Data Type: experimental
Version:
Version Date: 2016-12-15

Project
» Persistent Virus Infections in Marine Phytoplankton (Marine Chronic Viruses)

Contributors	Affiliation	Role
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Dataset Description

This dataset contains temporal dynamics information for viruses co-infecting *Pleurochrysis carterae*, a coccolithophorid alga. Parameters included in this dataset are the number of virus droplets used, number of virus genomes per milliliter of culture, droplet normalization factors used, and the normalized number virus droplets used.

Related datasets:
* Pleurochrysis carterae growth
* Virus dPCR assay primers
* Accession numbers (P. carterae viruses and field samples)
* TEM Pleurochrysis carterae thin section images
* TEM Pleurochrysis carterae virion images

Methods & Sampling

Pleurochrysis carterae CCMP645 cells in mid-exponential growth phase were transferred (10% v/v) into flasks containing either F/2 (Guillard, 1975) [1] or L1 (Guillard and Hargraves, 1993) [2] media, in duplicate. Virus production in each flask was monitored by digital PCR (dPCR) for a 106-day period. The frequency of sampling varied throughout the period of the study as indicated in the excel data file. At each sampling point, 0.5 ml samples were collected and stored at -80C without the addition of any fixatives for further quantification of viral abundances. Prior to analysis, the samples were thawed at room temperature, diluted 1:1 in nuclease-free water. Cellular debris was removed by centrifugation at maximum speed for 5 seconds. Two microliter aliquots were taken from the supernatant and put directly into digital PCR (dPCR) reactions to quantify the abundance

of co-infecting virus genotypes. Specifically, probes and primers were designed to target genotypes: *P. carterae* endemic virus genotypes 2 and 1b (PsEV2 and PsEV1b, respectively; dsDNA viruses); *P. carterae* Polinton-like viruses (PleuroPLV; dsDNA viruses); and *P. carterae* CRESS viruses (PcCV1, ssDNA viruses). For the latter, we designed probes for both the REP and the CAP genes to discriminate viral particles with partial (i.e., amplification for only the REP or the CAP markers from a single dPCR intact drop) or complete genomes (i.e., amplification with both molecular markers from a single dPCR intact drop). For more information about primers used in these experiments see the Virus dPCR assay primer dataset.

Specific primer/probe assays for all samples were multiplexed as follows:
* Multiplex Assay 1 – PsEV2 and PsEV1b probes were TET-labelled and multiplexed with PcCV-1 Rep and Cap probes (FAM labelled).
* Multiplex Assay 2 – For the second dPCR multiplex assay, the same samples were thawed again, diluted and spun out as before, and 2 ul of the supernatant was put into multiplex reaction with PleuroPLV assay (probe was FAM-labelled) and same 2 PsEV assays as an internal control to see how the repeated freeze-thaw cycles impacted the counts.

dPCR reactions contained 900 nM of each primer set and 200 nM probe, except for PsEV1b-TET and PcCV1-REP-FAM, which contained 450 nM primers and 100 nM probe. PCR reaction volumes were parsed into 5 million droplets per sample (RainDrop Source, RainDance Technologies) and then amplified in a C1000 Touch deep-well thermal cycler (Bio-Rad) with the following thermal protocol: 95C for 10 min; 95C for 15 s and then 60C for 60 s, with a ramping rate of 0.5C s−1 for 50 cycles; and final in-activation at 98C for 10 min. The droplets were enumerated on the RainDrop Sense (RainDance Technologies).

Reactions were run on a Bio Rad icycler and data was analyzed following Bio Rad's Instruction Manual, Catalog Number 170-8740.

References

[1] Guillard, Robert RL. "Culture of phytoplankton for feeding marine invertebrates." *Culture of marine invertebrate animals*. Springer US, 1975. 29-60.

[2] Guillard, R. R. L., and P. E. Hargraves. "Stichochrysis immobilis is a diatom, not a chrysophyte." *Phycologia* 32.3 (1993): 234-236. https://doi.org/10.2216/i0031-8884-32-3-234.1

Data Processing Description

Standard procedures based on Cq value were followed to quantify gene copies in each sample. We followed general guidance from Bio Rad's Instruction Manual, Catalog Number 170-8740.

BCO-DMO Data Manager Processing Notes:
* Added date column from start date 2015-10-07 and date information in sample ID.
* added a conventional header with dataset name, PI name, version date
* modified parameter names to conform with BCO-DMO naming conventions
* blank values replaced with no data value 'nd'
* Date format converted to ISO Date format

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Data Files

File	
PleuroViruses.csv	(Comma Separated Values (.csv), 22.46 KB) MD5:b11f753e6b02b02f4504bce3b0496f53
Primary data file for dataset ID 670442	

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Parameters

Parameter	Description	Units
assay_id	Assay identifier (1 or 2)	unitless
date_start	Start date of experiment	unitless
date	Date of sample	unitless
media	Type of media used (L1 or F/2)	unitless
replicate	Replicate (A or B)	unitless
drops	Number of droplets counted by RainDrop Sense out of 5 mill formed drops	drop
drops_CAP_FAM	Number of droplets with partial P. carterae CRESS virus genomes; CAP-gene target only	drop
drops_REP_FAM	Number of droplets with partial P. carterae CRESS virus genomes; REP-gene target only	drop
drops_REP_and_CAP_FAM	Number of droplets with complete P. carterae CRESS virus genomes; CAP- and REP-gene targets	drop
drops_PsEV2_TET	Number of droplets with P. cartera endemic virus genotype 2 genomes	drop
drops_PsEV1b_TET	Number of droplets with P. cartera endemic virus genotype 1b genomes	drop
drops_PLV_FAM	Number of droplets with P. carterae Polinton-like virus	drop
drops_norm	Droplet normalization factor calculated as intact drops (Count divided by 5 mill total droplets)	drop
PsEV2_norm	Normalized number of droplets with P. cartera endemic virus genotype 2 genomes	drop
PsEV1b_norm	Normalized number of droplets with P. cartera endemic virus genotype 1b genomes	drop

REP_norm	Normalized number of droplets with partial P. carterae CRESS virus genomes; REP-gene target only	drop
CAP_norm	Normalized number of droplets with partial P. carterae CRESS virus genomes; CAP-gene target only	drop
REP_and_CAP_norm	Normalized number of droplets with complete P. carterae CRESS virus genomes; CAP- and REP-gene targets	drop
PLV_norm	Normalized number of droplets with P. carterae Polinton-like virus genomes	drop
PsEV2_conc	Estimated P. cartera endemic virus genotype 2 genomes per milliliter of culture	genomes per milliliter
PsEV1b_conc	Estimated P. cartera endemic virus genotype 1b genomes per milliliter of culture	genomes per milliliter
REP_conc	Estimated P. carterae CRESS virus REP-gene partial genomes per milliliter of culture	genomes per milliliter
CAP_conc	Estimated P. carterae CRESS virus CAP-gene partial genomes per milliliter of culture	genomes per milliliter
REP_and_CAP_conc	Estimated P. carterae CRESS virus complete genomes per milliliter of culture	genomes per milliliter
PLV_conc	Estimated P. carterae Polinton-like virus genomes per milliliter of culture	genomes per milliliter
sample_id	sampling day number (1-106) culture media (F/2 or L1) replicate (A or B)	unitless

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Centrifuge
Generic Instrument Description	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

Dataset-specific Instrument Name	RainDrop Source
Generic Instrument Name	dPCR
Dataset-specific Description	RainDrop Source from RainDance Technologies
Generic Instrument Description	Digital Polymerase Chain Reaction (dPCR)

Dataset-specific Instrument Name	Bio Rad icycler
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Reactions were run on a Bio Rad icycler and data was analyzed following Bio Rad's Instruction Manual, Catalog Number 170-8740.
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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Deployments

Bigelow_Martinez_2015-2016

Website	https://www.bco-dmo.org/deployment/670437
Platform	lab Bigelow
Start Date	2015-01-01
End Date	2016-12-30
Description	Bigelow Laboratory for Ocean Sciences **Methods & Sampling** laboratory experiment

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Project Information

Persistent Virus Infections in Marine Phytoplankton (Marine Chronic Viruses)

Description from NSF award abstract:
Viruses are prevalent in every part of the environment of our living planet, and yet our understanding of type, distribution, and function is the least well-known aspect of biodiversity. In recent years we have developed an increased appreciation for the role viruses play in driving host evolution in the environment, but fundamental knowledge about the mechanisms involved remain lacking. Additionally, viruses may influence diversity indirectly through "kill the winner" scenarios, as well as through cell lysis and subsequent release of dissolved

nutrients, which facilitate restructuring of microbial communities. The majority of research on marine viruses to date has focused on combinations of acutely susceptible host strains with highly virulent virus isolates. However, it is likely that marine viruses also employ a persistent infection life strategy, arguably preferring it to the more widely recognized lytic cycle. The objective of this project is to demonstrate that persistent virus infections occur in marine phytoplankton, and that these are a crucial component of ocean ecosystem function and a key evolutionary driver in primary producers. Using a range of persistent virus:host systems, this project will investigate:
1) how pervasive persistent virus infections are in marine systems; and
2) the role of non-coding RNAs in maintaining host:virus symbiosis.

This is a high risk-high pay research as it involves a radically different approach to the analysis of viruses in marine systems. The investigators plan to apply a suite of molecular (transcriptomics, genomics and development of novel diagnostic markers) techniques to include the analysis of microRNAs to determine the functional importance of persistent viruses in the ocean. The results of this project will be potentially transformative for our understanding of virus-driven phytoplankton evolution and its potential impact on biodiversity in marine phytoplankton, a vital component of the global carbon cycle.

Note: William Wilson (Bigelow Laboratory) was the Former Principal Investigator on this project award.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1346272

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