Symbiont titers in Bugula neritina colonies grown under different conditions

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Project
» Biogeography of a marine defensive microbial symbiont: relative importance of host defense vs. abiotic factors (BiogeogDefensiveSymb)

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Abstract
This dataset includes symbiont titers in Bugula neritina colonies grown under different conditions.

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Acquisition Description

Adult *B. neritina* colonies were collected from Oyster, VA, and Beaufort, NC. Larvae were collected from individual colonies and settled into 6-well plates. Samples of the adult colonies were preserved in DNA/RNA stabilization solution. After settlement, the plates were transferred to mesocoms maintained at different temperatures (16°C and 22°C). In addition half of the mesocosms had 1-2 filter-feeding invertebrates, the solitary tunicate, Styela sp., that could potentially on larvae. The colonies were fed cultured *Rhodomonas* sp. phytoplankton. Individual colonies were collected at different time points (18 w, 23 w), and preserved in DNA/RNA stabilization solution. Metagenomic DNA was extracted from preserved colonies using the Qiagen QIAamp DNA mini kit following the manufacturer's directions. The final eluate was tested through endpoint PCR, using BnCOI primers (see "Table 1" Supplemental File). The PCR conditions were: initial denaturation at 95°C for 15 min, 30 cycles of 95°C denaturation for 30 s, 50°C annealing for 30s, 72°C extension for 1 min, and a final extension at 72°C for 5 min. The reaction setup per 25 uL was 12.5 uL Apex Mastermix, 2.5 uL each 10 uM forward, reverse primers, 4.5 uL DI water, and 3 uL template. Visualization of amplicons after agarose gel electrophoresis confirmed successful metagenomic DNA extraction and amplification.

The metagenomic DNA from *B. neritina* colonies underwent multiplex (host and symbiont targets) quantitative PCR using the Applied Biosystems StepOne Plus Real-Time PCR System to determine the absolute quantity of the symbiont in the DNA using a symbiont target, bryA, and a host target, VDAC. The reaction mix was as follows (20 uL reaction): 10 uL Tonbo BioSciences Fastprobe/Cyberfast qPCR Mastermix, 0.8 uL 10 uM each host forward, reverse primer, 0.8 uL 10 uM each symbiont forward, reverse

primer, 0.4 uL each 10 uM host, symbiont probe, 4 uL DI water. The reaction conditions were: initial denaturation for 20 s at 95°C, 40 cycles of 95°C denaturation for 5 s, followed by annealing and extension at 60°C for 20 s.

The same symbiont primers and probe were used for both Type N and S colonies, but the host VDAC primers and probe differed between the two host genotypes. Each sample was run in triplicate, and a standard curve was generated for each run using copy number standards (see below). The efficiency for each run and for each target ranged between 91% to 98%. The R-squared values for the standard curve ranged between 97% and 99%. The mean number of copies were calculated for each sample by fitting the CT value to the standard curve.

Quantitative PCR Copy Number Standards: Using DNA from parental samples, host VDAC (Type N and Type S) and symbiont *bryA* primers (see "Table 1" Supplemental File), and Apex Mastermix, PCR was performed with specific cycling parameters mentioned previously. Agarose gel electrophoresis followed by visualization of the amplicons was performed to confirm amplification. The PCR amplicons were purified using the Qiagen MinElute PCR Purification kit following the manufacturer's directions. The samples were concentrated to create a stock solution for both host VDAC and symbiont *bryA* amplicons. A Nanodrop spectrophotometer was used to quantify the concentration of amplicons (average of three readings). Serial dilutions using nuclease-free water as a diluent were performed to produce standards ranging from 32 to 4,000 copies of DNA per mL for both VDAC and *bryA* stocks. Dilutions were aliquoted, stored at -80oC until use, and were never refrozen.

Processing Description

BCO-DMO Processing:
- renamed fields;
- rounded values to 2 decimal places as specified by data provider.

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Parameters

Parameter	Description	Units
Colony	Offspring of specific colony used	unitless
Genotype	Host genotype (N or S), symbiotic status (+ or -)	unitless
Parent	Ratio of symbiont gene copy number to host gene copy number of parent	unitless
Cont_18w_16	Ratio symb/host gene copy number colonies grown at 16°C, no predators, after 18 weeks	unitless
Pred_18w_16	Ratio symb/host gene copy number colonies grown at 16°C, with predators, after 18 weeks	unitless
Cont_18w_22	Ratio symb/host gene copy number colonies grown at 22°C, no predators, after 18 weeks	unitless
Pred_18w_22	Ratio symb/host gene copy number colonies grown at 22°C, with predators, after 18 weeks	unitless
Cont_23w_16	Ratio symb/host gene copy number colonies grown at 16°C, no predators, after 23 weeks	unitless
Pred_23w_16	Ratio symb/host gene copy number colonies grown at 16°C, with predators, after 23 weeks	unitless
Cont_23w_22	Ratio symb/host gene copy number colonies grown at 22°C, no predators, after 23 weeks	unitless
Pred_23w_22	Ratio symb/host gene copy number colonies grown at 22°C, with predators, after 23 weeks	unitless

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Instruments

Dataset-specific Instrument Name	Applied Biosystems StepOne Plus Real-Time PCR System
Generic Instrument Name	qPCR Thermal Cycler
Generic Instrument Description	An instrument for quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction (Real-Time PCR).

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Project Information

Biogeography of a marine defensive microbial symbiont: relative importance of host defense vs. abiotic factors (BiogeogDefensiveSymb)

Coverage: Western Atlantic coast, ranging from latitudes 38.61283 to 29.753272

Recent research has shown that microorganisms can be very important to their eukaryotic hosts, by providing nutrition or contributing to host defense against enemies, such as pathogens or predators. In many cases, however, hosting a bacterial symbiont imposes a physiological cost on the host organism, resulting in reduced growth or reproduction in the presence of the symbiont. Further, these costs may be

more pronounced in some habitats than others, causing natural selection to act in eliminating symbiont-containing hosts from the population. In this project, the investigators are studying the relationship between the marine bryozoan invertebrate, Bugula neritina, and its uncultured symbiont. The symbiont produces natural products with activity against cancer, Alzheimer's disease, and HIV. Interestingly, these compounds also are distasteful and protect larvae from predators, indicating that this symbiotic relationship is defensive in nature. Along the East Coast of the US, the investigators have found a much higher proportion of individuals that have the defensive symbiont at lower latitudes, while the symbiont is absent in individuals collected at higher latitudes. This pattern is consistent with the theory that higher predation pressure exists at lower latitudes. Other environmental factors, such as temperature, can also vary over a wide geographical area, and may also play a role in influencing the relationship. In this project, the investigators will evaluate the ecological and environmental parameters that influence the distribution of a defensive symbiont, including predation pressure and temperature. Defensive symbionts represent another level of ecological complexity, and likely play an important role in structuring marine communities. This study will provide insight into how environmental factors can influence host-symbiont interactions and drive partner co-evolution. Furthermore, the bioactive products have pharmaceutical potential, and understanding how environmental factors influence the relationship between B. neritina and its symbiont may improve bioprospecting for novel compounds that could be developed into drugs.

In this research, the investigators will determine the ecological and environmental parameters that influence the distribution of a defensive symbiont in the marine bryozoan, Bugula neritina. The goal of this research is to determine the mechanism that results in the defensive endosymbiont being restricted to hosts that inhabit lower latitudes. This pattern of symbiont distribution could be the result of differing levels of costs and benefits at different latitudes: where predation pressure is low, the costs of hosting the symbiont outweigh the benefits, and aposymbiotic individuals outcompete their symbiotic conspecifics. In areas of higher predation, the defensive benefit outweighs the cost, and symbiotic individuals have higher survival rates than their undefended, aposymbiotic conspecifics. An alternative, but not mutually exclusive hypothesis, is that symbiont growth is inhibited at higher latitudes, where it is not as beneficial, and growth is induced in areas of higher predation. Specific goals are to determine if (1) a biogeographical cline in predation pressure corresponds to a gradient of symbiont frequency associating with the host, (2) symbiotic hosts have a higher fitness at low latitudes, and aposymbiotic hosts have a higher fitness at high latitudes, and (3) symbiont growth is promoted at low latitudes and inhibited at high latitudes. A combination of field and laboratory-based experiments will be conducted using ecological and molecular biology techniques. Bioactive compounds produced by symbionts of marine invertebrates can mediate multi-trophic interactions and potentially influence benthic community structure. There has been almost no research, however, on how ecological and environmental parameters influence the distribution of marine defensive endosymbionts.

Related Reference:

Linneman J, Paulus D, Lim-Fong G, Lopanik NB (2014) Latitudinal Variation of a Defensive Symbiosis in the Bugula neritina (Bryozoa) Sibling Species Complex. PLoS ONE 9(10): e108783. doi:10.1371/journal.pone.0108783

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Funding

Funding Source	Award
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