

Potent cytotoxins produced by a microbial symbiont protect host larvae from predation

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Hypotheses

- Bryostatins are found in *B. neritina* and provide protection from predator fish
- This protection is only associated with the larvae
- *E. sertula* produces these bryostatins



Why is this important?

- First example of symbiont producing anti-predator defense in marine environment
- Bryostatins are being considered for cancer treatment

Background-*Bugula neritina*

- Sessile marine invertebrate
- Three populations
- Suspension feeder
- Hermaphroditic
- Byrostatins
- Lives on piers, vessel hulls, buoyies, sea grass leaves and rocky reefs



Courtesy of California
Academy of Science
[http:// www.exoticguide.com](http://www.exoticguide.com)

Background-Ovicells

- Ovicells are white globules
- Fertilized egg in ovicell
- Within ovicell becomes large, brown larvae
- Nutrition to larvae from ovicell
- Released at dawn



Courtesy California Academy of Science
<http://www.exoticguide.com>



Background-*Endobugula sertula*

- Member of gamma-proteobacterium
- Symbiont of *B. neritina*-in pallial sinus
- Extracellular rod-shaped
- Active polyketide synthase gene cluster
- Currently not able to cultivate
- Identified by Haygood and Davidson (University of California, San Diego) using molecular techniques



Materials and Methods

- Organisms used-*B. neritina* and the pinfish (*Lagodon rhomboides*) from North Carolina coastal waters
- *B. neritina* were kept in flow-through water tables and stimulated to release larvae
- Larvae and adults were harvested for chemical and DNA extractions
- Two methods of collection compared
 - *Sieve collection vs. pipette method*



Materials and Methods-continued

- Chemically extracted *B. neritina* were mixed with squid paste and used for feeding assays
- Bryostatin concentration in extracts determined by HPLC
- *Above experiment repeated used gentamicin treated B.neritina*
- *B. neritina* adults and larva treated with gentamicin were compared to control population using molecular techniques
 - *Used universal COI primers to check for contamination*
 - *Checked for 16s rDNA using primers specific for E. sertula*

Larval vs. Adult Palatability and Bryostatin Concentration

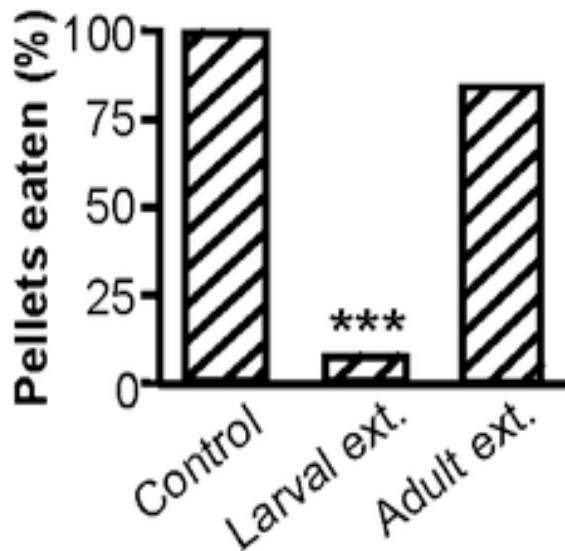


Fig. 2 Palatability of larval and adult lipophilic extracts of *Bugula neritina* to pinfish ($n=13-15$). Asterisks indicate significant differences between percentage of responses to control and extract pellets (McNemar's test; $***P<0.001$)

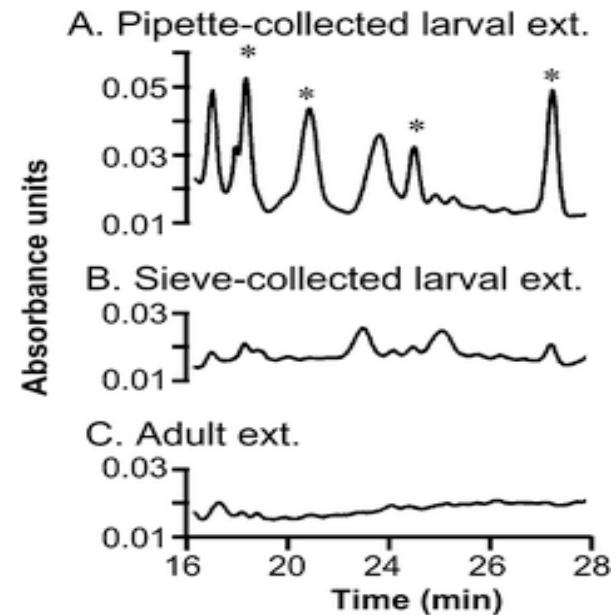


Fig. 3 Representative HPLC chromatograms (recorded at 229 nm) of **A** pipette-collected larval, **B** sieve-collected larval, and **C** adult lipophilic extracts obtained from identical volumes of tissue. Peaks in these chromatograms with asterisks are bryostatins

Treated *B. neritina* vs. Control

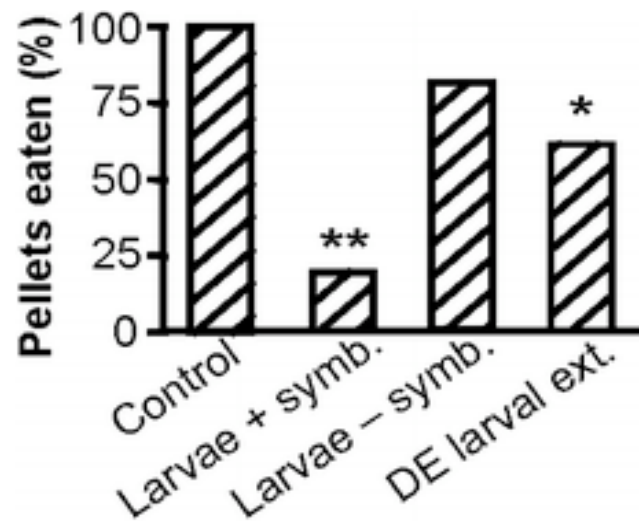


Fig. 4 Palatability of extracts of larvae obtained from North Carolina *Bugula neritina* with *Endobugula sertula* (*larvae+symb.*), North Carolina *B. neritina* without *E. sertula* (*larvae-symb.*), and Delaware *B. neritina*; $n=10-13$ for each assay. Asterisks indicate significant differences between percentage of responses to control and extract pellets (McNemar's test; * $P<0.05$, ** $P<0.01$)

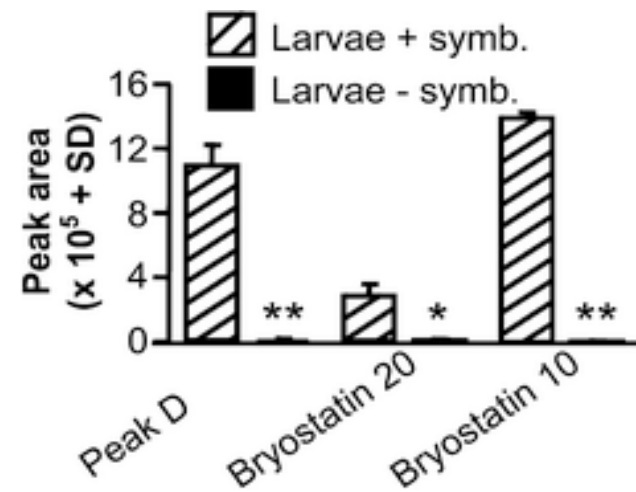
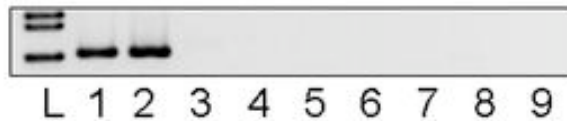


Fig. 5 Peak areas (+1 SD) of the three deterrent bryostatins in identical volumes of larvae from North Carolina *Bugula neritina* with *Endobugula sertula* ($n=2$) and without *E. sertula* ($n=3$). Asterisks indicate significant differences between peak areas for each compound (ANOVA, log transformed data; * $P<0.05$, ** $P<0.01$)

Molecular Identification of Symbiont

A. Amplification of *E. sertula* 16S rDNA



B. DGGE of 16S rDNA extracted from larvae from antibiotic experiment

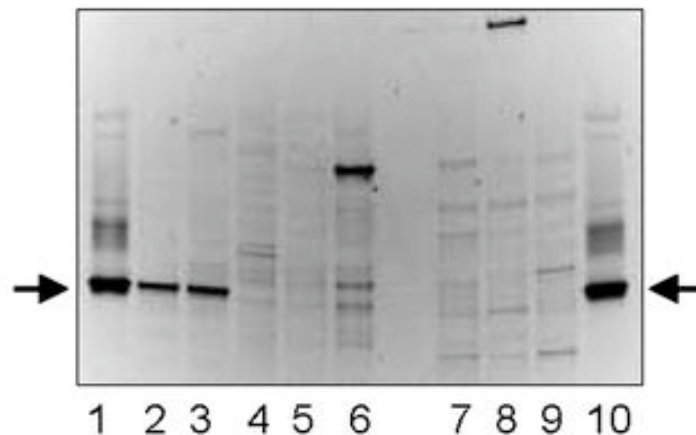


Fig. 6 **A** PCR products of DNA from larvae of North Carolina *Bugula neritina* colonies with *Endobugula sertula* (lanes 1, 2) and from colonies lacking *E. sertula* (lanes 3–5), and from larvae from colonies of Delaware *B. neritina* (lanes 6–8) amplified using 16S rDNA primers specific to *E. sertula* (Haygood and Davidson 1997); lane 9 no template control, lane L molecular weight ladder. **B** Denaturing gradient gel electrophoresis (25–55% denaturant) of PCR-amplified DNA [universal eubacterial primers 338f-gc and 519r-c (Muyzer et al. 1993)] from larvae of North Carolina *B. neritina* colonies with *E. sertula* (lanes 2, 3), North Carolina colonies lacking *E. sertula* (lanes 4–6), and Delaware *B. cf. neritina* (lanes 7–9). Lanes 1 and 10 are *E. sertula* standard. Arrows indicate the *E. sertula* bands.



Conclusions

- Larvae are protected from predators by bryostatins
 - Larvae have higher concentrations of bryostatins than adult
- Bryostatins are near the surface of larvae
- Non-reproductive adults do not contain detectable levels of bryostatins



Conclusions-continued

- There is a correlation between antibiotic treatment and the level of bryostatins present
- *E. sertula* is the true source of bryostatins
- First example in marine environment of symbiont providing chemical defense against predator



Take Home Message

- Research provides insight into chemical ecology of defensive symbionts in the marine environment
- On the biomedical level-insight into better collection methods for an increase in bryostatin yield



Ideas for further study

- Investigation of the Delaware species larval defense mechanism
- Work on isolation and cultivation methods for *E. sertula*
- Try to determine the method of transference from adult to larvae of the symbiont