UNIVERSITY OF CALIFORNIA, SAN DIEGO

Biology of the Bryostatins in the marine bryozoan *Bugula neritina*: Symbiosis, cryptic speciation and chemical defense

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Doctor of Philosophy in Marine Biology

by

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ABSTRACT OF THE DISSERTATION

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Abstract

This dissertation investigates the identity and function of a bacterial symbiont described in the marine bryozoan *Bugula neritina* by R.M. Woollacott in 1981. *B. neritina* is the source of bryostatins, unique cytotoxins suspected to have a bacterial source, and is considered a single species throughout its cosmopolitan temperate range. Bryostatins found from different collections of *B. neritina* vary, and only certain populations produce bryostatins that possess an octa-2,4-dienoate substituent.. In this dissertation the bacterial symbionts of the larvae are identified by small subunit ribosomal rRNA (SSU) gene sequences and named "*Candidatus* Endobugula sertula." The variable regions of these genes were used to design oligonucleotides specific to the symbiont. These specific oligonucleotides were used for *in situ* hybridization to the bacteria in the pallial sinus to confirm the origin of the sequence, and for specific

amplification of symbiont SSU rRNA genes by PCR. Then the mitochondrial cytochrome *c* oxidase subunit I gene was used to identify two distinct species of *B. neritina* each harboring a different symbiont as determined by SSU rRNA sequence. Variation in the bryostatin profiles is associated with this genetic difference. Only one *B. neritina* / "*E. sertula*" association can produce bryostatins with an octa-2,4-dienoate substituent (bryostatins 1-3, 12 and 15). In order to elucidate the possible involvement of the symbiont in production of bryostatins, experiments were conducted to eliminate "*E. sertula*" from *B. neritina* to determine whether *B. neritina* can continue to grow normally without the symbiont, and/or continue to produce equivalent levels of bryostatins. Symbiont levels were estimated using a symbiont-specific PCR assay, then bryostatin activity levels were compared between control and treated *B. neritina* colonies. When symbiont levels were greatly reduced, bryostatin activity declined by approximately 50%. Genetic evidence was discovered that indicates "*E. sertula*" has the potential to synthesize complex polyketides like bryostatin. Finally evidence was gathered to address the hypothesis that bryostatins serve as defensive compounds for the bryozoan host. The distribution of bryostatins in the colonies is suggestive of a defense, and it was found that predatory nudibranchs of *B. neritina* sequester bryostatins and concentrate them in their egg ribbons. In summary, the symbiont's most likely function is to provide a chemical defense, bryostatins, for the host

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