

Abstract

Microbial consortia confer important benefits to animal and plant hosts, and model associations are necessary to examine these types of host/microbe interactions. The accessory nidamental gland (ANG) is a female reproductive organ found among cephalopod mollusks that contains a consortium of bacteria, the exact function of which is unknown. To begin to understand the role of this organ, the bacterial consortium was characterized in the Hawaiian bobtail squid, *Euprymna scolopes*, a well-studied model organism for symbiosis research. Transmission electron microscopy (TEM) analysis of the ANG revealed dense bacterial assemblages of rod- and coccus-shaped cells segregated by morphology into separate, epithelium-lined tubules. The host epithelium was morphologically heterogeneous, containing ciliated and nonciliated cells with various brush border thicknesses. Hemocytes of the host's innate immune system were also found in close proximity to the bacteria within the tubules. A census of 16S rRNA genes suggested that Rhodobacterales, Rhizobiales, and Verrucomicrobia bacteria were prevalent, with members of the genus *Phaeobacter* dominating the consortium. Analysis of 454-shotgun sequencing data confirmed the presence of members of these taxa and revealed members of a fourth, Flavobacteria of the Bacteroidetes phylum. 16S rRNA fluorescent in situ hybridization (FISH) revealed that many ANG tubules were dominated by members of specific taxa, namely, Rhodobacterales, Verrucomicrobia, or Cytophaga-Flavobacteria-Bacteroidetes, suggesting symbiont partitioning to specific host tubules. In addition, FISH revealed that bacteria, including *Phaeobacter* species from the ANG, are likely deposited into the jelly coat of freshly laid eggs. This report establishes the ANG of the invertebrate *E. scolopes* as a model to examine interactions between a bacterial consortium and its host.