

Abstract

**Various aspects of the
interactions between the
symbiotic bacterium *Cardinium*
and its insects host**

Thesis

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By

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Abstract

Researchers in many fields are now becoming aware of the importance of symbiotic microorganisms in every aspect of invertebrate biology. Some of these bacteria benefit their host's fitness directly, while others manipulate the reproduction of their hosts in ways that enhance their own transmission. The best known of these 'reproductive manipulators' is *Wolbachia*, a Proteobacterium that is widespread in arthropods and nematodes and has been implicated in all of the reproductive alterations discovered to date. A previously unknown bacterial symbiont, *Cardinium hertigii* has recently been described and characterized. Molecular phylogeny showed that the bacterium belongs to the Bacteroidetes bacterial group, and is phylogenetically distant from *Wolbachia*. Currently, three major phenomena are known to be associated with the presence of *Cardinium*: 1) Cytoplasmic incompatibility, which occurs between infected and uninfected sexual lines of the parasitoid wasps *Encarsia pergandiella*. 2) Feminization, the diversion of genetic males into phenotypic female, a phenomenon which has been described in two acari species. 3) Parthenogenesis, which has been found in one species of *Encarsia* and some populations of the armoured scale *Aspidiotus nerii*. The aim of this project was to study various aspects of the interactions between *Cardinium* and several of its host insects. For that purpose, the following goals have been set: 1) to identify the symbiotic bacteria in the members comprising a tri-trophic food chain; 2) to test for possible horizontal transmission in that system and 3) to identify genes associated with symbiont-host interactions.

The tri-trophic system chosen consists of the cactus scale insect, *Diaspis echinocacti*, a common herbivore feeding on cactus (*Opuntia*) worldwide. Several natural enemies are reported from the cactus scale, including nine species of *Aphytis* and the nearly host-specific species *Plagiomerus diaspidis*. Hyperparasitoid wasps in the genus *Marietta* represent the highest trophic level. A denaturing gradient gel electrophoresis (DGGE) technique was used to identify the symbionts among the insects comprising the cactus food chain. It was found that each of these insects hosts one symbiont only, except

Marietta which did not show any stable infection. *D. echinocacti* was found to carry a symbiont from the Flavobacteria group, apparently a primary symbiont. *Cardinium* was the only bacterium found in *P. diaspidis*, reinforcing the assumption that this symbiont is responsible for the parthenogenetic reproduction in that species. *Wolbachia* was the only symbiont present in *Aphytis sp.*, while no particular symbiont was associated with the *Marietta*. The distribution of *Cardinium* in *P. diaspidis* was studied using fluorescence *in situ* hybridization (FISH). The use of *Cardinium*-specific fluorescence probes revealed high concentration of that bacterium mainly in the ovaries of *P. diaspidis*, suggesting high frequency of the maternally-inherited symbiont and obligate association between the symbiont and its host.

To identify genes which are specific for the host-symbiont interactions, it was important to work on a system in which the bacterium could be removed from the host. Because the model organism *Drosophila melanogaster* is an important genomic tool, finding *Cardinium* in that insect could have been useful for searching for meaningful genes. However, the screening of 64 *Drosophila* lines in a PCR using *Cardinium*-specific primers showed no evidence for the presence of *Cardinium*. As an alternative, parasitic wasps from the species *Encarsia pergandiella*, known hosts for *Cardinium*, were used to isolate symbiont-associated candidate genes that are involved in the insect/symbiont interaction. Antibiotic treatment of *E. pergandiella* resulted in two populations: an uninfected line- *Encarsia*^{rif} and an infected control line- *Encarsia*^{ddw}. A combination of suppression subtractive hybridization (SSH) and microarray techniques was used to identify differential gene expression between these two lines. Some genes from the SSH library showed similarity to symbiotic bacteria and insects, while others showed similarity to plant and human genes. Further research is needed in order to verify the role of these genes and find more specific genes.