Bacterial Endosymbionts of *Adelges tsugae* Annand: Potential Targets for Biocontrol?

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ABSTRACT

We report the existence of four morphological forms of endosymbiotic bacteria in *Adelges tsugae* Annand and describe their ultrastructure. The endosymbionts are both intracellular and free-living in the hemocele. Systemic treatment of adelgid-infested hemlock seedlings with the antibiotic rifampicin resulted in degradation of adelgid endosymbionts and eventual death of the insects.

KEY WORDS

Bacteria, endosymbiont, ultrastructure.

INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is a devastating non-native pest of eastern hemlocks (*Tsuga canadensis* (L.) Carrière and *T. caroliniana* Engelm.) and a major threat to the health and sustainability of hemlock as a forest resource in eastern North America. HWA is native to Asia, where it infests Asian hemlocks but causes no particular damage. It was first reported in western North America in the 1920s on western hemlocks (*T. heterophylla* (Raf.) Sarg. and *T. mertensiana* (Bong.) Carrière), but these species, like those in Asia, appear to tolerate the effects of adelgid feeding. It is not known with certainty whether native predators in Asia and western North America mitigate impacts of HWA on hemlock in these areas or if genetic differences make eastern hemlocks more susceptible to adverse impacts of adelgid feeding. However, a recent study of terpenoids in worldwide *Tsuga* species found a possible linkage between terpenoid profiles and hemlock susceptibility to HWA (Lagalante and Montgomery 2003).

Terpenoids are volatile compounds found in conifers; they serve in chemical communication between plants and insects and are known to have antibiotic properties. There is evidence of terpenoid interference with the host-symbiont relationship in a number of insects, including Aphidoidea (aphids, phylloxerans, adelgids) (Van den Heuvel et al. 1998; Raguraman and Saxena 1994). Symbiotic microorganisms are common in Aphidoidea, but little is known about such in the Adelgidae. Here we describe bacterial endosymbionts in HWA, and speculate that host plant terpenoids in western and Asian hemlocks may adversely affect these microorganisms and inhibit HWA development on these *Tsuga* species.

METHODS

Hemlock woolly adelgid-infested foliage was collected from *T. canadensis* located in urban and forested settings in CT, PA, and VA, and from *T. heterophylla* in UT and OR. Collections were made over a period of several years and included all developmental stages of the three adelgid morphs (sistens, progrediens, and sexuparae). In addition, a group of eastern hemlock seedlings, naturally infested with aestivating HWA sistens, was treated in the fall with the antibiotic rifampicin via root soak for five days. Control seedlings were treated with water. All specimens were examined using light microscopy and transmission and scanning electron microscopy.

RESULTS AND DISCUSSION

Adelges tsugae harbors bacterial organisms that are found free living in the hemocele as well as bacteria in specialized cells known as mycetocytes or bacteriocytes. Based on their ultrastructure, *A. tsugae* endosymbionts appear to be prokaryotic bacteria with several distinct morphological forms. There are no apparent differences in the forms of bacteria found in the sistens, progrediens, or sexuparae morphs.

Form "A" bacteria are exclusively intracellular, residing in mycetocytes. They are 2.1-2.9 µm in diameter and variable in shape. The bacteria are densely packed in the mycetocyte and their cytoplasm is uniformly distributed and relatively dense. Form "A" endosymbionts are not present in oocytes or eggs prior to formation of mycetocytes, but they are found in all other *A. tsugae* stages except the post-oviposition adult, which lacks mycetocytes. Form "B" endosymbionts occur both intra- and extracellularly. They are spherical, 2.3-3.1 µm in diameter, and less electron-dense than the "A" form. They also are in mycetocytes, both separately and in combination with the "A" form. The "B" form often is seen in mycetocytes with disrupted cell membranes. Form "C" endosymbionts are rod-shaped, 1.5-2.3 µm in diameter and 8.7-14.6 µm long. They are prevalent in the hemocele of all *A. tsugae* nymphs and adults, and in oocytes and eggs, but do not appear in mycetocytes. The "C" form penetrates oocytes, transmitting the organism to the next adelgid generation. As oocytes develop, another morphological form, "D", appears along with "B" and "C" in a bacterial syncytium, which is incorporated into the embryo. As mycetocytes develop, the "D" form disappears and the "A" form appears in the new mycetocytes.

Treating infested hemlock seedlings with the antibiotic rifampicin resulted in inhibition of HWA development while changes occurred concurrently in the appearance of the endosymbionts. Treated adelgids eventually became aposymbiotic, gut tissues became increasingly necrotic, and nymphs died in the second instar. Anomalies similar to those seen in the endosymbionts of antibiotic-treated HWA (i.e., vacuolization, ruptured cell membranes, and necrosis) were occasionally observed in adelgids collected from *T. canadensis* and often observed in HWA on *T. heterophylla.*

Lagalante and Montgomery (2003) speculated that the significant differences in terpenoid profiles in the two eastern North American *Tsuga* species and the Asian and western North American species might correspond to the susceptibility/resistance of *Tsuga* species to HWA. We speculate that the mode of action for resistance may be terpenoid-induced degeneration of the endosymbiont population in HWA resulting in reduced development and fecundity of the insect population feeding on western North American and Asian *Tsuga* hosts. There could be subtle differences between the terpenoid profiles of individual *T. canadensis*, which could account for the degeneration observed in endosymbionts of some HWA and could reflect tolerance of some hemlocks to HWA feeding. Thus, the bacterial endosymbionts of HWA could serve as appropriate targets for biocontrol through systemic inhibition of metabolic pathways or by selecting resistant cultivars based on terpenoid profiles.

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