Gut Bacteria of Bark and Wood Boring Beetles

Archana Vasanthakumar [1]
Yasmin Cardoza [1]
Italo Delalibera [1]
Patrick Schloss [2]
Jo Handelsman [2]
Kier Klepzig [3]
Kenneth Raffa [1]

Bark beetles are known to have complex associations with a variety of microorganisms (Paine and others 1987; Ayres and others 2000; Six and Klepzig 2004). However, most of our knowledge involves fungi, particularly external species. In contrast, we know very little about their associations with bacterial gut symbionts (Bridges 1981). Similarly, work with wood colonizing insects such as termites reveals a diverse and functionally important community of gut bacteria (Hongoh and others 2005). Yet our knowledge of the gut microbiota of wood boring beetles is rudimentary. Our work is aimed at addressing these gaps, as bark and wood boring beetles include important forest pests, natural disturbance agents, and invasive species.

This report describes the composition, variation among life stages, and ecological roles of beetle gut communities. The study systems include three bark beetles—the southern pine beetle, pine engraver, and spruce beetle—and three wood borers—the Asian longhorned beetle, linden borer, and emerald ash borer. Insects were evaluated by a combination of culture-independent and culture-dependent approaches. Culture-independent analyses involved extraction of total DNA from entire guts, amplification of bacterial 16S rDNA, and sequencing (Broderick and others 2004). Culture-dependent approaches involved plating dilutions of gut extracts, periodic counts of colonies, and molecular identification by the above processes. Sampling efficiency in culture independent studies was estimated by rarefaction curves.

The predominant bacteria in these six beetle species were members of the alpha-proteobacteria, beta-proteobactaeria, gamma-proteobactaeria, Firmicutes, and Actinobacteria. The diversities of gut communities varied dramatically. At one extreme, the gut bacteria of linden borer consisted entirely of gamma-proteobactaeria (Schloss and others 2006). At the other extreme, only half of Asian longhorned beetle's most common bacteria were in one group, Firmicutes. Overall, the gut communities of wood borers tended to be more diverse than those of bark beetles at the level of bacterial genera. The emerald ash borer contained 26 bacterial genera in larvae and 27 in adults (Vasanthakumar, in prep). Of these, 15 morphotypes were present in both stages. Bacterial densities were higher in adults than larvae. Asian longhorned beetle larvae contained 24 bacterial genera (Schloss and others 2006). The southern pine beetle contained 13 genera in larvae and nine genera in adults, with only three species in common (Vasanthakumar and others 2006). The

---

[1] Department of Entomology, University of Wisconsin, Madison, WI

[2] Department of Plant Pathology, University of Wisconsin, Madison, WI

[3] USDA Forest Service, Southern Research Station, Pineville, LA

pine engraver had six genera in larvae, three genera in pupae, and three genera in adults, with two genera common to all life stages (Delalibera and others, subm.). Densities of bacteria were higher in pine engraver larvae than in adults, and higher in adults than pupae. Although linden borer guts have a low diversity of gut bacteria, the densities of these bacteria are much higher than in pine engraver (Delalibera and others 2005). Variation in bacterial members varied widely among these six insects. No genera were detected in all six, only four were detected in three, and only seven genera were detected in even two beetle species.

Bacteria have been shown to confer a broad array of benefits and detriments to their host insects (Cazemier and others 1997). To date, we have evaluated two roles in bark- and wood-boring beetles. We observed cellulolytic activity in gut bacteria of both wood borers tested—linden borer and emerald ash borer, but in neither of the two bark beetles tested—southern pine beetle and pine engraver (Delalibera and others 2005; Vasanthakumar and others, in prep.). Secondly, bacteria in oral egestions from spruce beetle can defend against gallery invading fungi that otherwise reduce oviposition and increase adult mortality (Cardoza and others 2005). These bacteria appear almost entirely responsible for the highly fungistatic activities of the spruce beetle's oral egestions.

Ongoing work involves potential roles of gut bacteria in detoxification of tree defense compounds and analyses of the stability and resilience of the gut community. Understanding the functioning of gut microbiota can offer promising insights into novel approaches to pest management (Broderick and others 2006).

Acknowledgments

We thank Debbie Miller, Leah Bauer, Robert Haack, Jolie Mahfouz, Ed Holsten, Ken Zogas, and Barbara Bentz, (USDA FS) for sending insects, to the Milwaukee City Foresters for trees, and Amelia Barber, Ted Ehlert, Ben Burwitz, and Amelia Barber for lab assistance. This work was supported by USDA FS, Southern Research Station, USDA FS North Central Research Station, NSF DEB-9629776, DEB-0314215 and DEB-0080609, USDA-NRI WIS04746, McIntire-Stennis, the Howard Hughes Medical Institute, and UW CALS.

References

Ayres, M. P.; Wilkens, R. T.; Ruel, J. J. ; Lombardero, M. J.; Vallery, E. 2000. Nitrogen budgets of phloem-feeding bark beetles with and without symbiotic fungi. Ecology 81: 2198-2210.

Bridges, J. R. 1981. Nitrogen-fixing bacteria associated with bark beetles. Microbial Ecology 7: 131-137.

Broderick, N. A.; Raffa, K. F.; Goodman, R. M.; Handelsman, J. 2004. Census of the bacterial community of the gypsy moth larval midgut using culturing and culture-independent methods. Applied and Environmental Microbiology 70: 293-300.

Broderick, N. A.; Raffa, K. F.; Handelsman, J. 2006. Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. Proceedings of the National Academy of Sciences USA. 103: 15196-15199.

Cardoza, Y. J.; Klepzig, K. D.; Raffa, K. F. 2006. Bacteria in oral secretions of an endophytic insect inhibit antagonistic fungi. Ecological Entomology 31(6)

Cazemier, A. E.; Hackstein, J. H. P.; Op den Camp, H. J. M.; Rosenberg, J.; van der Drift, C. 1997. Bacteria in the intestinal tract of different species of arthropods. Microbial Ecology 33: 189-197.

Delalibera, I., Jr.; Handelsman, J.; Raffa, K. F. 2005. Cellulolytic Activity of microorganisms isolated from the guts of *Saperda vestita* (Coleoptera: Cerambycidae), *Ips pini*, and *Dendroctonus frontalis* (Coleoptera: Scolytidae). Environmental Entomology 34: 541-547.

Delalibera, I., Jr.; Vasanthakumar, A.; Burwitz, B. J.; Schloss, P. D.; Klepzig, K. D.; Handelsman J.; Raffa, K. F. Submitted. Gut bacterial composition of the pine engraver beetle, *Ips pini* (Say) (Coleoptera: Curculionidae: Scolytinae). Submission to Envrionmental Entomology.

Hongoh Y.; Deevong, P.; Inoue, T. 2005. Intra- and interspecific comparisons of bacterial diversity and community structure support coevolution of gut microbiota and termite host. Applied Environmental Microbiology 71: 6590–6599.

Paine, T. D.; Raffa, K. F.; Harrington, T. C. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annu. Rev. Entomology 42: 179-206.

Schloss, P. D.; Delalibera, I., Jr.; Handelsman, J.; Raffa, K. F. 2006. Bacteria associated with the guts of two wood-boring beetles: *Anoplophora glabripennis* and *Saperda vestita* (Cerambycidae). Environmental Entomology 35: 625-629.

Six, D. L.; Klepzig, K. D. 2004. *Dendroctonus* bark beetles as model systems for studies on symbiosis. Symbiosis 37: 207-232.

Vasanthakumar, A.; Delalibera, I., Jr.; Handelsman, J.; Klepzig, K. D.; Schloss, P.; Raffa, K. F. 2006. Characterization of gut-associated microorganisms in larvae and adults of the southern pine beetle, *Dendroctonus frontalis* Zimmerman. Environmental Entomology.

Vasanthakumar, A.; Handelsman, J.; Raffa, K. F. In preparation. Exploring gut microbial diversity in an invasive wood-boring beetle, the emerald ash borer.

---

current addresses:

Y. Cardoza: USDA-ARS/CMAVE, Gainesville, FL.

I. Delalibera: Univ. São Paulo, Piracicaba, SP, Brazil.

P. Schloss: Department of Microbiology, University of Massachusetts, Amherst, MA.