

Composition of the bacterial community in the gut of the pine engraver, *Ips pini* (Say) (Coleoptera) colonizing red pine

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Abstract

The gut bacterial community of a bark beetle, the pine engraver *Ips pini* (Say), was characterized using culture-dependent and culture-independent methods. Bacteria from individual guts of larvae, pupae and adults were cultured and DNA was extracted from samples of pooled larval guts. Analysis of 16S rRNA gene sequences amplified directly from the gut community suggests that the gut bacterial communities associated with *I. pini* are relatively simple, compared to many other systems. Six bacterial genera from four classes were detected by culturing gut bacteria from larvae, pupae and adults. Two genera, *Pantoea* and *Stenotrophomonas* (γ -Proteobacteria) were found in all life stages of *I. pini*, consistently in larvae and adults, and less commonly in pupae. Sequences that affiliate with the Enterobacteriaceae of the γ -Proteobacteria were found in 95% of the clones sampled. The Enterobacteriaceae genera, *Pantoea* and *Erwinia*, accounted for 88% of all clone sequences. These results are consistent with previous work indicating that another bark beetle, the southern pine beetle, *Dendroctonus frontalis* Zimmerman, also has a relatively simple gut flora, compared with wood colonizing insects such as wood borers and termites. The composition and abundance of bacteria associated with different life stages of *I. pini* are possibly associated with specific functions of the gut bacterial communities of larvae, pupae, and adults.

Keywords: Bark beetle, gut microbiota, 16S ribosomal RNA, rarefaction analysis

1. Introduction

Bark beetles (Coleoptera: Curculionidae: Scolytinae; altern. Scolytidae) have a variety of relationships with symbiotic microorganisms (Paine et al., 1997). Some microorganisms serve as food for larvae (Six and Klepzig, 2004), some assist in digestion, and some increase nitrogen concentration in phloem surrounding the larvae (Ayres et al., 2000). Symbionts also detoxify plant compounds (Dowd, 1992) and assist in overcoming host defenses (Krokene and Solheim, 1998). Some symbionts produce compounds that are used as aggregation pheromones by bark beetles (Brand et al., 1975), whereas others produce

volatile metabolites that can be exploited by natural enemies of bark beetles (Sullivan and Berisford, 2004).

Despite the large body of knowledge on microbial symbionts of bark beetles, most of this information concerns external and mycangial fungi. In contrast, relatively little is known about the composition and function of the gut microbiota, especially bacteria. Moreover, most previous studies on the diversity of gut microbiota of bark beetles relied on culture dependent techniques and microscopic observations. Those studies revealed nitrogen-fixing bacteria (Bridges, 1981), fungi such as the blue-stain fungus, *Ophiostoma ips* (Rumbold) Nannfeldt (Furniss et al., 1995) and yeasts (Moore, 1972; Brand et al., 1977). More recently, gut bacteria of the southern pine beetle, *Dendroctonus frontalis* Zimmermann (Vasanthakumar et al., 2006), and bacteria in oral egestions

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