Bacterial Protection of Beetle-Fungus Mutualism

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The pervasiveness of beneficial associations between symbiotic microbes and plants and animals in every ecosystem illustrates how the acquisition of a microbe’s physiological capacity confers substantial fitness benefits to hosts (1). However, dependence on mutualistic microbes becomes a liability if antagonistic microbes attack or outcompete beneficial ones (2). Therefore, mechanisms to preserve beneficial microbes must be widespread, although poorly understood, component of host-microbe mutualisms. We show that a beetle uses a bacterium to protect its fungal food source from a competitor fungus.

Southern pine beetles, Dendroctonus frontalis, engage in a beneficial symbiosis with the fungus Entomocorticium sp. A, which provides nourishment for their developing larvae. Adult beetles carry Entomocorticium sp. A in a specialized storage compartment called a mycangium (Fig. 1A), excavate ovipositional galleries within the inner bark and phloem of host pine trees, and inoculate these galleries with Entomocorticium sp. A (3, 4). The success of the D. frontalis–Entomocorticium sp. A mutualism is challenged by an antagonistic fungus, Ophiostoma minus, which can outcompete Entomocorticium sp. A and thereby disrupt beetle larval development (3, 4). Our results indicate that successful maintenance of the D. frontalis–Entomocorticium sp. A mutualism is likely mediated by an actinomycete bacterium that produces antibiotics that selectively inhibit O. minus.

The presence of previously unknown actinomycetes within the D. frontalis–Entomocorticium sp. A mutualism was established by scanning electron microscopy (SEM) and enrichment culture isolations (5). SEM revealed unexpected and profuse growth of actinomycetes within the galleries of D. frontalis, as well as inside the mycangia (Fig. 1B and fig. S1A). Isolations from 110 beetle individuals yielded 846 colony-forming units (CFUs) of actinomycetes, including at least one CFU from each of 92 individuals. Out of 164 actinomycete CFUs selected to be transferred to pure culture, 99 isolates had a red morphotype, whereas 65 isolates had a white morphotype. DNA sequence analyses confirmed the visual morphotype distinction, and within each of the two morphotypes there was complete 16S rDNA sequence identity. The two morphotypes form a monophyletic clade closely related to Streptomyces mosacchari. Furthermore, we also isolated the same red morphotype from 5 of 10 mycangia sampled.

We explored the potential role of the actinomycetes in mediating the D. frontalis fungal community by using symbiotic pairing bioassays and chemical analyses. The bioassays, which crossed all possible combinations of the two actinomycete morphotypes with Entomocorticium sp. A and O. minus, revealed that isolates of the red morphotype produced a diffusible activity that inhibits the beetle’s antagonistic fungus, O. minus, but only slightly affects the beneficial fungus, Entomocorticium sp. A (Fig. 1C and fig. S1, B and C). Extensive chemical and spectral analyses on strains of the red morphotype revealed the antifungal molecule responsible for selective inhibition to be a polynye peroxide, which we named mycangimycin. Mycangimycin (C_{20}H_{24}O_{8}), which has not been previously reported, is a linear 20-carbon carboxylic acid with an endoperoxide linking C-3 and C-5 to form a 1,2-dioxolane and a conjugated cis, cis, trans, trans, cis, trans-heptaeanae 2C-7 to C-20 (Fig. 1D). Liquid culture antifungal assays using purified mycangimycin showed O. minus to be almost 20 times more susceptible [minimal inhibitory concentration (MIC) = 1.0 μM] than Entomocorticium sp. A (MIC = 19.0 μM) (fig. S1D). The identification of an actinomycete that is localized in the mycangium and galleries, which produces an antibiotic that selectively suppresses the antagonistic fungus, O. minus, indicates that D. frontalis engages in an additional mutualism with bacteria to regulate the Entomocorticium sp. A–O. minus fungal community. Because other bark-beetle species also depend on successfully maintaining beneficial fungi, tripartite beetle-fungus-bacterium mutualisms may be widespread.

Our study parallels earlier work on fungus-farming ants, which use actinomycetes to help protect their fungal gardens from pathogens (6). Taken together, these findings suggest that the use of antibiotic-producing actinomycetes may be a common method for maintaining beneficial microbes. Indeed, considering the importance of pathogens as a driving force in the evolution of all hosts, the benefit of such associations may extend to helping protect plants and animals from pathogens to which they themselves are susceptible (7, 8). If, as seems likely, these associations are widespread, targeting them could be an effective strategy for locating novel biologically active natural products.

References and Notes

5. Materials and methods are available on Science Online.
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We have a patent application submitted on mycangimycin.

Supporting Online Material

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Materials and Methods

Fig. S1

References

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