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 a 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 actinomycetous 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 thermosacchari*. 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 symbiont 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 polyene peroxide, which we named mycangimycin. Mycangimycin ($C_{20}H_{24}O_4$), 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*-heptaene spanning C-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.

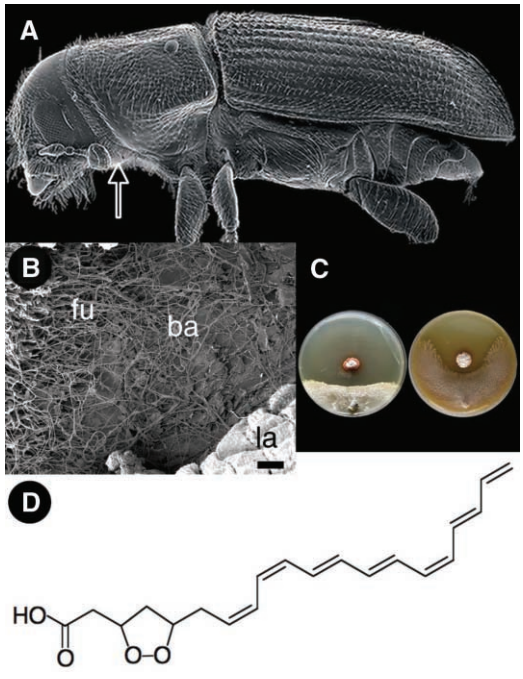

Fig. 1. (**A**) SEM micrograph of adult *D. frontalis* showing the location of a mycangium (arrow), which is used to transport *Entomocorticium* sp. A. (**B**) SEM micrograph from the *D. frontalis* gallery showing the actinomycetous bacterium (ba), fungus (fu), and beetle larva (la). (**C**) Representative examples of pairwise bioassay challenges illustrating inhibition of the fungal antagonist, *O. minus* (left), by a *D. frontalis* symbiotic actinomycete (strain SPB074). In contrast, the southern pine beetle's fungal mutualist, *Entomocorticium* sp. A, is relatively resistant (right) (see SOM for more details). (**D**) The structure of mycangimycin contains a seven-conjugated double bond chain and a five-membered endoperoxide ring.

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

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Supporting Online Material

www.sciencemag.org/cgi/content/full/322/5898/63/DC1
Materials and Methods
Fig. S1
References

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