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Symbionts, Peptides, and (No) Iron: How Ants Defend Their Fungal Crop

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A nonribosomal peptide having a unique relationship with iron is used by ants to protect their fungal gardens.

Humans are not the only species on Earth that have developed agriculture. Numerous insects, including ants, termites, beetles, and bees, grow their major food source, fungi, on "farms". Like human farmers who deal with insects and pathogens that destroy crops such as wheat and maize, these insects must also deal with pests and pathogens that attack their fungal crop. One powerful farming strategy that has evolved is the development of symbiotic relationships between these insects and bacteria. The insects harbor the beneficial bacteria, which in turn produce natural products that selectively kill fungal pathogens and predators, but leave the cultured fungal crop intact. In other words, these insects have established an in situ supply chain of agrichemicals for crop protection for themselves.

In this issue of *ACS Central Science*, Pupo, Clardy, and co-workers[1] study the fungus growing attine ants, an ant tribe that has been farming for 50 million years. Attine ants rely on symbiotic relationships with *Pseudonocardia* and *Streptomyces* bacteria for the production of compounds that kill the fungal parasites that infest their crops.[2] Notably, the natural products used by these insects have always shown limited geographical distribution, thereby suggesting that geographically distinct insect-symbiotic pairs each evolve a unique molecular solution for crop protection. This is typical of natural products. In fact, natural products are also called "specialized metabolites" precisely because these molecules have so often uniquely evolved in the context of a specific geographical and ecological niche.[3]

Since attine ants are dispersed widely throughout the Neotropics, the authors were surprised to discover that a single nonribosomal peptide, which they named attinimicin, is produced by more than 70% of all *Pseudonocardia* spp. symbionts isolated across a two-million square kilometer area in Brazil. Thus, the authors report here the first widespread production and use of a natural product important for ant fungal farming.

The authors made this discovery by first isolating 42 ant-associated *Pseudonocardia* bacteria strains from geographically disparate regions. These bacteria were cultured and then screened for activity against pathogenic fungi that are known to invade ant fungal crops. Of the 16 isolated strains that selectively inhibited growth in this screen, metabolomic analysis showed that more than 60% of these cultures produced the same natural product. A subsequent, broader examination of ant-associated symbiotic bacteria showed that this compound is prevalent in symbionts from ants located across Brazil. Isolation of this compound showed that it was a nonribosomal peptide type natural product. The biosynthetic gene cluster for this natural product could be readily identified in the genome sequence of eight representative strains of *Pseudonocardia* that were subjected to whole genome sequencing.

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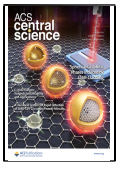

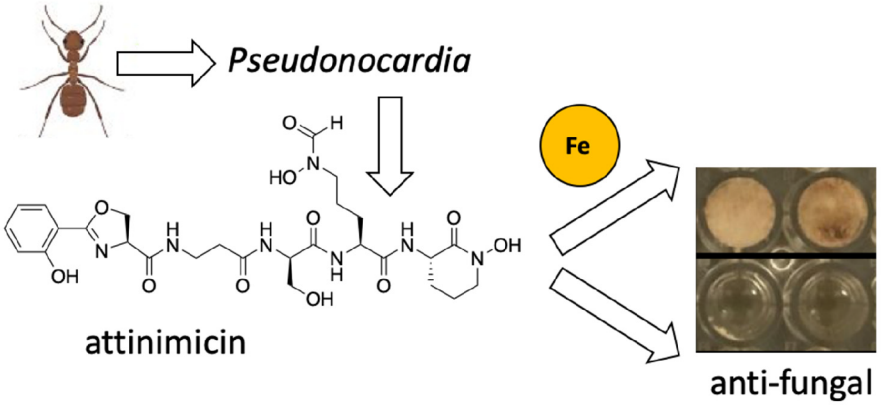

Figure 1. Through associated symbiotic bacteria, attine ants produce the molecule attinine, which has antifungal activity only in the absence of iron. Photo on the right reproduced from ref 1. Copyright 2020 The Authors. Published by American Chemical Society.

Understanding how a molecule performs in nature is much more complicated than identifying a specific biological activity in a laboratory setting. Nevertheless, the *ex vivo* biological activity of a natural product can provide important clues to the actual ecological function. Based on the growth inhibition screens of the isolated *Pseudonocardia* strains, the authors proposed that attinimicin acts as a selective antifungal agent toward the species of pathogenic fungi that infect ant crops. However, the authors could not help but notice that the chemical structure of attinimicin resembles a siderophore, a type of natural product that evolved to chelate iron.[4] Thus, the authors hypothesized that iron chelation might also play some role in the ecological function of this molecule.

The authors measured the affinity of attinimicin toward iron and found that it is significantly lower than established siderophores, though attinimicin selectively bound iron in the presence of other metals. Nevertheless, this modest iron-chelating activity prompted the authors to evaluate the activity of attinimicin against pathogens both in the presence and absence of iron. While iron-bound attinimicin had no inhibitory activity against *Escovopsis*, a parasitic fungus that attacks ant fungal crops, iron-depleted attinimicin showed moderate activity. The authors reasoned that this dependence on iron depletion may be the key for the efficacy of the natural product in a natural context, since iron in vivo is almost exclusively bound to iron carrier proteins (e.g., hemoglobin, transferrin), all of which have higher iron-binding affinities than attinimicin. Supporting this hypothesis, in a test of *in vivo* efficacy, the authors found that attinimicin significantly reduced the fungal burden in mice infected with *Candida albicans* at a comparable efficacy compared to clinically used antifungal drugs.[5]

Much work remains to be done to elucidate the complete mechanism by which attine ants protect their fungal crops against parasites. Here, the authors report a key piece of the puzzle. Analysis of a geographically broad selection of bacteria allowed the identification of an unusually conserved defense molecule named attinimicin. Although the *in vitro* activity of this compound was modest, attinimicin was detected in ants in their natural habitat—a strong indication that this compound has an ecological function. The authors discovered that the absence of iron "switched on" the activity of this molecule, and this switch is likely responsible for potent *in vivo* activity, an environment in which most iron is chelated. The *in vivo* antifungal activity of attinimicin was also demonstrated in a mouse model, thereby highlighting how molecules initially identified strictly for ecological interest may be translated for use in human medicine.

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