Beekeeping With Bacteria

By Alison McAfee

Microbes are giving us a new wave of defenses against hive pests and pathogens

Bacteria are not something to be cringed at. Villains like *E. coli*, *Listeria*, and *Staphylococcus aureus* have given the whole bacterial kingdom a bad rap, but we can capitalize on beneficial microbes to work for us instead of against us. Probiotic supplements are some of the first "good bacteria" products aimed at promoting honey bee health, but the fast-paced world of biotechnology is leading to increasingly creative applications, from genetically engineering gut bacteria to have better weapons against honey bee pathogens, to creating an anti-wax moth bacterial spritz to preserve stored comb.

Super *Snodgrassella*

Sofia Romero, a master's student in Leonard Foster's laboratory (where I completed my PhD), is looking for new ways to prevent American foulbrood infections without using antibiotics. Other research has focused on delivering AFB-fighting *Lactobacillus* bacteria to honey bees as part of a protein patty supplement (see Scott McArt's article from January 2020, for more on this[1]), which is a promising approach.[2] But instead of giving bees new bacteria, Romero is refurbishing an old one.

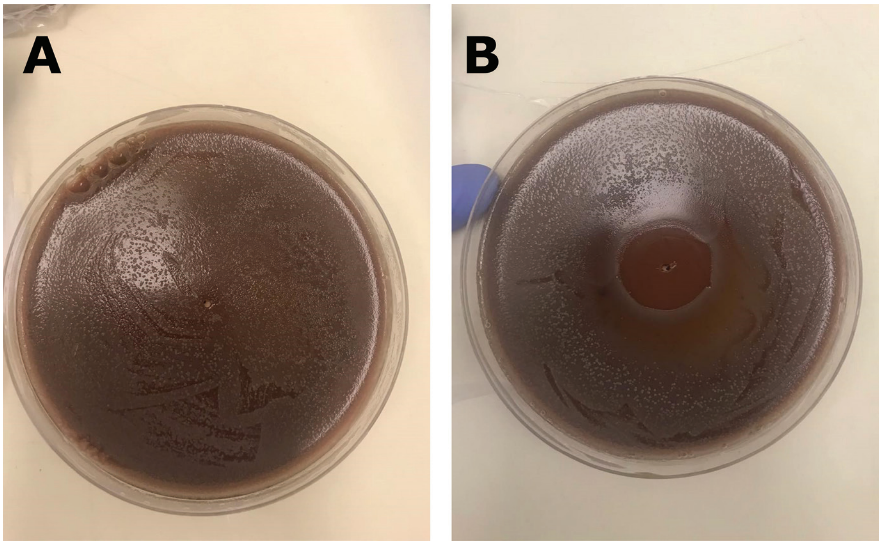
The goal is to engineer natural honey bee gut bacteria to manufacture antimicrobial peptides (small, bactericidal protein fragments), ramping up their potency against AFB. Romero has isolated the bacterium *Snodgrassella alvi*, which is one of the most abundant commensal bacteria in the honey bee's gut, and is engineering it to produce and secrete the peptides melittin and jelleine, which kill AFB bacteria but have minimal effects on the bee's natural microbiota. "I think having a probiotic with the ability to produce, deliver and secrete antimicrobial peptides is a highly rewarding approach," Romero says. "It decreases the costs of the chemical synthesis of the peptides and provides a very convenient and safe method of delivery."

Unlike animals, bacteria are very easy to genetically engineer — it's so easy, this is routinely done in a single afternoon in a typical molecular biology laboratory. And since *Snodgrassella* is a normal part of the bee gut microbiome and gets passed on from worker to worker, a single inoculum could potentially maintain perpetual AFB resistance in the colony.

Romero's research is still in early stages of development, and is not expected to lead to field trials any time soon. She is currently working on tweaking the bacteria's secretion system to make sure the peptides actually become expelled. Romero recently won the Foundation for the Preservation of Honey Bees graduate student scholarship for her work on engineering *Snodgrassella*.

Lactobacillus BioPatties

Other probiotic approaches for fighting disease, like the BioPatty formula developed by PhD candidate Brendan Daisley and his colleagues at Western University,[2] are making rapid



Romero cultured Snodgrassella alvi *on agar supplemented with sheep's blood, which seems to be what it prefers in the lab setting. This allowed her to conduct "zone of inhibition assays" to quickly measure the potency of bactericidal molecules. The antibacterial compound is touched to the center of a plate covered in bacteria, then a zone devoid of bacteria grows around the contact point with time. A) The compound has no antibacterial effect; B) The compound has strong antibacterial effect, with a large zone of inhibition.* Photos by Sofia Romero

Daisley and his colleagues mix their BioPatties in the laboratory (left). BioPatties are intended to be used just like regular pollen patties, placed across the top bars of a hive (right). Photos by Brendan Daisley

progress. Daisley's BioPatties, which include three *Lactobacillus* species (*L. rhamnosus*, *L. kunkeei*, and *L. plantarum*) that secrete AFB-fighting molecules, don't use bacteria that have been genetically engineered. Rather, the researchers carefully selected specific strains that they previously demonstrated to have immunomodulatory properties. "Our strain selection for the *Lactobacilli* is very targeted," Daisley says. "All the strains we test in bees have first been tested extensively in our *Drosophila* [fruit fly] model to determine their safety and ability to modulate insect detoxification and immunity. We're trying to use microorganisms that have shown a benefit in model organisms and see if they can show the same benefit in honey bees as well."

The researchers found that a *Lactobacillus*-supplemented diet reduced AFB pathogen loads in symptomatic colonies and increased larval survival in laboratory experiments, while other, independent work has shown that the bacterial secretions are the active ingredient.[3] Daisley and his colleagues are currently expanding this work to test if the BioPatties can prevent European foulbrood infection and protect against pesticide toxicity to boot. Daisley's ultimate goal is to "make specific formulas to address specific diseases and pesticides for different times of the year."

Daisley and his colleagues were originally planning to test if the BioPatty could protect honey bees against pesticide toxicity, but an inadvertent AFB outbreak gave them an unexpected opportunity to investigate this disease instead. Unfortunately, that also meant their experimental colonies, shown here, had to be burned after taking samples, precluding long-term, follow-up monitoring. Photo by Brendan Daisley.

Although several *Lactobacillus* species are commercially available (such as *L. brevis* and *L. acidophilus*, which are commonly used for brewing sour craft beers), the different species have variable effects on honey bees and even the same species can have a dramatically different impact depending on the strain, environmental conditions, and how it's administered. Much care must be taken when selecting species, strains, and delivery methods. For example, *L. rhamnosus* and *L. kunkeei* (two of the species in the BioPatty) can actually increase honey bee mortality under some conditions,[4,5] whereas *L. acidophilus* can improve honey production,[6] and *L. brevis* can stimulate the immune system.[7] And the specific *L. plantarum* strain Daisley used (Lp39) is better at stimulating the immune system than other strains from the same species. The variable results reported by different studies, and sometimes negligent labelling of specific strains on probiotic products, suggests that these products still need prudent testing and verification.

"Most of the probiotics available for honey bees are untested or don't include information on their specific strains," Daisley says. Though it might be tempting, my gut says that the desperate beekeeper should not leap-frog product approval and reach for a supplement, or make a homebrew, before it's on the shelf. However, many products that *are* on the shelf have not been sufficiently tested, either.

Daisley and his colleagues are not about to rush the BioPatty into commercial production. "We're trying to make sure the science is there first. We don't want to make a product based off only a few studies — we're trying to repeat everything and develop specific formulations to address specific problems."

Bacillus thuringiensis versus wax moth

While the science of supplements has been historically lax, the Environmental Protection Agency (EPA) is taking some other bacteria-based beekeeping products quite seriously. One biological pesticide — called B402 — for preventing the destruction of stored comb by wax moths is now being proposed for registration. The pesticide formulation, which is meant to be sprayed on combs before storage in the off-season, includes live *Bacillus thuringiensis* (Bt, for short) bacteria which produce a protein that is highly toxic to moth and butterfly (lepidopteran) caterpillars, such as wax moth larvae.

B. thuringiensis naturally occurs in the soil, and different bacterial subspecies produce proteins that are toxic to different insect species. Some are toxic to lepidopterans, while others are toxic to mosquitoes or beetles. This particular *B. thuringiensis* is the *aizawai* subspecies (strain ABTS 1857), which is not toxic to mammals or bees. Since the bacterium is naturally occurring, some Bt spray-on products are approved for use in organic agriculture, whereas crops that have been engineered to product their own bacteria-derived toxin are not.

The idea is that by spraying stored comb with active *B. thuringiensis* spores, any wax moth larvae would inadvertently consume the spores while happily chomping and meandering through the comb. Inside the basic (high pH) wax moth gut, the toxic protein contained within the spores activates and binds to specific receptors on the gut cell lining, causing the larva to stop eating and grow leaky holes in the gut. The bacteria can then proliferate in the rest of the body and kill the larva. Honey bees have a slightly acidic, rather than basic, gut environment, and don't have the same gut epithelial receptors for the toxin to bind to. What's more, the spores lose viability at high temperatures (> 30 C, or 86 F), so when the stored combs are returned to hives in the spring, the spores will die and the bacteria shouldn't have the opportunity to compete with or disturb the natural bee gut microbial community.

This is not the first time that such a Bt-containing bacterial spray has been proposed to be registered in the U.S. According to Jerry Hayes, the former columnist for this magazine's "The Classroom" section, the previous generation of the product (B401, also known as Certan) was registered years ago but became de-registered when the fees grew too expensive for the manufacturer, Vita-Europe, to maintain.[8] It is unclear how B402 and B401 effectively differ, although one is labelled "serotype 7" and the other is "strain ABTS 1857."

While the product is currently available in Canada and Europe, with de-registration it became illegal to purchase or use B401 in the United States for wax moth control. However, another product, called XenTari, is also composed of *B. thuringiensis* subspecies *aizawai* and is available in the U.S., but is intended for crop applications (not beehives). I imagine there is ample off-label use in beekeeping. But with the EPA's public consultation period for B402 closed as of January 24, 2020, and the fact that an almost identical product has been registered before, having to sneak around to use this bacterium on stored comb may soon become a thing of the past.

Bee-free honey

Fighting pests and pathogens isn't the only thing bacteria are being used for in the bee world. A group of undergraduate students from the Israel Institute of Technology began developing a bacterial system for synthesizing artificial honey as part of the International Genetically Engineered Machine (iGEM) competition last year. They engineered the bacteria *B. subtilis* to produce and secrete enzymes (catalase, invertase, and glucose oxidase) that achieve important reactions needed to convert nectar (or in this case, a sucrose solution) to "honey." They also designed a convenient capsule to house their "synthetic honey stomach" to allow for easy separation of the bacteria from the finished honey product. The students formed one of 353 iGEM teams from around the world and competed internationally in Boston to win a gold medal for their project.

To be clear, this system does not produce actual honey. It is completely devoid of phytochemicals — the molecules that give honey its wide-ranging colors, flavours, and many of its beneficial properties. However, with how cheap and easy it is to engineer bacteria to execute specific chemical reactions, the alarmist in me worries that synthetic biology might lead to a new wave of honey adulteration schemes. If bacteria can be coaxed to produce a bioequivalent mixture of honey sugars, what is to stop someone from using that mixture to cut their honey, diluting the phytochemicals but leaving just enough for it to appear "normal" to the most sophisticated tests?

Whether we like it or not, bacteria are an integral part of our lives. Quite literally, there are more bacterial cells on and in our bodies than our own human cells (of course, we don't look like a mound of cocci and bacilli because bacteria are much smaller than animal cells). The more we learn about these tiny life forms, for better or worse, the more we can exploit them.

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