Can probiotic supplements protect colonies from AFB?

No beekeeper wants their bees to develop infections of *Paenibacillus larvae*, the bacteria causing American Foulbrood (AFB). It's deemed such a threat that colonies must be destroyed if positive detections are found. In addition, neighboring colonies in an apiary or operation must be quarantined, which can significantly impact beekeepers, especially those who need to move their colonies on a schedule.

While few formal surveys have been conducted, it's well-known that some beekeepers prophylactically treat their colonies with antibiotics to ward off AFB. But this practice comes with a cost. Similar to how several antibiotics are losing their efficacy to combat human bacterial diseases due to improper use, antibiotic-resistant strains of AFB have been detected for the past several years, presumably due to the prophylactic treatments applied by beekeepers. In other words, new strains of AFB are evolving that can survive antibiotics, and those strains pose an even larger threat to beekeepers.

But what if instead of using prophylactic *anti*biotics, beekeepers could use prophylactic *pro*biotics to combat AFB? That could be a simple, elegant, and potentially cheap way to protect colonies from AFB while avoiding the development of resistant strains. But is it possible? This is the topic for our twenty-sixth "Notes from the Lab," where we highlight **"Novel probiotic approach to counter *Paenibacillus larvae* infection in honey bees,"** written by Brendan Daisley and colleagues and published in the **ISME Journal (2019).**

For their study, Daisley and colleagues first took advantage of a fortuitous natural outbreak of AFB in one of their apiaries. (Yes, most beekeepers would consider an AFB outbreak anything but fortuitous, but remember these beekeepers are scientists studying AFB!) Next, they also complemented this observational study with manipulative lab experiments isolating the influence of microbes on *P. larvae* and larval survival. This combination of field observations and controlled laboratory studies can be an excellent 1-2 punch for advancing scientific knowledge on a topic, in this case the impact of probiotic bacteria on AFB.

Specifically, the field experiment followed six colonies, two of which were controls (no treatment), two that were supplemented with vehicle controls (pollen patty and a buffered solution without probiotics), and two of which were supplemented with the probiotic treatment (pollen patty and the buffered solution containing probiotics, which the authors call a BioPatty). The authors cultured three bacteria to make their probiotic BioPatty: *Lactobacillus plantarum* Lp39, *Lactobacillus rhamnosus* GR-1, and *Lactobacillus kunkeii* BR-1.

Previous studies in fruit flies found these three strains of bacteria could improve innate immunity, pesticide detoxification, and protection against harmful microorganisms, hence the interest in these specific bacteria by the researchers. The colonies were administered their respective treatments twice (day 0 and day 7) and nurse bees were collected for later screening on each of these days. Colonies were identified as positive for AFB on day 12 and were thus destroyed on this date as per government regulations after larval samples were collected from the infected hives. The nurse bees and larvae were subsequently tested for differences in bacterial communities depending on whether they were taken from treatment or control colonies.

Co-authors Graham Thompson, Anna Chernyshova and Brendan Daisley (L-R) preparing to apply the BioPatty treatment to the experimental colonies

Collecting larvae from an experimental colony for subsequent microbial characterization in the lab

Next, the authors conducted two sets of follow-up laboratory experiments. First, they isolated and cultured the *P. larvae* strain from their infected colonies and challenged this strain in Petri dish assays with probiotic bacteria, bacteria isolated from honey bee guts, and antibiotics. If you're unfamiliar with Petri dish assays, think about that container of food that you've accidentally left in the back of your fridge for several months at some point in your life (this seems to happen all the time in my house right now … I'd like to blame the 7-year-old, though the culprit is surely myself more often than not). Anyways, when you open the newly-found container, you've probably seen different types of mold, right? Well, some of that mold has "won" in the competition with other molds and is therefore what you see. Similarly, Petri dish assays assess which bacteria "win" in competition with others.

Co-author Andrew Pitek showing consumption of the BioPatty during the field experiment

Finally, in their last set of experiments, the authors experimentally infected honey bee larvae with *P. larvae* and assessed survival of larvae that were fed the buffered solution not containing probiotics compared to the buffered solution containing probiotics (analogous to the BioPatty). These experiments occurred in small plastic dishes so the microbial environment could be closely controlled.

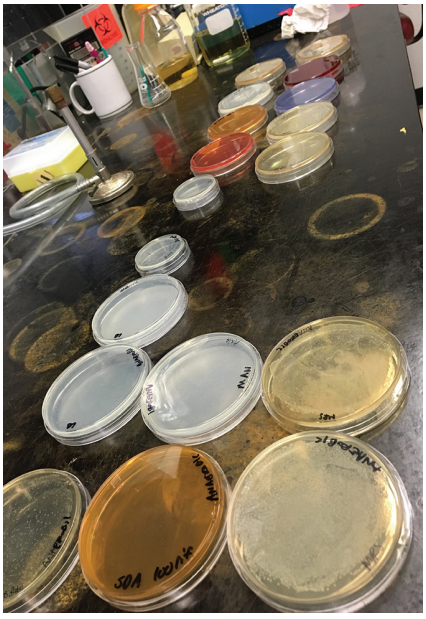
So, what did they find? Did the probiotics protect the colonies from AFB in the field? Yes and no. Larvae sampled from colonies receiving the BioPatty had nearly 100-fold less *P. larvae* and the pathogenic activity of those *P. larvae* was marginally lower compared to control colonies. Furthermore, the bacterial community of the bees was distinctly different between the controls and bees fed the BioPatty. Specifically, the bees that consumed the BioPatty had greater amounts of beta-proteobacteria and actinobacteria, in addition to having higher levels of the three probiotics. These are very intriguing and promising results. That said, the BioPatty colonies still developed AFB disease symptoms and by regulatory standards needed to be destroyed.

What about the lab? Did the probiotics ward off *P. larvae* and increase larval survival? The laboratory results were indeed very encouraging. First, the Petri dish assays showed that growth and cell viability of *P. larvae* were reduced by each of the three probiotics. Even more promising, two of the probiotics (*Lactobacillus plantarum* Lp39 and *Lactobacillus rhamnosus* GR-1) and the combination treatment of all three probiotics were as efficient at inhibiting *P. larvae* compared to the most popular commercial antibiotic, oxytetracycline (Terramycin). Finally, when fed to developing larvae in the lab, the combination treatment of all three probiotics significantly reduced *P. larvae* levels and there was a trend for increased survival. Similar to the field study, the bacterial communities of the larvae were different in the probiotic treatment compared to controls.

Well this seems super promising. Is this proof that probiotics will prevent my colonies from getting AFB? Unfortunately, no. The field results and especially the lab results are very promising, but they don't provide proof that probiotics will prevent your colonies from getting AFB. That said, the results are tantalizing, further work is underway, and a company has taken notice. If you're interested in learning more about the new BioPatty product and ongoing research, including the extensive field trials that are being conducted to robustly test efficacy in real beekeeping operations, contact information can be found here: **https://seed.com/seedlabs/**.

As you've probably seen (including via advertisements in this magazine) there are many companies who are starting to produce and sell probiotic products for bees. Just like the probiotic products you see in your local grocery store, some have science behind them and some don't. If you

One of the experimental colonies that tested positive for Paenibacillus larvae *(American Foulbrood) and exhibited clinical symptoms of AFB during the field experiment*



Some of the Petri dish inhibition assays used to assess competition between Paenibacillus larvae*, probiotic bacteria, and antibiotics*

have a question about a particular product, do your homework and ask the manufacturer! As Daisley and colleagues' study shows, the potential for probiotics to benefit bees (and a lot of other things) is clear. But there's also research showing that some probiotic products can increase bee diseases and mortality, ultimately reducing the productivity of your colonies. So, perhaps the prudent advice on this topic is obvious: Bee informed!

Until next time, bee well and do good work,

Scott McArt

Reference:

Daisley, B. A., A. P. Pitek, J. A. Chmiel, K. F. Al, A. M. Chernyshova, K. M. Faragalla, J. P. Burton, G. J. Thompson and G. Reid. 2019. Novel probiotic approach to counter *Paenibacillus larvae* infection in honey bees. ***ISME Journal***. **https://doi.org/10.1038/s41396-019-0541-6**

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