Testing a novel honey bee probiotic formulation

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Introduction Like all animals, honey bees rely on beneficial symbiotic microbes to digest food, to provide critical essential nutrients, and to crowd out pathogenic microbes. One group that appears to have particular importance to honey bees are thirteen different lactic acid bacteria (LAB) from the genera *Lactobacillus* and *Bifidobacterium*, which are located in the honey stomach and fresh food stores of honey bees worldwide. LAB protect nectar and pollen from spoilage by other microbes during honey and bee bread formation. LAB have also been shown to suppress the growth of colony pathogens such as *Paenibacillus larvae*, the causative agent of American Foulbrood. While LAB are essential to colony function and survival, these bacteria are vulnerable to local extinction because they do not form persistent spores. Honey bee colonies must constantly maintain and pass around live, active inoculates of these beneficial bacteria in the honey stomach and food stores to benefit from LAB activities.

Unfortunately, antimicrobial treatments that beekeepers use to kill colony pathogens can also affect beneficial colony microbes. Beekeepers routinely use antimicrobial treatments to suppress or eliminate pathogens responsible for significant colony diseases. In particular, beekeepers have successfully used the fungicide fumagillin against Nosema (*Nosema apis* and *Nosema ceranae*) and the antibiotics terramycin and tylosin against American Foulbrood (*Paenibacillus larvae*). However, the effects of these antimicrobial treatments on beneficial microbes and honey bee nutrition is relatively unknown. The purpose of our study is to determine the effects of three common colony antimicrobials (fumagillin, terramycin, and tylosin) on honey bee beneficial microbes, nutrition, and health. We also wish to determine if the negative effects of antimicrobials on beneficial microbes can be reduced by feeding back the missing beneficial microbes to the bees in supplemental probiotics.

Project objectives1) Determine the effects of antimicrobial treatments on beneficial Lactic Acid Bacteria (LAB)
2) Determine if feeding LAB back to bees as a probiotic can increase or restore LAB levels in bees
3) Determine the effects of antimicrobial treatments and probiotic LAB supplementation on honey bee mortality, nutrition, and health

We present preliminary results addressing part of Objective 3 (effects of antimicrobial treatments on bees) in caged bee experiments.

Figure 1. A) Lactic acid bacteria isolated from a honey stomach. Newly emerged adult bees (emerging from capped cells) lack colony microbes and must acquire the microbes from outside sources, including B) open nectar and bee bread (stored pollen) and by C) food sharing (trophallaxis) with older adult bees.

Methods *Caged Bee Studies.* Newly-emerged adult bees were collected from capped brood frames isolated in a deep box with newly-drawn frames containing fresh food stores (open nectar, honey, and bee bread). Because newly-emerged adult bees lack gut microbes, we exposed the new adults to three common sources of colony microbes in the first two days of their lives (Figures 1A-1C). Newly-emerged bees were given frames containing open nectar and bee bread (stored pollen) and allowed to be fed food by older adult bees by trophallaxis (food-sharing, Figure 1C) across a screen. Approximately 250 two-day old bees were then placed into each Plexiglas and screen cage (Figures 2A) and placed in an incubator room maintained at 30°C and 40-45% relative humidity. Bees were provisioned with 1:1 sugar syrup and DI water in bottles and pollen patty in rubber plugs (Figure 2B). Bee mortality and food and water consumption (sugar syrup, pollen, and water) were checked every two days. Food supplies were changed and 10 bees were sampled for later nutritional and microbe analysis every 8 days (8 days being considered a "week").

Bees were initially fed untreated sugar syrup, pollen patty, and water from day 0 to day 8. Antimicrobial treatments were administered by the method and dose recommended on the label on days 8 and 16 (Figure 2C). Terramycin and tylosin were applied as a powdered dusting as 10 mg in 1 g of powdered sugar. Fumagillin-b was introduced in the sugar syrup solution as 87 mg in 70 mL sugar syrup. Bees were exposed to fumagillin in the feeder for 8 days after initial treatment and to fumagillin-treated sugar syrup in comb cells for some time more. Seven cage replicates were performed per treatment.

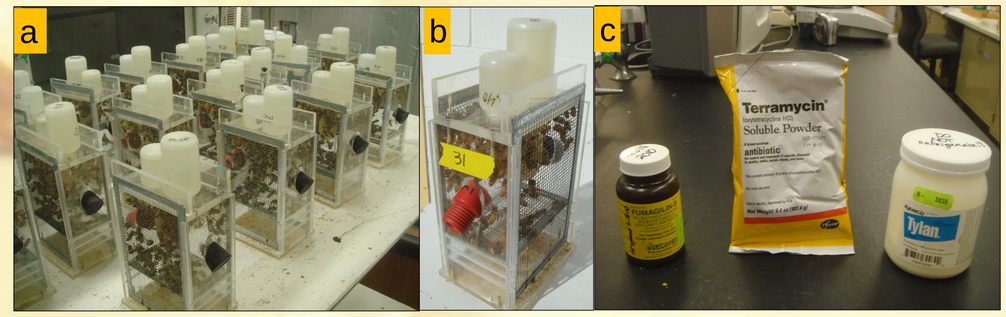

Figure 2. A) Plexiglas and screen cages used to house approximately 250 adult bees in the caged bee bioassays. B) Closer view of cages with sugar syrup, water, and pollen patty feeders visible. Bees readily made comb and filled the cells with sugar syrup stores. C) The three colony antimicrobials used in this experiment were fumigillin, terramycin, and tylosin (L-R). Each antimicrobial was applied at a dose and in a form according to label.

Results Honey bees experienced no significant difference in cumulative mortality until after the second treatment (Figure 3A). Bees fed fumagillin-b displayed higher mortality than control bees fed untreated sugar syrup. When sugar syrup consumption was examined, fumagillin-fed bees consumed significantly less sugar syrup than control bees fed untreated sugar syrup, a trend that appeared to increase as the treatment continued (Figure 3B). Honey bees are exposed to antimicrobials administered in food solutions longer than topical applications because treated sugar syrup is stored in cells until consumed.

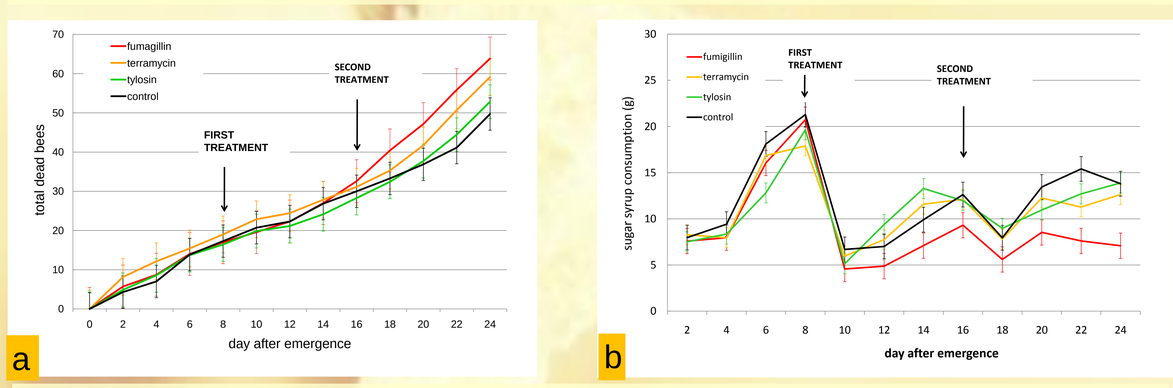

Figure 3. A) Average cumulative mortality and B) two-day sugar syrup consumption of honey bees in caged bee experiment. Antimicrobial treatments were applied twice at day 8 and day 16 either in a powdered sugar dusting (terramycin and tylosin) or in sugar syrup (fumagillin). The early peak in sugar consumption occurred during construction of the cage comb. Error bars are standard error .

Conclusions/Future Studies Fumagillin may affect colonies by acting as an antifeedant to honey bees. The experiment presented here is still ongoing (to 48 days) with microbial and chemical analysis still needed to detect the impact of antimicrobials on beneficial LAB microbes and the nutritive state of the bees. Further analysis of the microbial contents of sampled bees will show whether antimicrobial-treated bees lack LAB and other beneficial microbes. A second caged bee experiment is scheduled for early 2011 to determine whether the affects of antimicrobial treatments can be reversed with probiotic supplementation.

Day 0 to 8	no treatment
Day 8 to 16	antimicrobial treatment
Day 16 to 24	antimicrobial treatment
Day 24 to 32	no treatment (latent period)
Day 32 to 40	probiotic supplementation treatment
Day 40 to 48	no treatment (second latent period)

pictures courtesy of USDA and Vita Europe

The first and second latent periods will allow us to gauge the effects of the antimicrobial and probiotic treatments after the treatment period. We will also conduct similar set of controlled feeding studies on nucleus colonies ("nucs") isolated in flight arenas. Ultimately, we hope to reduce the negative impact of antimicrobials on honey bee colonies at vulnerable times of the year, such as emergence from late winter dearth during the almond pollination season.

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