Experiment One: Bee Gut Colonization

September 4 - 8, 2015
Goal: To prepare for the gut colonization experiments
Methods: LB media was inoculated with *E. coli* transformed with pSB1C3-RFP and incubated for a day. Approximately 10^6 or 10^7 cells were used to inoculate 10 mL of 50% sucrose-water solutions to feed to the bees. 10^3 and 10^2 dilutions of the liquid culture were plated on 25μg/mL chloramphenicol LB plates. A frame of emerging bees was removed from the hive the day before and kept in an incubator at 33°C with 80% relative humidity.
Results: The 10^3 and 10^2 dilution plates displayed much larger growth than expected indicating the bees were fed more bacteria than originally planned. The bees survived in the incubator and many new bees emerged the day after showing that the conditions were similar to the hive conditions.

September 9 - 13, 2015
Goal: To colonize *E. coli* in the bee gut.
Methods: There were three groups of bees in this study: one negative control group fed with 50% sucrose-water, another group fed with the 10^6 cells in 50% sucrose-water, and the last group fed with 10^7 cells in 50% sucrose-water. There were ten bees in each group, totalling the study to thirty emerging bees. All the bees were hand fed 5 μL each with their respective bacterial density and then kept in an incubator at 33°C with 80% relative humidity to mimic hive conditions. To analyze whether the bacteria colonized the bee gut, the alimentary canal was dissected from three live honeybees after two days and two live honeybees after four days. The alimentary canals were homogenized with a bead mill at 5500 rpm for twenty seconds. Homogenized mixtures, and 100X and 10,000X dilutions of the mixture, were plated onto 25 μg/mL chloramphenicol LB plates and colony forming units (cfu) were determined.
Results: There was no correlation with the number of dead bees and the bacterial concentration (Figure one). It was assumed that the bees died of natural causes or rough handling while hand feeding. There was no bacterial growth from the negative control group and one 10^6 group after two days. For the rest of the 10^6 and all three of the 10^7 bees, there was a lawn of red bacterial growth on the plates, indicating that our bacteria of interest successfully colonized the honeybee gut (Figure two). After four days, there was a limited number of living bees in both the 50% sucrose-water control and 10^6 cage. As a result, one dead bee from the control and two dead bees from the 10^6 cage were included in the dissection. The bees fed with 50% sucrose-water and one bee from the 10^6 cage had no bacterial colonies. The bees fed with 10^7 cells and one bee fed with 10^6 displayed red bacterial growth, similar to that seen after two days (Figure two). The colonized bee from the 10^6 group had 1×10^6 cfu. Two colonized bees from the 10^7 group had 4×10^6 cfu and 6×10^5 cfu, respectively. We continued experiments with a 10^7 cell count in the 50% sucrose-water solution, since it had the most success in colonizing in the bee gut.

September 13

September 11

50% sucrose-water solution

10^6 bacterial cells in 50% sucrose-water solution

10^7 bacterial cells in 50% sucrose-water solution

alive honeybees

dissected honeybees that have do not have *E. coli*

dead honeybees

dissected honeybees that have the *E. coli* with pSB1C3-RFP

dead honeybees dissected that have no *E. coli*

Figure One: Honeybee survival data for Experiment 1. The relative position from right to left indicates the time of bee death, from September 11th to 13th.

Figure Two: LB chloramphenicol plates with the different dilutions of the bee gut bacteria. The left image displays the bacteria from a honeybee fed with *E. coli* transformed with pSB1C3-RFP and the right image displays the bacteria from a honeybee fed with *E. coli.* The large white mass seen on the right plate is due to mould present in the bee gut.

Experiment Two: Resistance to Imidacloprid

Table One: The data collected from the twelve different bee feeding conditions. The *E. coli* constructs and the honeybee feeding solutions are listed.

		rfp	BBa_K1813073	BBa_K1813074	BBa_K1813075
50% sucrose-water solution	d2	28	26	25	28
	d3	28	20	23	28
	d4	27	19	18	28
15.7 μM of imidacloprid	d2	29	26	28	29
	d3	22	21	25	26
	d4	5	4	5	15
1.57 μM imidacloprid	d2	28	27	25	29
	d3	28	12	19	19
	d4	N/A	5	8	N/A

*notations; d2= day two of the experiment with the number of alive bees after only *E. coli* feedings, d3= day three of the experiment with the number of bees alive after the first day of imidacloprid feeding, d4= day four of the experiment with the number of bees alive after the second day of imidacloprid feeding, N/A= data not yet available because these experiments are still ongoing.

September 13 - 14, 2015
Goal: To prepare for the resistance to imidacloprid experiments.
Methods: *E. coli* transformed with BBa_K1813074, BBa_K1813073, and BBa_K1813075 were inoculated into liquid media and grown for a day. Approximately 10^7 cells were added to 10mL of 50% sucrose-water solutions to feed to the bees. Dilutions of the liquid culture were plated on 25μg/mL chloramphenicol LB plates. 15.7μM and 1.57μM concentrations were used for the imidacloprid sugar solutions. A frame of emerging bees was pulled the day before feeding and kept in an incubator at 33°C with 80% relative humidity.

September 14 - ongoing, 2015
Goal: To test whether the constructs gave the honeybees a greater resistance to imidacloprid.
Methods: As illustrated in table one, there were twelve different cages, each containing thirty newly emerged honeybees. Thirty newly emerged bees were fed *E. coli* transformed with pSB1C3-RFP for two days. Approximately 10^7 of the cells were put into the 50% sucrose-water solution as this number of cells displayed the best results in the gut colonization experiment.

After two days, they were fed 15.7μM imidacloprid in 50% sucrose-water solution, based on literature LD50 values. The literature LD50 value of imidacloprid is 40 ng per bee and assuming that a honeybee eats 10 uL daily, the concentration of imidacloprid for a LD50 after 2 days is 4 ug/mL and 0.4 ug/mL (1). Since imidacloprid is not water soluble, it was dissolved in DMSO. Following literature procedures, only 1% of DMSO was added to the sugar solutions due to the toxicity of DMSO (1).
The sugar solutions were fed to the bees through falcon tubes that contained a small hole at the tip. Since imidacloprid is light sensitive, these falcon tubes were wrapped in aluminum foil with only the tip exposed. The bees were kept in an incubator at 33°C with 80% relative humidity. The living and dead number of bees were monitored daily. The number of living bees in each cage were monitored and dead honeybees were removed daily. Not all of the honeybee trials started on the same day due to the availability of the honeybees. As illustrated in table one, the pSB1C3-RFP trials with 15.7 μM imidacloprid and 1.57 μM imidacloprid, as well as the BBa_K1813075 trial with 1.57 μM imidacloprid were started a day later. Therefore, data is not yet available because these experiments are still ongoing.
Results: Bee deaths after the initial bacterial colonization feedings were assumed to be due to natural causes because it was shown in experiment one that the *E. coli* has no toxic effect on the bees. 50% sucrose-water solution was fed to the bees as a control to ensure nothing in the solution was poisoning the bees. As a negative control, the honeybees were fed with the *E. coli* transformed with pSB1C3-RFP. When fed imidacloprid, more than half of the bees died after two days indicating that the LD50 concentration of imidacloprid for two days is lower than expected from the literature value. Moreover, the honeybees fed with *E. coli* transformed with BBa_K1813073 and BBa_K1813074 displayed the same amount of honeybee deaths from the imidacloprid indicating the constructs did not contribute any resistance to imidacloprid. The honeybees fed with *E. coli* transformed with BBa_K1813075 displayed a greater amount of survival than the negative control. 15 honeybees were still alive after 48 hours, compared to the 5 surviving bees with the negative control, suggesting that the construct could be aiding honeybee survival.

Experiment three: resistance to 6-chloronicotinic acid

Table Two: The data collected from nine different conditions. The *E. coli* constructs and the honeybee feeding solutions are listed.

		rfp	BBa_K1813001
50% sucrose-water solution	d2	28	26
	d3	28	20
	d4	27	15
maximum concentration of 6-CNA (50 µM)	d2	28	27
	d3	28	25
	d4	28	21
minimum concentration of 6-CNA (5 µM)	d2	26	4

*notations; d2= day two of the experiment with the number of alive bees after only *E. coli* feedings, d3= day three of the experiment with the number of bees alive after the first imidacloprid feeding, d4= day four of the experiment with the number of bees alive after second imidacloprid feeding

September 13 - 14, 2015
Goal: To prepare for the resistance to 6-CNA experiments.
Methods: Liquid media was inoculated with *E. coli* transformed with pSB1C3-RFP and BBa_K1813001 and cultured for a day. Approximately 10^7 cells were added to 10mL of 50% sucrose-water solutions to feed the bees. Dilutions of the liquid culture were plated on 25µg/mL chloramphenicol LB plates. For the 6-CNA 50% sucrose-water solutions, the maximum amount of 6-CNA was dissolved in DMSO, since 6-CNA is not soluble in water. Following previous procedures, only 1% of DMSO was added to the sugar solutions due to the toxicity of DMSO (1). This dilution resulted in a concentration of 50µM for the maximum amount of 6-CNA sucrose solutions and 5µM for the minimum amount of 6-CNA sucrose solutions. The maximum concentration of 6-CNA is below the tested lethal dosage in literature (1). A frame of emerging bees was removed from the hive a day before beginning the experiment and kept in an incubator at 33°C with 80% relative humidity.

September 14 - ongoing, 2015

Goal: To test whether the constructs gave the honeybees a greater resistance to imidacloprid
Methods: As illustrated in table 2, there were twelve different cages, each containing thirty newly emerged honeybees. Similar to experiment two, the bees were fed with *E. coli* transformed with different constructs for two days through a falcon tube and then fed with respective concentrations of imidacloprid in 50% sucrose-water solutions. The number of living bees in each cage were monitored daily.
Results: Unfortunately, before feeding the honeybees the lower concentration of 6-CNA sugar solution, more than half of the bees fed with the BBa_K1813001 died. However, the deaths are not correlated with the construct fed because other cages fed with the same construct displayed a normal death rate. The minimum amount of 6-CNA trial was not further investigated due to time constraints and the lack of available replacement honeybees. Moreover, the maximum concentration of 6-CNA did not cause any significant damage to honeybee survival. Therefore the function of the part at the minimum 6-CNA concentration could not be tested.

References:

1. Suchail, S., Guez, D., and Belzunces, L.P. (2001). *Environmental Toxicology and Chemistry*, 20, 11, 2482-2486.