# What are the Microbiome Profiles of Bees in a Nonproductive Hive?

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**Abstract**

Human agriculture depends on honeybee pollination. Our hypothesis was that dysfunctional hives may be the result of a change in honeybee microbiome. Honeybee samples from a dysfunctional hive were used to prepare DNA for sequencing to detect microbiome variations, using next-generation sequencing. Our results showed significant differences across three types of bees from a dysfunctional (non-honey producing) hive in Cutchogue, NY. Samples from the queen of the dysfunctional hive as well as a worker bee and drone were compared for their microbiome profiles. There were notable differences across the microbiome profiles of each sample type. There was more diversity overall noted in the queen bee samples, and the least diversity was seen in the drone samples. Analysis of these results, and comparison to existing literature, will be significant not only in understanding the dysfunction of this particular hive but also may lead to better understanding of underlying issues concerning honeybee decline worldwide. This increased understanding is critical to human health, given that honeybee pollination is essential for human agriculture.

**Introduction**

Honey bees are vital in all aspects of human agriculture, as well as pollination for most plant species (1). Recent studies involving honey bee microbiomes have begun to illustrate the link between healthy bees and successful honey production. One study suggests that honey bees may be deficient in "good" bacteria because of monoculture farming and other current agricultural practices (2). A recent study has outlined greater understanding of the role of specific gut bacteria in worker bees (3), and another study examined how social status affects the microbiome and fungal communities in honey bee guts (4). Our project focuses on the microbiomes of a group of bees collected from a nonproductive hive at an apiary located at Salt Air Farm in Cutchogue, New York. The queen, some female workers, and some male drones were donated by the owner of the apiary following the failure of the hive to produce honey during a season in which all other hives were productive. Interestingly, when the rest of the workers and drones were then transplanted to a different hive with a new queen, that hive was productive. Our aim is to use microbiome data from the bees to investigate whether the failure of the hive may have been associated with abnormal microbiomes in any of the bees.

**Materials and Methods**

**Sample Collection**: Bees were collected by Ms. Prudence Heston, owner of Salt Air Farm. Bees were euthanized by freezing and kept at -20°C until DNA processing. Both MoBio Kit protocols and conventional silicon binding protocols were used to prepare the DNA. For PCR, 2 ul of the DNA was combined with prepared primer mix and Taq polymerase. The PCR temperatures and number of cycles optimum for producing the amplified DNA were entered into a program that ran the cycler, as instructed by our Open Lab leaders. The primer used was designed to amplify a 16S ribosomal DNA region in the samples. A small portion of the amplified DNA was checked for quality by separating it via agarose gel electrophoresis. Samples of high enough quality were sent to a laboratory for Illumina next-generation sequencing.

**Results**

The total bacterial species count for all samples was 436,025. The total families represented were 496. The counts/sample were 0.188. For the queen bee samples, we observed a number of differences in represented species compared to the worker bee and drone bee (Fig's 1,2). We observed Acidobacteria, Bactereoidetes, and Cyanobacteria groups in the queen bee, while these were much reduced or almost absent in the worker and drone bees (Fig. 2). The queen bee samples also exhibited greater diversity of species represented than the other groups (Fig. 1). All bee samples contained Proteobacteria and Firmicutes, but these levels were lower in the drone bees and higher in the queen bee and worker bee samples (Fig. 1). The worker bee samples had higher variability than either the queen bee or drone samples. Worker bee samples had high levels of Proteobacteria and Firmicutes (Fig. 1). Drone bee samples had the highest levels of Firmicutes bacteria, and reduced diversity overall (Fig. 1). The queen bee showed low levels of Lactobacillus bacteria compared to the other bees (Fig's 1,2). Pathogenic bacterial genuses were detected in the workers and drones, but not in the queen bee (Fig. 2).

**References**

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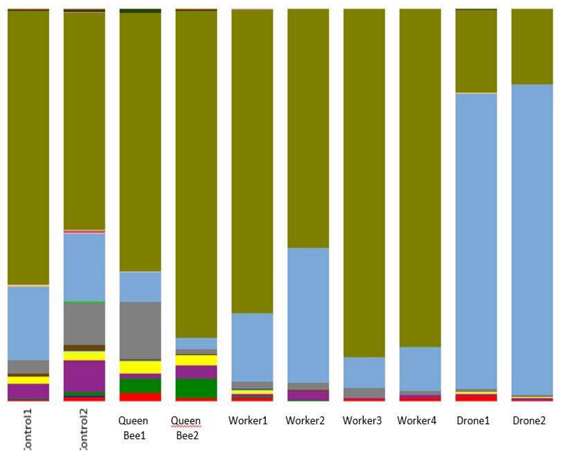



**Figure 1**: This figure shows the taxa plot generated for the honeybee microbiome samples in our study. Note the high levels of Firmicutes (blue) in drones and Proteobacteria (green) in workers and queen bee. Also note the increased diversity of taxa in queen bee samples.

| Legend | Taxonomy | Total % | II1 % | II2 % | II3 % | II4 % | II5 % | II6 % | II7 % | II8 % | NCBN % | NCSP % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unassigned;Other | 0.5% | 1.2% | 0.6% | 0.8% | 0.1% | 0.3% | 0.5% | 0.2% | 0.1% | 0.3% | 1.1% |
| | k__Archaea;p__[Parvarchaeota] | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% |
| | k__Bacteria;p__AD3 | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| | k__Bacteria;p__Acidobacteria | 1.1% | 3.7% | 5.2% | 0.1% | 0.1% | 0.0% | 0.0% | 0.1% | 0.0% | 0.3% | 1.0% |
| | k__Bacteria;p__Actinobacteria | 2.2% | 1.4% | 3.3% | 0.9% | 2.3% | 0.3% | 0.6% | 0.5% | 0.5% | 3.8% | 8.1% |
| | k__Bacteria;p__Bacteroidetes | 1.1% | 3.1% | 2.7% | 0.9% | 0.2% | 0.0% | 0.0% | 0.4% | 0.2% | 1.8% | 2.0% |
| | k__Bacteria;p__Calditrhix | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% |
| | k__Bacteria;p__Chlamydiae | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| | k__Bacteria;p__Chlorobi | 0.0% | 0.1% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| | k__Bacteria;p__Chloroflexi | 0.3% | 0.5% | 0.1% | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.7% | 1.6% |
| | k__Bacteria;p__Cyanobacteria | 3.9% | 14.5% | 1.3% | 1.9% | 1.8% | 2.5% | 1.1% | 0.7% | 0.4% | 3.5% | 11.1% |
| | k__Bacteria;p__Elusimicrobia | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% |
| | k__Bacteria;p__Fibrobacteres | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| | k__Bacteria;p__Firmicutes | 27.2% | 7.5% | 2.9% | 17.3% | 34.5% | 7.9% | 11.3% | 75.2% | 79.2% | 18.7% | 17.0% |
| | k__Bacteria;p__Fusobacteria | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.3% | 0.0% | 0.4% | 0.3% |
| | k__Bacteria;p__GN02 | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| | k__Bacteria;p__GN04 | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| | k__Bacteria;p__Gemmatimonadetes | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% |
| | k__Bacteria;p__Hyd24-12 | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| | k__Bacteria;p__Nitrospirae | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% |
| | k__Bacteria;p__OP9 | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% |
| | k__Bacteria;p__Planctomycetes | 0.1% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.4% |
| | k__Bacteria;p__Proteobacteria | 62.8% | 66.0% | 83.0% | 77.3% | 60.9% | 88.9% | 86.4% | 20.9% | 19.2% | 69.8% | 55.3% |
| | k__Bacteria;p__SAR406 | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| | k__Bacteria;p__Spirochaetes | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.2% |
| | k__Bacteria;p__TM6 | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| | k__Bacteria;p__TM7 | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| | k__Bacteria;p__Tenericutes | 0.5% | 2.0% | 0.5% | 0.0% | 0.0% | 0.0% | 0.0% | 1.4% | 0.3% | 0.3% | 0.4% |
| | k__Bacteria;p__Verrucomicrobia | 0.1% | 0.0% | 0.2% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 0.2% |
| | k__Bacteria;p__WS3 | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% |

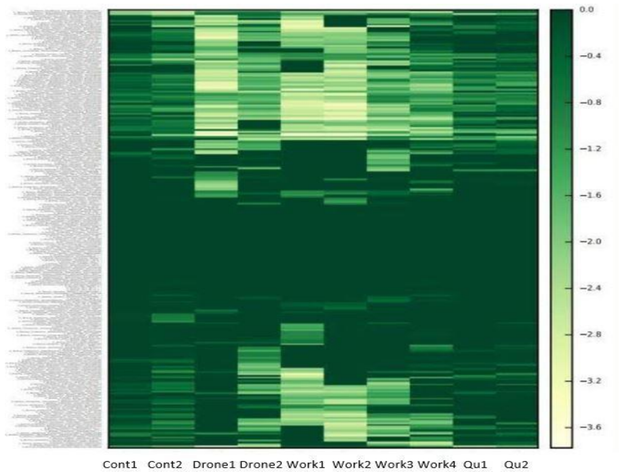
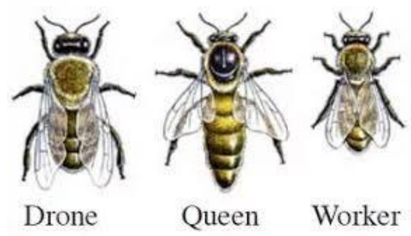



**Figure 2**: The figure to the left shows the Level-5 alpha diversity plot for the honeybee samples in our study. Note the low levels of Lactobacillus in the queen bee samples, as well as the presence of possibly pathogenic strains of Serratia genus in worker and drone bees.




**Discussion**

A normal honeybee hive with a functional queen is expected to make at least some honey. However, the hive that we studied was normal in all outward respects, but it produced no honey. Other hives in the same apiary produced honey normally that season. When the worker and drone bees from this hive were transferred to a new hive with a new queen, honey production was normal. Some recent studies have indicated that low-level hive stress caused by predators, the timing of flower blooming, and even exposure to electromagnetic waves from cell phones can lead to decreased honey production (5). The data indicates that the workers and the drones have a high level of Firmicutes and Proteobacteria. This agrees with other published reports (4). Typically, the queen's gut is comprised of a high level of Lactobacillus (4). However, the queen in our study showed low Lactobacillus levels (Fig's 1,2) which may be associated with poor queen health. The pathogen Serratia genus was present in the workers and the drones but absent in the queen. This may indicate a problem with overall hive health. Even though recent studies indicate a low bacterial diversity in the queen bee versus the workers (3), our data demonstrate the highest diversity in the queen bee (Fig's 1,2). It is interesting that the queen had the highest variability as they do not leave the hive (3).