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Honey Bee Microbiome



Malcolm T. Sanford

The honey bee gut microbiome is fast becoming a focus in terms of honey bee health. A general description of this important area can be found [here](#): At Apimonida 46, the following abstracts were presented, providing further insight into study on this significant arena.

The honey bee microbiome in health and disease

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The honey bee gut microbiota is integral to host physiology, serving a critical role in host signaling and protection. It is intimately associated with host metabolism and longevity, and functions in host disease prevention, parasite susceptibility, detoxification, immunity and oxidative balance. Constituent or 'core' gut microbiota is hypothesized to mediate susceptibility to disease and ultimately even shape the ecology and evolution of disease states. The stability of the worker microbiota suggests a healthy ecosystem, but like all well-structured communities, the microbiota is beset with cryptic cheaters and opportunists.

A variety of biotic and abiotic factors can alter gut microbial balance, leading to decreased host function, decreased longevity, and disease progression. Environmental perturbation or natural variation in the core gut or hive microbiota may contribute to, or even represent the point source for many opportunistic and cryptic disease states associated with honey bee colony decline. Further insight into microbiota structure and function exploring host gene expression, queen and worker aging, floral contributions, larval development, and disease prevalence will further define the critical role of microbiota in honey bee health. Honey bee gut microbiota regulate the expression of host cytochrome P450 enzymes and contribute to host detoxification ability.

Honey bee gut microbiota regulate the expression of host cytochrome P450 enzymes and contribute to host detoxification ability

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Gut microbiota is known to affect host physiology and a growing number of researches have demonstrated a close relation between insect gut microbiota and insecticide resistance. However, the contribution of honey bee gut microbiota to host detoxication ability has yet to be investigated, especially the effect to host endogenous detoxification pathways.

In order to address this question, we compared the expression of CYP6AS1, CYP6AS3, CYP6AS4, CYP6AS10, CYP9Q1, CYP9Q2 and CYP9Q3 in midgut and hindgut between gut microbiota deficient (GD) workers and conventional gut community (CV) workers at mRNA level. The mortality rates of GD and CV workers treated with sublethal dosage thiacloprid or fluvalinate were also measured. Our results show that gut microbiota could promote the expression of CYP6AS1, CYP6AS3, CYP6AS4, CYP6AS10, CYP9Q2 and CYP9Q3 in midgut, and the survival rate of GD workers are significantly lower than CV workers when treated with pesticide.

In addition, we investigated the impact of gut dysbiosis caused by antibiotic treatment on host detoxification ability. Comparisons between antibiotic treated workers and normal workers indicated that gut dysbiosis leads to attenuated expression of cytochrome P450 enzymes (P450s) and reduced survival rate. Taken together, our findings demonstrate a direct relationship between the presence of symbiotic bacteria and the expression of P450s, suggest that honey bee gut symbiont could contribute to bee health through modification of host xenobiotics detoxification pathways and reveal a potential negative impact of antibiotics to honey bee health.

The BeeBiome Data Portal: a web-based resource to facilitate bee microbiome studies by making data findable, accessible and reusable

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Bees are home to a diverse set of microorganisms ranging from protozoa to bacteria to viruses. They can collectively be referred to as the bee microbiome, include beneficial, commensal, and pathogenic species, and play important roles for bee health and disease. With the recent advent of sequencing technologies, scientists have been able to reveal the outstanding genetic and functional diversity of microbial life associated with bees.

While these datasets are publicly available from different sources, they have never been catalogued or made accessible from a single resource. Most of these datasets are deposited in different formats and are associated with different meta-data, making it difficult to systematically search for sequence resources and hindering cross-study analysis that would push the field forward.

We believe that the scientific community would profit from a web-based platform to share publicly available datasets associated with bee microbiome research. With the financial support of the Eva Crane Trust, we will build the web-based BeeBiome Data Portal.

The main aims of this resource will be to (i) catalogue all open-access bee microbiome datasets (mainly sequence data), (ii) enable the mining and integration of these datasets for cross-study analyses, (iii) provide common formats and procedures to deposit novel datasets in repositories like for example the European Nucleotide Archive ENA, and (iv) propose standard methods and terminology. This portal will facilitate collaborative efforts with the ultimate goal to understand the impact of associated microbes and viruses on bees, including managed species as well as wild pollinators. Moreover, the BeeBiome Data Portal will serve as a means to reach out to a diverse audience, including beekeepers, the general public, policy-makers and the private sector to inform about bees and their microbes. Information on the BeeBiome consortium can be found on [here](#):

Impact of nutrition on honey bee immunity, gut microbiota and *Nosema ceranae* infection

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Apis mellifera colony loss episodes have been reported worldwide and have been associated with parasites and pathogens, pesticides and nutritional stress. Nutritional problems are associated to the increase in monoculture areas and the reduction of pollen availability and/or diversity for bees, among others. These conditions may favor the infection by different pathogens, as the microsporidia *Nosema* spp. In this study, we examined whether the diet influences bee immunity, gut microbiota and the development of *Nosema* spp., under laboratory conditions. Newly emerged bees were caged and fed ad libitum with two different diets: *Eucalyptus grandis* (monofloral bee bread) or a mix of at least 18 botanic families (polyfloral bee bread). The experiment was carried out by triplicate and two independent trials were performed.

Bees fed on monofloral and polyfloral diets survived almost 60 days under laboratory conditions. No differences between groups were observed. However, at ten days of feeding, *E. grandis* bee bread decreased the expression level of vitellogenin and genes involved in social and individual immunity (glucose oxidase, hymenoptaecin, lisozym) compared to polyfloral one. The diet also influenced the abundance of some members of the gut microbiota, as bees fed on *E. grandis* bee bread showed a lower abundance of Lactid Acid Bacteria (*Lactobacillus* spp. or *Bifidobacterium* spp.) and higher abundance of *Bartonella apis*, compared to bees feed on polyfloral diet.

In the second trial, bees subjected to different diets were infected with *Nosema ceranae* spores, and we found that *E. grandis* pollen favored the multiplication of the microsporidia. These results suggest that nutrition influences social and individual immunity, the abundance of lactic acid bacteria and the development of *Nosema* spp., contributing to the understanding of the influence of agriculture intensification on colony health.

Engineered symbionts immunize honey bees against colony collapse pathogens

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Honey bees support global agriculture, but also suffer numerous health threats and high hive mortality each year. Pathogens, climate change, and hive management practices all likely contribute to these hive deaths. Deformed wing virus (DWV) and parasitic *Varroa destructor* mites interact to be among the most significant factors causing these hive losses, and they remain difficult to treat.

Here I describe a new approach: engineered symbiotic bacteria from the bee gut. The bee gut microbiome is a simple community of bacteria that plays an important role in host health and nutrition. In this work we have engineered the natural bee gut bacteria *Snodgrassella alvi* as a platform to express compounds that support bee health while *S. alvi* lives inside bees. *S. alvi* producing double-stranded RNA in situ persistently activate RNA-interference, an important component of the honey bee antiviral immune response. We demonstrate these engineered bacteria can be used to control bee gene expression and behavior. Further, we show these engineered symbionts can lower replication of deformed wing virus, thereby improving bee survival, and even kill *Varroa* mites parasitizing bees. I will discuss how this work will support reverse genetic studies in honey bees, and may one day be used to support the health of bees used in agriculture.

Clothianidin induces microbial gut dysbiosis to honey bees, *Apis mellifera*

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Honey bees are facing a wealth of synergistically interacting stress factors affecting their lifespan, health, and productivity. Clothianidin acts on the central nervous system of insects, specifically targeting the nicotinic acetylcholine receptors inducing behavioral, memory and immunity alterations.

It is now well documented that functions associated with immune response and behavior are controlled by the intestinal microbiota in insects. Given that clothianidin is persistent in the environment, there is an urgent need to develop alternative and sustainable strategies to mitigate its toxic effects on bee health. The first objective was to identify the host microbiota functional interactions impacted by clothianidin.

In vivo studies have been conducted to measure impact of clothianidin exposure on bee survival, behavior, syrup consumption and eubiosis/dysbiosis condition of the gut microbiota. Three concentrations (0.1; 1 and 10 ppb) have been tested. Strikingly, the lowest concentration (0.1 ppb) exerted the most negative impact on bees, showing the highest mortality rate compared to 1 and 10 ppb experimental groups. Moreover, phenotypes changes were recorded in all exposed groups and differences in syrup consumption were observed across experimental groups. Then, transcriptomic analyses were performed to identify which bee midgut microbiota strains were impacted in terms of functional activity by sublethal concentration of clothianidin.

The second objective was to select endogenous probiotic candidates being able to degrade clothianidin into innocuous metabolites. Endogenous microbial communities from host gut microbiota were observed to improve host's resistance against bacterial pathogens and intracellular parasites, both in mammals and insect models. Our pesticide quantification reported a complete degradation of clothianidin in contact with some probiotic candidates.

The third objective was to measure under in vivo conditions effect of select endogenous probiotic candidates administrated to bees exposed to clothianidin to test if the selected strains will help to restore the impaired functions. Our results highlighted promising endogenous bacterial probiotic candidates to develop a probiotic formulation mitigating the negative impact of neonicotinoid exposure on bee colonies.

More specifically, administration of one of our probiotics significantly improved the survival rate of bees exposed to clothianidin compared to control bees (i.e. supplemented with sugar (1:1)).

Sensitivity of honeybee gut microorganisms to antimicrobial substances present in beekeeping

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The honey bee beneficial microbiota represents a key variable in honey bee colony health. Antimicrobial and chemical treatments wipe out microbial communities and can cause dysbiosis in bees. Lactic acid bacteria, including *Lactobacillus*, are most abundant in honey bee hind gut where they mitigate toxin exposure, nutrient absorption, and stimulate innate immune responses. Synergistic or antagonistic interactions between microbial and antimicrobial factors present in beekeeping warrants greater exploration. Sensitivity of honeybee gut lactic acid bacteria to antimicrobial substances in bee environment: antibiotics tetracycline, tylosin, lincomycin, fumagillin, pesticides glyphosate and fipronil, and plant extracts, is addressed in this work.

An array of scholarly articles on the honey bee microbiome can be found on [World Wide Web](#).