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The Role of Honeybee Gut and Honey Microbiome in Sustainable Bee and Human Health

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Abstract

Honeybees are significant to agriculture and global ecosystems due to their role as key pollinators. The honeybee's gut microbiota is necessary for maintaining their health, providing nutrition and protection against pathogens. The objective is to develop effective strategies to promote the well-being of honeybee species. This paper comprehensively reviews current literature utilizing advanced genomic techniques to characterize bee gut microbial diversity. It examines culture-independent and culture-dependent methods to classify microorganisms inhabiting the bee gut. Their symbiotic relationships and contributions to critical bee physiological processes are also explored. The gut microbiome holds an indispensable role in bee health by regulating immune function, nutrient absorption and defense against pathogens. Specific bacterial taxa like *Lactobacillus, Bifidobacterium, Snodgrassella, Apibacter, Frischella* and *Gilliamella* exhibit probiotic, antimicrobial and symbiotic properties that safeguard bee gut homeostasis. The unique microbial composition of honey, influenced by the bee gut microbiota and environment, holds potential prebiotic and probiotic benefits for human health. Maintaining a balanced bee gut microbiome through microbiome engineering could strengthen bee resistance to diseases, thereby addressing worldwide bee population declines. Further unravelling the health impacts of honey microbes could uncover novel therapeutic applications and advance sustainable apiculture and human nutrition initiatives.

Keywords: Honeybee, Microbiome, Probiotics, Symbiosis

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INTRODUCTION

Honeybees are social insects from the Hymenoptera order and are classified under the genus *Apis*, are renowned for their remarkable ability to produce and store honey and other beneficial substances. The microbiome is crucial for maintaining health and well-being of both bees and humans, comprising all microorganisms inhabiting a specific area, including bacteria, archaea, viruses, fungi and protozoans.[1,2] It has recently been demonstrated that honeybees have a unique and species-specific microbiome, which may offer protection from parasites and pathogens.[3,4] Extensive studies have shown the pivotal role of the gut microbiome in bees, influencing critical functions such as digestion, immune system development, and antimicrobial protection.[5] Similarly, in humans, gut microbes perform crucial functions in managing immune responses and vital metabolic processes.[6,7] Genetic analysis of bee gut microbiomes provides valuable insights into their composition, diversity, and potential functions, utilizing sophisticated metagenomic techniques.[8,9] This approach enables scientists to identify specific bacterial taxa crucial for maintaining bee health.

The gut microbiome is indispensable for bee health, contributing significantly to carbohydrate digestion, infection defense, and overall immune system enhancement.[10] Moreover, factors such as nutrient content and secondary chemicals present in pollen influence the bee gut microbiome, potentially impacting bee survival and well-being.[11] In human health, the gut microbiome serves a pivotal role in metabolizing essential nutrients and maintaining intestinal barrier function.[12] Dysbiosis, a disruption in the balance of the gut microbiome, poses risks to both human and bee health.[13] In bees, dysbiosis is induced by American and European Foulbrood which lead to impaired immune function, reduced nutrient absorption, and heightened susceptibility to illnesses.[14] Similarly, dysbiosis in humans is associated with health issues such as Inflammatory Bowel Disease, Irritable Bowel Syndrome, Metabolic Syndrome and Obesity, Type 2 Diabetes and clostridium difficile Infection.[15-19]

Understanding the intricate interplay between the honeybee gut and honey microbiome and their impact on sustainable bee and human health is necessary for devising effective strategies to promote the well-being of both species.

Strategies for the study of honeybee gut microbiome

The majority of current methods for classifying microorganisms in honeybee gut are genomic in nature. This means that they depend on identifying a certain DNA sequence to a particular microbial group at different levels of resolution, such as species, phylum, or even strain.[20] The amplicon-based technique is characterized by standard protocols that begin with complete genomic DNA isolation sourced from a microbial ecological sample and continue with the sequencing of the whole genome, specific genes, or genomic regions. The two types of genomic methodologies are culture-dependent and culture-independent, depending on where the microbial sample came from Figure 1.[21]

Culture dependent method

The term “culture-dependent methods” describes microbial sampling techniques wherein gut homogenates undergo particular culture conditions prior to genomic analysis. The primary approaches for establishing the taxonomy of gut bacteria in honeybees before the widespread adoption of Sanger sequencing and next-generation sequencing were culture-based techniques. This led to limited identification because certain species were preferred under certain circumstances and those hard to culture in the laboratory were not represented.[21,22] The honeybee core microbiota species may now be characterized genetically, morphologically, and biochemically, including distinct strains of the species, through culture-based methods.[23] Through culture dependent method, the gut bacterial flora can also be identified by culturing in the Luria Bertani (LB) agar for Gram-negative bacteria, Nutrient agar (NA), Brain Heart Infusion (BHI) agar, a general-purpose non-selective media suitable for a broad range of organisms. *Gluconobacter* (GB) agar media is more selective for acetic acid bacteria like *Gluconobacter*, and De Man, Rogosa and Sharpe (MRS) agar is highly selective for lactic acid bacteria, particularly *Lactobacillus* species.[24]

Culture-independent method

Culture-independent methods analyze DNA and RNA of microorganisms directly obtained from samples, bypassing the need for intermediary laboratory culture processes required by culture-dependent methods. This mitigates species-related biases that can be cultured. The utilization of amplicon sequencing for a universally conserved marker gene, such as the 16S ribosomal RNA (rRNA), represents the predominant methodology for delineating the microbial taxonomy within a specified ecological niche. Total nucleic acid sequencing, or metagenomics, can yield more detailed information, including information about the metabolic and functional capacities of the microbiota. Through the application of culture-independent methodologies, it has become achievable to determine both the structural composition and the relative prevalence of the microbiome.[25,26]

For the purpose of taxonomic identification of honeybee microbiota species using sequencing, there are two primary methods available: marker gene sequencing based on amplicon analysis and shotgun metagenomics.[3,27] The initial phase involves the application of high-throughput sequencing techniques on metagenomic samples, which is traditionally followed by the alignment of sequencing reads to a recognized reference database abiotic stressor. The utilization of the amplicon-based marker gene methodology is prevalently observed in both culture-dependent and culture-independent techniques, attributable to its comparative simplicity. This method primarily involves amplification of gene through PCR and sequencing of specific fungal and bacterial markers. In bacterial studies focusing on taxonomy and phylogeny, amplifying the 16S rRNA gene is the prevailing choice. This gene is favored due to its widespread presence across bacterial species, and the entirety of its sequence contains a sufficient degree of variation to differentiate among the majority of bacterial species.[28-30] The 16S ribosomal RNA gene encompasses nine distinct "hypervariable regions" (V1-V9), which are bordered by conserved sequences. This configuration facilitates the enhancement of amplification through the utilization of universal primers.[21,31]

The study of identifying microbial eukaryotes in the bee gut has not been extensively explored. Amplicon sequencing, utilizing the 18S rRNA gene and Internal Transcribed Spacer (ITS) regions, is a powerful approach to identify and classify eukaryotic communities in environmental samples. The 18S rRNA gene is highly conserved across eukaryotes, making it an ideal target for universal primers, while the ITS regions provide higher taxonomic resolution for fungi and other microeukaryotes.

In this method, specific primers are designed to amplify regions of the 18S gene (often the V4-V6 regions) or ITS, allowing the sequencing of eukaryotic DNA. High-throughput sequencing platforms, such as Illumina, are frequently used due to their ability to process large amounts of data efficiently. This approach offers insights into the diversity and relative abundance of eukaryotic taxa within a given community. Recent studies have optimized primer sets and protocols to minimize bias and improve taxonomic resolution when using the 18S rRNA and ITS regions. For instance, a study highlighted the development of degenerate primers that capture a broad range of eukaryotic taxa, which were then applied using Illumina sequencing for diverse environmental samples. This method is widely used in microbial ecology and environmental monitoring to assess eukaryotic diversity in ecosystems ranging from marine environments to terrestrial soils. This approach, while efficient for taxonomic profiling, is often complemented by metagenomics to gain insights into the functional potential of the microbial community.[3,32] ITS (Internal Transcribed Spacer) approach have revealed a diverse fungal microbiome associated with honeycomb and the gut of different honeybee species, which play distinct ecological roles within the hive. In honeybee guts, *Ascomycota* and *Basidiomycota* dominate, with species such as *Kodamaea*, *Zygosaccharomyces*, and *Wallemia* showing higher abundance. In contrast, the honeycomb exhibits a different fungal profile, featuring *Bipolaris*, *Metschnikowia*, *Trichoderma*, and *Starmerella*, suggesting environmental adaptation of fungi within the hive ecosystem. The fungal diversity plays roles in nutrient cycling, pathogen defense, and symbiosis with the bees themselves.

Interestingly, some fungi in the honeybee gut and honeycomb, such as *Aspergillus flavus* and *Fusarium solani*, are linked to both honeybee health and environmental interactions, underscoring the intricate relationship between the fungal microbiome and the bees' physiology. Further insights have also identified bacterial-fungal symbioses, such as *Bacillus velezensis*, present in fungi from honeybee-associated samples. This highlights the complexity of microbial interactions within the hive ecosystem, contributing to overall hive health and productivity.[33,34] In conclusion, the authors noted that ITS regions provided adequate resolution for identifying eukaryotic elements at the species level with high precision.[35,36]

Exploring bacterial diversity within the honeybee gut is a captivating field of research.[8,37] Recent investigations indicate that gut microbial community of honeybees comprises a diverse array of bacteria, yeasts, and fungi.[38] These microorganisms engage in intricate interactions with the bee host and among themselves, contributing to the bee's developmental processes.[39] Exposure to pesticides or other environmental stressors has the potential to disrupt the bee gut microbiome, detrimentally affecting bee health and ultimately causing declines in honeybee populations.[37] The gut bacteria within honeybees play a crucial role in breaking down complex carbohydrates, such as pollen and nectar into simpler forms, immune system modulation, defense against pathogens, behavioral regulation and protection against environmental stress.[40-43] Figure 2 illustrates the different regions of the honeybee gut and their associated bacterial communities.[44]

The well-being and metabolic functions of honeybees rely heavily on their gut microbiome. A substantial part, around 95%, in the gut microbial community is comprised of a select group of phylotypes, including *Actinobacteria* (such as *Bombiscardovia* and *Bifidobacterium*), *Bacteroidetes* (like *Apibacter*), *Gammaproteobacteria* (including *Gilliamella* and *Frischella*), *Firmicutes* (such as *Bombilactobacillus, Apilactobacillus,* and *Lactobacillus*), *Betaproteobacteria* (like *Snodgrassella*), and *Alphaproteobacteria* (including *Commensalibacter, Bartonella,* and *Bombella*). Research indicates that certain bacterial taxa, such as *Lactobacillus, Bombilactobacillus, Snodgrassella, Bifidobacterium,* and *Gilliamella*, considered core microbiomes of honeybees, play vital roles such as defense against pathogens and carbohydrate digestion, highlighting a symbiotic relationship (Table 1).[10]

In Homeostatic signalling, Short Chain Fatty Acids (SCFAs) are a key energy source for

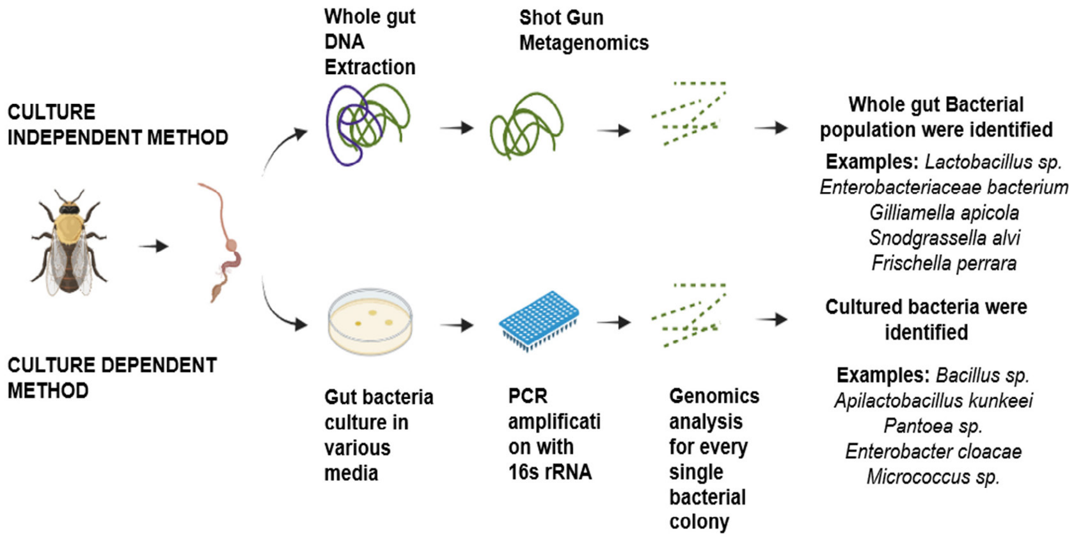

Figure 1. Culture-independent method and culture-dependent for study of honey bee gut microbiome

honeybees, especially for cells lining the gut. These acids can regulate metabolic pathways and energy balance, crucial for maintaining gut health. SCFAs have been shown to enhance immune responses by modulating signalling pathways involved in immune cell activity. In honeybees, they help regulate inflammation and immune homeostasis. SCFAs promote the integrity of the gut epithelium by enhancing tight junction protein expression, helping protect against harmful pathogens and toxins.[45,46]

In defense Mechanisms, SCFAs lower gut pH, creating an inhospitable environment for pathogenic bacteria, thereby preventing infections. This acidic environment favors beneficial microbes and inhibits harmful ones. SCFAs can activate pathways like the NF-kB signaling, leading to enhanced production of antimicrobial peptides

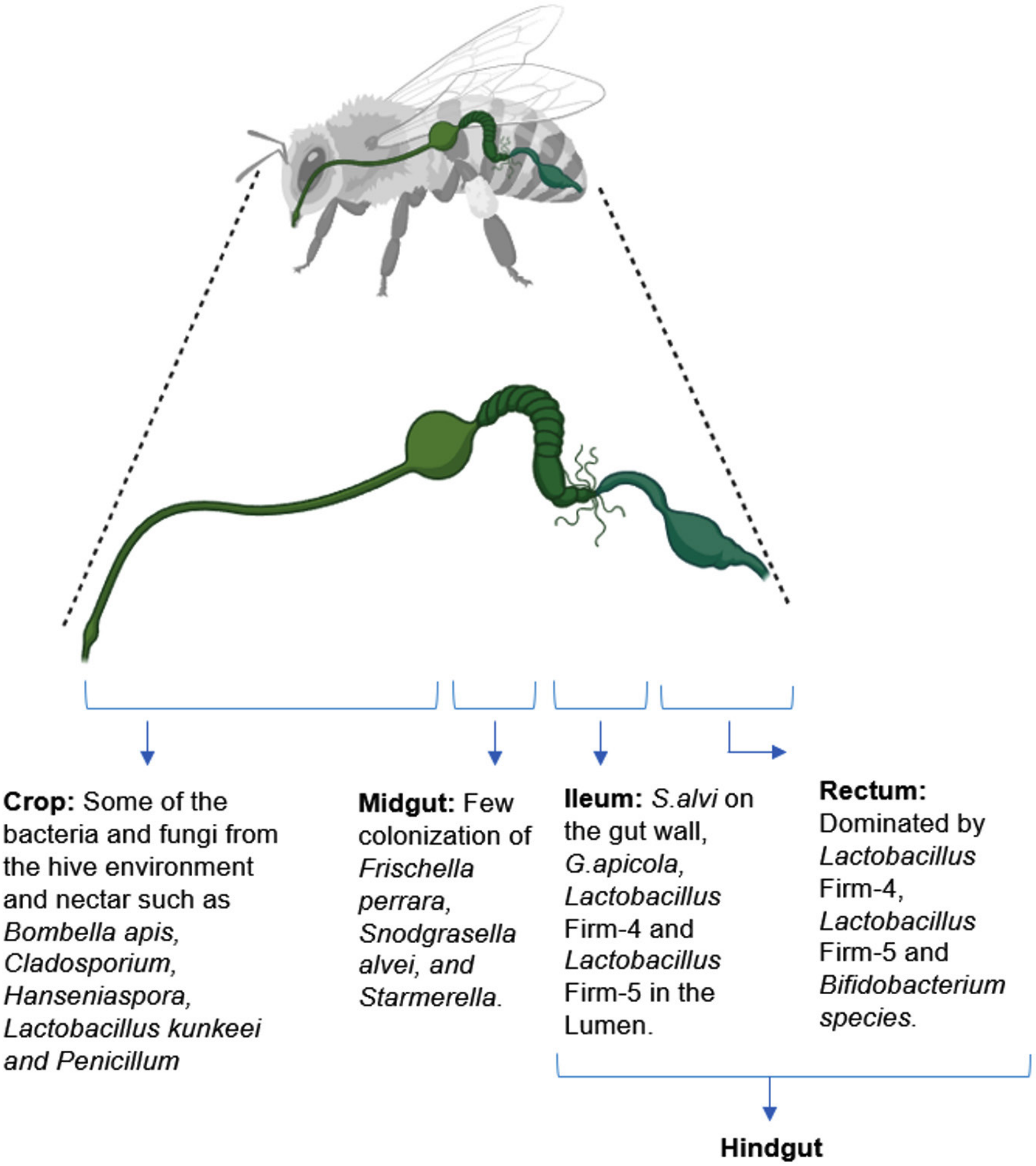

Figure 2. Bacterial Community in Honeybee gut[26]

and immune effectors, which play a defensive role against gut pathogens.[43,47]

Through 16S ribosomal RNA gene sequencing, it has been proven that the bee's gut microbiome incorporates only 8-10 phylotypes, with the majority belonging to the phyla *Proteobacteria, Firmicutes,* and *Actinobacteria* which together, they represent more than 95% of all 16S rRNA sequences.[48] Various samples have exhibited the presence of well-known pathogens such as *Nosema* sp*., Serratia marcescens, Ascosphaera apis* and *Paenibacillus* larvae affecting honeybees.[28]

Impact of honeybee gut microbiome on bee health

The gut microbiome in honeybees serves a role similar to that in mammals, contributing to host physiology such as metabolism and immunological functions.[41,56] In honeybees, the gut microbiome is comprised of a few bacterial phylotypes spread throughout the hindgut, midgut, and foregut.[48] Despite insects having fewer commensal bacteria in their guts compared to mammals, these bacteria can still exert a significant influence on insect health. Understanding the gut microbiome's role in bee health is essential, as it can greatly reduce the prevalence of bee diseases.

A metagenomic investigation uncovered the capability of *Gilliamella apicola* to degrade pectin, an important constituent of the inner pollen wall known as intine.[57] Current studies employing untargeted metabolomics have characterized the metabolic processes within the honeybee gut microbiome. Specifically, the gut region primarily inhabited by *G. apicola* is where galacturonate, the primary product of pectin breakdown, accumulates.[57] The honeybee gut microbiome utilizes a diverse range of substrates derived from pollen. These results indicate that the bee gut microbiome has evolved to utilize food components that the host does not metabolize and absorb, resulting in their accumulation in the hindgut.[47] The microbiome-induced metabolic changes alter the physical and chemical characteristics of the gut environment. In the presence of the microbiome, both pH and redox potential decrease along the bee gut, with a specific reduction in oxygen levels observed in the ileum.[58] Understanding how the microbiome influences specific host phenotypes is crucial, although currently, the transmission of metabolites to the host remains unknown. In mammals, metabolites like butyrate move from the intestines to various host tissues, impacting colon cell function. Similar functions have been proposed in bees, though they require validation.

In recent research, the honeybee gut microbiome has been shown to play a crucial role in bee health through metabolic processes that enhance nutrient absorption and protection from pathogens. For example, *Snodgrassella alvi*, a core honeybee gut symbiont, helps colonize the bee gut using host-derived organic acids, such as citrate and glycerate, while modulating tryptophan metabolism to produce protective compounds like anthranilate. This symbiotic relationship supports the immune system, gut health, and overall resilience of bees to environmental stresses.[59]

Additionally, honeybee gut bacteria, including *Gilliamella apicola* and *Snodgrassella alvi*, form biofilms that offer a defense against pathogens, and these bacteria have also demonstrated resistance to antibiotics commonly used in apiculture, such as tetracycline, highlighting their role in antimicrobial defense. These findings underscore the importance of the honeybee gut microbiome in maintaining colony health, resilience to pathogens, and possible environmental impacts through horizontal gene transfer of antibiotic-resistant genes.[60]

Lactobacillus species in the honeybee gut influence behaviors related to learning and memory by modulating tryptophan metabolism. Specifically, *Lactobacillus* Firm-5 converts tryptophan into indole derivatives, activating the host aryl hydrocarbon receptor (AhR), which promotes learning and memory abilities. Additionally, antibiotic treatments disturb gut microbiota, impairing olfactory memory and behavioral performance under field conditions. This shows that a healthy, conventional gut microbiota is crucial for both physiological and cognitive processes in bees.[61]

Recent research highlights the crucial role of honeybee gut microbes in supporting bee health by regulating metabolic processes, behavioral functions, and brain chemistry. Specific bacterial species like *Gilliamella apicola* aid in carbohydrate and glycerophospholipid

Table 1. Microorganisms found in honey bee gut

No.	Microbe	Function	Symbiosis	Examples	References
1.	*Gilliamella apicola*	Digestion of pollen andhoney, production of lactic acid	Mutualistic-benefits both the bee and the bacteria	Aids in breaking down complex sugars in pollen and honey, providing the bee with essential nutrients	49
2.	*Bifidobacterium asteroideum*	Synthesis of vitamins and short-chain fatty acids, immune system support	Mutualistic	Contributes to the bee's vitamin B production and gut health, potentially boosting its immune response	50
3.	*Lactobacillus Firmontanus*	Production of lactic acid, inhibition of harmful bacteria	Mutualistic	Creates an acidic environment in the gut that protecting the bee from pathogens	22
4.	*Snodgrassella alvi*	Nitrogen fixation, production of essential amino acids	Mutualistic	Fixes atmospheric nitrogen into a form usable by the bee, and synthesizes essential amino acids that the bee cannot produce itself	49
5.	*Bombella intestini*	Production of B vitamins, degradation of complex carbohydrates	Mutualistic	Provides the bee with B vitamins, and helps break down complex carbohydrates into simpler sugars that the bee can absorb	51
6.	*Candidatus Bartonella apis*	Transmission of bee viruses, potential role in queen development	Symbiotic-complex relationship with benefits influences carbohydrate metabolism, specifically aiding in the digestion of plant-derived polysaccharides	May play a role in the transmission of certainbee viruses, but also potentially contributes to queen development and reproduction	52
7.	*Melissococcus pluton*	Production of lactic acid, inhibition of harmful bacteria	Mutualistic	Creates an acidic environment in the gut that protecting the bee from pathogens	53
8.	*Frischella perrara*	Synthesis of vitamins and short-chain fatty acids, immune system support	Mutualistic	Contributes to the bee's vitamin B production and gut health, potentially boosting its immune response	54
9.	*Apibacter invictus*	Production of antimicrobial compounds, protection against pathogens	Mutualistic	Produces compounds that prevent the growth of harmful bacteria, safeguarding the bee's gut health	55

metabolism, while *Lactobacillus* Firm-4 and Firm-5 enhance amino acid pathways. These gut bacteria also influence neurotransmitter levels such as dopamine and serotonin, which impact sensory sensitivity and taste-related behaviors. Additionally, *Lactobacillus* species play a role in modulating gene expression related to neural functions like olfactory perception, essential for foraging and social behaviors in bees.[61]

Since the bee gut microbiome can significantly lower the prevalence of bee diseases, understanding the function of the gut microbiome in maintaining bee health is essential. In order to keep bees safe from diseases and pathogens, maintaining their gut microbiome is essential.[37] In bumblebees, it was demonstrated that the gut microbiome leads to a notable decrease in the presence of the gut parasite *Crithidia bombi*.[62] Both microbiome transplants and monocolonization experiments revealed that the gut bacteria in bees trigger the host immune system. *F. perrara* has an impact on the pylorus's homeostasis and gut immunity. In addition, the incidence of *F. perrara* varies throughout colonies and that higher concentrations of this bacteria have been linked to altered diets and compromised host development, this could have an impact on bee health.[48]

Research has shown that the gut microbiome of bumblebees and honeybees contributes in a certain manner as a resistance mechanism. Microbiome-free *B. terrestis* inoculated with wild-type workers faecal matter showed greater resistance to the *Crithidia bombi* gut parasite than with uninoculated bees in two different experiments.[63,64] Instead of the colony from which the bees originated, the colony source had a greater influence on the microbiome transplant's ability to provide protection, indicating that varying gut microbiome compositions can offer different levels of protection. *Bombus terrestris* maintains a social core gut microbiota, similar to honeybees. Further research is necessary to determine whether individual members of the community provide pathogen protection to bumble bees, as these investigations were unable to identify the strains that underlie the protection.[64] Fungi may help bees by competing with harmful pathogens for resources, thereby reducing the growth of spoilage microbes in bee provisions. Some studies have shown that certain fungi can inhibit the growth of bee pathogens. For example, fungi like *Aspergillus*, *Cladosporium*, and others isolated from honeybee provisions have demonstrated the ability to inhibit the growth of *Ascosphaera apis*, a pathogen affecting bee larvae. However, the effectiveness of different fungal strains can vary, and not all fungi exhibit this inhibitory ability.[65]

Antibiotic properties rendered by the gut microbes

Bacillus species provide benefits to honeybees by producing a range of enzymes and antibiotic-like substances. These substances play a dual role, aiding in carbohydrate digestion and inhibiting the proliferation of harmful organisms within the bee gut.[66] Additionally, pathogens have been observed in the honeybee gut, with the predominant segment of these grouped contigs associated with the trypanosomatid parasite *Lotmaria passim*.[67] *Gilliamella apicola* and *Snodgrassella alvi* have been studied extensively for their roles in antibiotic resistance. These bacteria form biofilms in the honeybee gut, aiding in the defense against pathogenic bacteria and contributing to antimicrobial resistance (AMR). Recent studies have demonstrated that *Gilliamella* species exhibit resistance to tetracycline, commonly used in apiculture, leading to the accumulation of antibiotic-resistant genes (ARGs) within bee gut microbiomes.[68] *Lactobacillus* spp. and *Bifidobacterium* spp. possess antimicrobial properties that inhibit harmful pathogens, not only within the bees but also in the hive and external environments. These microbes produce bacteriocins and other antimicrobial compounds that protect the colony from diseases like American foulbrood, caused by *Paenibacillus larvae*.[69]

Probiotic properties rendered by the gut microbes

The bacteria residing in the honeybee gut are predominantly categorized as probiotics, playing a crucial role in maintaining bee health.[69] American foulbrood (AFB) and European foulbrood (EFB) are significant ailments affecting honeybee broods, leading to substantial economic losses in the apiculture sector worldwide due to declines in bee populations and honey yields. These diseases can be prevented

Table 2. Yeast, moulds and bacteria found in honey

Yeast	Functions	References
Ascophaera	Ascosphaera apis is a significant pathogen for the Apis mellifera, the Western honey bee leading to chalkbrood disease, which holds economic importance	80,81
Nematospora	Phytopathogen against honey bee larvae which is found in honey	82
Debaryomyces hansenii	Probiotic property in gut microbiome modulation	83
Zygosaccharomyces rouxii	Fermentation processes, overall flavor and preservation of the honey	84
Zygosaccharomyces mellis	High sugar tolerant yeast that leads to deterioration of the honey	18,81
Aureobasidium pullulans	A very high pullulan producing yeast-like fungus	85
Cryptococcus neoformans	Decomposer or breaking down organic matter	86
Moulds		
Aspergillus	Induces Stonebrood disease in honey bees	87
Bettsia alvei	Xerophilic fungi that thrive in low moisture content	88
Bacteria		
Bacillus sp.	Enterotoxin producer	81,89
Clostridium sp.	Enterotoxin producer	89,90

through the effectiveness of probiotic bacteria naturally present in the bee gut. EFB and AFB are caused by gram-positive bacteria, specifically *Paenibacillus* larvae and *Melissococcus plutonius*. Principal groups of probiotic bacteria, including *Bifidobacteria, Bacillus* species and Lactic Acid Bacteria (LAB) possess the ability to alleviate microbiome dysbiosis associated with antibiotics and immune deficiencies in adult worker bees. Certain *Lactobacillus* species found in the honeybee gut have demonstrated their probiotic properties and positive impact on host health.[70] Probiotics, as live bacteria ingested in appropriate amounts, significantly improve honeybee immunity in apiculture, resulting in enhanced disease resistance, increased honey production, wax gland development, colony loss, reduced mortality, and decreased nutritional stress.[71] Additionally, Probiotic bacteria in honeybees also provide significant advantages such as stimulating fat body development and food gland function.[72]

The gastrointestinal tract of Apis mellifera is the origin for the probiotic characteristics of LAB.[73] They discovered that LAB can thrive in the intestines of honeybees and can withstand pH fluctuations throughout the digestive system. Another study demonstrated that two *Lactiplantibacillus plantarum* strains, isolated from the Indian honeybee *Apis cerena indica*, possess probiotic properties. These included the ability to survive in simulated gastrointestinal conditions, the ability to withstand acid and bile tolerance and the ability to aggregate and be hydrophobic.[74] LAB species are among the many commensal bacteria found in honeybees' guts that have been identified as potentially effective probiotics. These bacteria may be used as a dietary supplement for humans and animals as well as to help honeybees recover from illness and become more resilient to it.[75,76] Other than LAB species, the *Bacillus* species also been identified to have the probiotic properties.[66] The evidence indicates that the gut of honeybees serves as a diverse repository for numerous LAB species, originating from a wide array of environmental origins. The predominant species in LAB are *Lactobacillus* species; however, sufficient numbers of *Bifidobacterium* and *Enterococcus* species are also present. The probiotic qualities of these bacteria have been thoroughly investigated, and it has been shown that they are essential for both the survival and healthy operation of the bee colony.

Antimicrobial (fungal/bacterial) properties rendered by the gut microbes

The gut microbiome of honeybees perform a crucial role in enhancing their overall well-being by bolstering their resistance to diseases through immune regulation and the production of diverse antimicrobial substances.[69] *Bacillus* species exhibit beneficial characteristics such as the formation of antimicrobial peptides (bacteriocins), immune stimulation, and adhesion.[77] Several studies have investigated the antibacterial properties of specific gut-associated *Bacillus* species against European foulbrood (EFB) and American foulbrood (AFB).[75] Recently, honey's antibacterial qualities are connected to the honey microbiome's effects in suppressing different human and foodborne pathogens.[25] The composition of microbial communities in honey samples exhibited significant antimicrobial activity, revealing traces of antimicrobial bacteria and a diverse origin of pollen in honeybee samples collected from various geographical locations in Greece. Various bacteria were identified in both honeybee guts and honey, with Bradyrhizobium exhibiting antagonistic effects against pathogens.[78]

Diversity of microbiome in honey

Microbes capable of thriving in honey must possess resistance to its high sugar concentration, acidity, and other antibacterial properties. Honey produced by bees may contain a diverse range of microbial entities, including bacteria like *Bacillus* and *Clostridium* species, as well as yeasts (Table 2). Certain lactic acid bacteria in honey have shown antibacterial activity and contribute to maintaining a healthy gut microbiome.[79] The presence of various microorganisms in honey not only contributes to its unique taste but also holds potential health benefits for humans.[37] *Saccharomyces*, *Bacillus*, and *Micrococcus* are easily extracted from honeycomb. While the mold *Aspergillus* may be dormant in honey, sugar-tolerant yeasts originate from flowers and soil. Microbes present in comb honey primarily result from pollen contamination and the feces of larvae fed by worker bees. However, these microbes struggle to thrive in honey for extended periods due to its high sucrose concentration and antimicrobial properties.

Study on microbial community structure among honey samples of different pollen origin such as fir, cotton, fir-oak, and Arbutus unedo honeys were done.[25] In fir honey, the most common taxon identified was *Lactobacillus kunkeei*. Cotton honey primarily harboured *Lysobacter*, *Meiothermus, Pseudomonas* and *Streptococcus* as the most abundant microorganisms. Conversely, fir-oak honey exhibited *Lonsdalea*, the microorganism associated with acute oak decline, and *Zymobacter*, known for fermenting under low oxygen and high osmotic pressure conditions, as predominant types. *Methylotrophic* bacteria were found abundantly across different geographic and pollen origins. Additionally, *Bacilli*/*anoxybacilli*, *sphingomonads, pseudomonads*, *paracocci*, and *lysobacters* were also identified in the honey samples. The analysis suggests that the microbial components detected in the honey samples mainly originate from the indigenous gut microbiome of honeybees and the microbiome present in the flowering plants they visit. This diverse range of bacteria includes potentially beneficial strains with probiotic properties, as well as pathogens capable of affecting both animals and plants.

Aside from the microbial taxa mentioned previously, additional Bacillus species such as *B. amyloliquefaciens, B. licheniformis, B. subtilis, B. cereus, B. pumilus, B. thuringiensis,* and *B. megaterium,* were also detected in honey samples. It's worth noting that bacterial strains of the *B. cereus* species are known for producing enterotoxins, whereas those from other *Bacillus* species are generally considered safe.[89]

Honey microbiome on human health

During the honey production process, honeybees ingest nectar and utilize enzymes to transform it. Additionally, they incorporate certain symbiotic microorganisms from their gastrointestinal tract, which may offer potential health benefits to humans.[91] Unlike the human microbiome, which typically remains stable over time, regular intake of new symbiotic microorganisms is essential to ensure their ability to colonize the human body and sustain their beneficial effects. Honey contains microorganisms which include probiotics, and consuming such microorganisms can provide nourishment and health benefits to humans.[92]

In honey harvested directly from bee hives, a variety of bacteria were isolated, including *Gluconobacter oxydans*, *Pseudomonas* spp*.,* and *Bacillus* spp. Notably, *G. oxydans* has demonstrated resistance to 2% bile salts and 100% survival at pH 5.0 and 2.0 after 3 hours of interaction, highlighting its resilience in challenging conditions.[93] Additionally, these bacteria possess the ability to assimilate cholesterol, potentially reducing its absorption by the body, making them promising probiotic candidates for use in food products. Moreover, given honey's high fructose content, certain bacteria residing in honey may rapidly break down fructose. These microorganisms, referred to as fructophilic lactic acid bacteria, exhibit a marked preference for the metabolic utilization of fructose in comparison to glucose. Notably, bacteria like *Lactobacillus kunkeei*, among *fructophilic* lactic acid bacteria, generate bacteriocins, functioning as a defensive barrier against competing microorganisms. *Lactobacillus* spp. was obtained from both the stomachs of honeybees and honey samples. They were then evaluated for their inhibitory effects against *Escherichia coli* and *Salmonella enterica*, showing significant inhibition.[94] A prebiotic is a dietary supplement that cannot be digested and alters the equilibrium of the gut microbiota by promoting the proliferation and function of helpful organisms while inhibiting harmful bacteria.[95] Honey has been identified as a suitable sweetening agent in fermented dairy products, facilitating the proliferation of essential bacterial strains including *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Bifidobacterium bifidum* and *Lactobacillus delbrueckii*, all of which are vital for maintaining gastrointestinal health, without obstruction.[96]

CONCLUSION

In conclusion, recent advancements in biotechnology offer promising avenues for improving the overall health of honeybees through microbiome engineering, strengthening their resistance to viral and bacterial infections. Extensive evidence underscores the critical role of the gut microbiome in honeybee health, with correlational analyses providing valuable insights and laboratory investigations driving experimental work. However, there remains a need for field research conducted in real apiary environments to fully understand the complexities of bee microbiomes. Furthermore, the role of honey microbiomes in both bee and human health cannot be understated. The unique microbial composition of honey, influenced by factors such as bee gut microbiota and environmental conditions, may contribute to the antimicrobial properties and potential health benefits associated with honey consumption for humans. As honey serves as a source of prebiotics and probiotics, its consumption may promote gut health and enhance overall well-being in humans. The evolving understanding of bee gut microbial communities, alike to those found in humans and other mammals, suggests significant advancements in research are on the prospect. Honeybee's pivotal role in pollinating a substantial portion of the world's crops and wild flowering plants, their declining populations have prompted an urgent examination of factors impacting their well-being, including their microbiomes. Thus, elucidating the details of this microbial community holds promise for enhancing bee health and addressing broader questions regarding the symbiotic relationship between hosts and microorganisms, thereby contributing to sustainable bee and human health initiatives.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SM conceptualized the study and applied methodology. VRS performed samples collection. KH performed data curation. MS visualization and investigated the study. KH wrote the original draft. KH, SM, MS, NS and VRS wrote the manuscript. NS and VRS reviewed the manuscript. NS edited the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in this manuscript.

ETHICS STATEMENT

Not applicable.

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