

Review Article

Role of Honey Bee Gut Microbiota in the Control of American Foulbrood and European Foulbrood Diseases

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Abstract

American foulbrood (AFB) and European foulbrood (EFB) are the two most important honey bee brood diseases which impose heavy economic losses to the apiculture industry worldwide by reducing bee population and honey production. Treatment with antibiotics has led to the emergence of antibiotic-resistant strains, calling for alternative safe treatment procedures that could control these diseases. Honey bee gut microbiota is known to affect the overall health of honey bees by enhancing their resistance to a number of diseases via modulation of the immune response and production of different antimicrobial metabolites. The majority of these gut resident bacteria are identified as probiotic bacteria and secure the health of these tiny insects. In the present review, we highlighted the significance of the honey bee gut microbial community and their probiotic potency for the prevention of AFB and EFB diseases in honey bees.

Keywords: American foulbrood disease, European foulbrood disease, probiotics, honeybee microbiota

1. Context

According to estimates, nearly 35% of human food consumption relies on insect pollination, and more than 90% of the crops depend on honey bee pollination services, highlighting the crucial importance of these insects. Honey bee gut is considered the reservoir of several microbes that most importantly affect their behavior, nutrition, immune homeostasis, and disease tolerance (1). Although the diversity of these microbes in the honey bee gut is less considerable than that in other animals, they are of major significance owing to their direct role in the enhancement of the immunity and resistance of honey bees to several bacteria and viruses (2, 3). Therefore, it is crucial to maintain the composition of the gut microbial flora in honey bees and any imbalance in the gut microbiota (dysbiosis)

could consequently lead to increased susceptibility of these insects to some infections.

A lack of foraging capacities, the use of insecticides in agriculture, and environmental stressors, such as high summer and low winter temperatures, are well-understood abiotic agents (4) which include a range of disease-causing organisms, such as bacteria, viruses, protozoa, fungi, and parasitic mites (5). Among these, two of the most economically important bacterial diseases affecting the brood are American foulbrood (AFB) and European foulbrood (EFB) diseases, both widely distributed and potentially lethal to infected colonies (6).

Melissococcus plutonius (*M. plutonius*) and *Paenibacillus larvae* (*P. larvae*), the gram-positive bacterium, are the causative agents of EFB and AFB,

respectively. Comparative genomics-based detected genes that might play a role in EFB pathogenesis and, ultimately, in the death of the honey bee larvae. They include bacteriocins, bacteria cell surface- and host cell adhesion-associated proteins, an enterococcal polysaccharide antigen, an epsilon toxin, proteolytic enzymes, and capsule-associated proteins (6). Once *M. plutonius* reaches the intestinal tract via the consumption of contaminated food, it quickly multiplies and can deprive the host of nutrients. The virulence of *M. plutonius* is highly variable, and some strains can kill a higher proportion of the host within a shorter time period, compared to other strains (7).

According to the enterobacterial repetitive intergenic consensus classification, five genotypes of AFB (i.e., I, II, III, IV, and V) are recognized, differing in their virulence and prevalence in colonies (8). The AFB disease affects the bee larvae during the early developmental stages, while adult bees only transmit the infectious spores of *P. larvae*. Bee larvae are infected with *P. larvae* endospores via the consumption of contaminated food sources, where these spores after consumption germinate in the larval intestine. The youngest larvae (12-36 h after egg hatching) are the most sensitive stage to the spores (9).

Lactic Acid Bacteria (LAB), *Bifidobacteria* and *Bacillus* species, are the major groups which could mitigate the antibiotic-associated microbiota dysbiosis and immune deficits in adult workers (2). The benefits of *Bacillus* species lie in their ability to produce a number of enzymes and antibiotic-like substances that could assist in the digestion of carbohydrates and suppress the growth of pathogenic or noxious organisms in honey bees, respectively (10, 11). Considering their health benefits, a number of these bacteria are considered probiotics and could exert desirable health effects on the host when consumed in adequate quantities (12, 13). To date, a large number of probiotic bacteria have been isolated, identified, characterized, and used

commercially in several products for human and animal use. Probiotic bacteria residing in the honey bee gut have been studied extensively for their antibacterial potential against the bacterial agents responsible for AFB and EFB (14).

2. Evidence Acquisition

The present study aimed to highlight the significance and role of gut microbiota in providing protection against the mentioned bacterial diseases in honeybees. For data retrieval, different keywords were used to gather important information using two widely used databases (i.e., Google Scholar and PubMed). The keywords used to search the relevant studies were "American foulbrood disease", "European foulbrood disease", "probiotics", and "honeybee gut microbiome". In addition to direct search, cross-referencing was also employed from the already reviewed and included studies to increase the search for more relevant articles. We just used published studies in this review, and all other articles were excluded. Data were collected from 2009 to 2021.

3. Results

3.1. American Foulbrood and European Foulbrood Diseases

P. larvae, a Gram-positive, spore-forming, and peritrichously flagellated bacterium is the causative agent of AFB which is a highly contagious disease affecting the larval and pupal stages of honey bees. It has been known that AFB disease is propagated and spread through various paths, such as the interchange of contaminated bee equipment between colonies and drifting bees. The *M. plutonius*, a Gram-positive bacterium, triggers European foulbrood disease in honey bees. It is a lanceolate non-spore-forming cocci with a close phylogenetic relationship to the genus *Enterococcus* (15).

3.2. Strategies for the Control of American Foulbrood and European Foulbrood Diseases

In the apiculture industry, disease prevention is the ultimate control strategy for preventing honey bee

colony losses. Several different approaches, including the use of antibiotics and destroying infected colonies via burning and antibiotics utilization, have been implemented to control AFB and EFB disease, respectively. For the control of EFB, primarily antibiotics, such as oxytetracycline hydrochloride, have been used extensively (16). Nonetheless, it has been a proven fact that the wide use of antibiotics could trigger disequilibria in the normal microbiota of the beehive, resulting in a decreased half-life of honeybees, increased risk of contamination of honey with the chemical residues (17) and, most important of all, could be toxic to honeybee broods and beneficial intestinal microflora of honey bees (18). It is reported that the veterinary drugs have negative impacts on the absolute abundance of several core taxa of the honey bee gut microbial community, causing microbial imbalance or dysbiosis, which in turn, leads to a decline in bee populations (19). Apart from all, the emergence of antibiotic-resistant pathogenic bacterial strains of *P. larvae* and *M. plutonius* has also been reported globally (20).

To date, a number of alternative non-contaminating natural and safe antimicrobial agents, including probiotics, essential oils, and propolis, have been studied for their role in the enhancement of honey bee health by preventing disease prevalence, avoiding the presence of undesirable residues in honeybee products, and consequently, enhanced survival of colonies and decreased mortality in honeybees.

3.3. Honeybee Gut Microbiota

The gut microbiota of honeybees plays as important a role as it does in mammals, including its involvement in host physiology, such as metabolism and immune functions (21). Some significant functions played by these gut microbes include nutrient biosynthesis and biomass deconstruction, digestion of proteins and lipids, detoxification of xenobiotics, inhibition of pathogen invasion, pesticide

resistance, colonization resistance against parasites, weight gain, and egg production (2, 22). In addition, these commensal microbes influence the transformation of nectar into honey and plant buds into propolis; moreover, they are responsible for the freshness of honey (23). Untargeted metabolomics studies revealed that a plethora of organic acids accumulates in the presence of gut bacteria, causing pivotal functional consequences in host physiology (24). Figure 1 displays the bacterial communities and localization of different bacteria within gut compartments in honey bees.

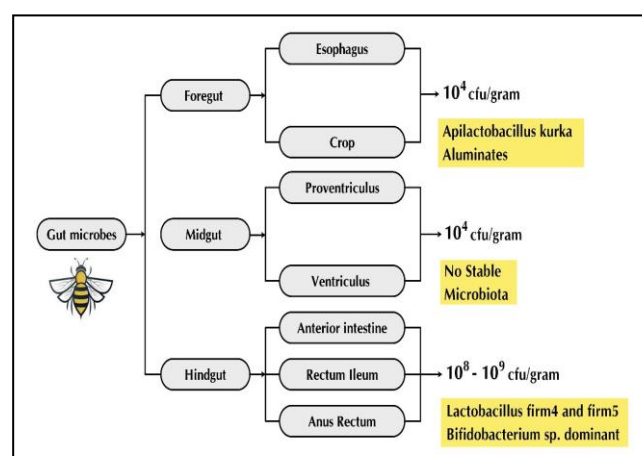


Figure 1. Bacterial communities and localization of different bacteria within gut compartments (foregut, midgut, and hindgut) in honey bees

Several abiotic and biotic factors are responsible for disturbing the gut microbial flora in honeybees (Figure 2). The microbiota of honey bees is distributed in different parts of the gut, including the foregut, midgut, and hindgut, being composed of a limited number of bacterial phylotypes (25). Although fewer commensal bacteria exist in the gut of insects, compared to mammals, they may exert more marked impacts on the health of insects. The simple and host-specialized gut microbiota of these insects, with 8 ~ 10 bacterial phylotypes, constitute approximately 95%-99% of the bacterial community residing in the gut of a bee (26).

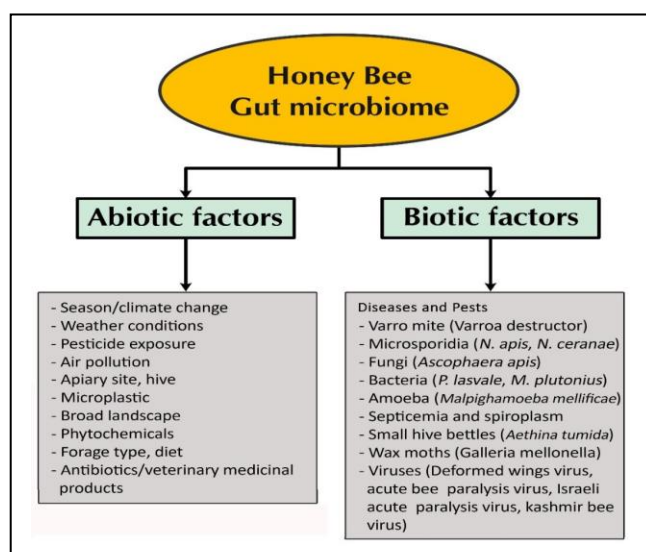


Figure 2. Abiotic and biotic stress factors affecting the gut microbiome composition of honey bees

The major phylotypes are nearly nine socially transmitted bacterial species, including *Gilliamella apicola*, *Snodgrassella alvi*, *Lactobacillus* Firm-4 and Firm-5, *Frischella perrara*, *Bartonella apis*, *Commensalibacter sp.*, and *Bifidobacterium sp.*, that comprise honeybee gut microbiome (27). While, *Proteobacteria* species, *Frischella perrara*, *Bartonella apis*, *Parasaccharibacter apium*, and a *Gluconobacter* are the less prevalent species (26, 28).

The LAB as a group of Gram-positive, non-sporulated, catalase-negative bacilli or cocci are vastly explored in the gut microbiome of honeybees (6, 29-31). It is reported that the LAB species evolved in honey bees have mutual dependence on each other. The LAB species by acquiring a nutritional niche protect honey bees and their honey against the pathogenic bacteria (31). A major role played by probiotic gut microbiota of honey bees is their ability to up-regulate the gene expression of the AMPs apidaecin and hymenoptaecin in gut tissue, suggesting that the microbiota exerts a systemic immune effect, rather than being only localized to the gut (26). Fructophilic lactic acid bacteria (FLAB) are also reported in honeybees fed diets rich in fructose. The main difference between LAB and FLAB is that the growth of later is poor or

delayed in the presence of glucose substrate. *L.kunkeei*, the most dominant species in the gut of these insects, is identified as a FLAB (32).

Apart from Gram-positive bacteria, a number of gram-negative bacteria also exist in the gut of honeybees. Acetic acid bacteria (AAB) are a large group of gram-negative bacteria within the clade of proteobacteria, most frequently found within the honey bee microbiome (31, 33). Among these, *Gluconobacter*, *Acetobacter*, *Gluconacetobacter*, and *Saccharibacter* are the bee symbionts belonging to AAB genera. Both LAB and AAB share some properties, for instance, both have the ability to grow and tolerate acidic pH, produce organic acids, such as lactic acid and acetic acids, and both genera have the ability to metabolize different sugars (3). Similar to LAB, the role of AAB residing in the honey bee gut in the control of pathogens has been also reported earlier (33).

3.4. Probiotic Microbiota of Honey Bees

Some of the *Lactobacillus* species residing in the gut of honeybees are shown to affect the health of the host by their probiotic potential (2, 6). As defined earlier, probiotic bacteria are live bacteria that have significant health effects on the host when consumed in sufficient quantity. In apiculture, probiotic bacteria are recognized to enhance honeybee immunity, leading to enhanced resistance to diseases, wax gland development, increased honey production, decreased mortality, colony loss, and nutritional stress (2, 32). Other major beneficial effects of probiotic bacteria reported in honeybees include growth stimulation of food glands and fat body (34).

Pachla, Ptaszynska (35) evaluated the potential probiotic features of LAB from *Apis mellifera* gastrointestinal tract. They found that LAB are able to tolerate pH changes throughout the honey bee intestinal tract and survive well in honey bee guts. According to them, a sucrose diet supplemented with LAB could decrease mortality and improve the lifespan of honey bees mainly through gut microbial homeostasis improvement, immune response stimulation, and intestinal pathogen elimination. In another study, two

Lactiplantibacillus plantarum strains isolated from the Indian honey bee, *Apis cerana indic*, were demonstrated to have probiotic properties, including acid and bile tolerance, survival in simulated gastrointestinal conditions, as well as hydrophobic and aggregative properties (36). To date, a vast number of commensal bacteria in the gut of honey bees, including LAB species, have been assessed as promising probiotic candidates that could be usefully applied as a health supplement not only for human and animal consumption but might also be used in honey bees to restore their health and increase their disease resistance (6, 29, 36).

In addition to LAB species, a number of *Bacillus* species with probiotic properties have been identified in honeybees (11, 37). Decades back, the relationships between honey bees and *Bacillus* bacteria have been extensively examined (38). Most of these studies have reported that the symbiotic *Bacillus* bacteria benefit honey bees by facilitating pollen fermentation, food

protection, and disease prevention. Along the same lines, Sabate, Carrillo (39) reported that *B. subtilis* strain Mori2 had beneficial effects on colony performance. Additional desirable features of *Bacillus* species include their ability to produce antimicrobial peptides (bacteriocins), stimulate immunity, and their adhesion abilities (40). *B.amyloliquifacien* HTI-19 and *B.subtilis* HTI-23 isolated from stingless honey bees in Malaysia demonstrated high survivability in the artificial modified digestive tract systems, wide antimicrobial spectra, high cell hydrophobicity, and auto-aggregation ability. The antibacterial potential of a number of gut-associated *Bacillus* species against AFB and EFB has been investigated by many (6). Furthermore, *Bacillus subtilis* strains were able to inhibit the growth of *P. larvae* and *A. apis* (39). Table 1 lists the bacterial species, including *Lactobacillus*, *Enterococcus*, *Bifidobacterium*, and *Bacillus* species, which have demonstrated antibacterial effects against AFB and EFB.

Table 1. Salient features of honeybee gut microbes and their activity against American foulbrood and European foulbrood

| Bacteria | Features | Effects against AFB and EFB | Ref |
|---|--|--|--------------|
| <i>Enterococcus faecium</i> SM21 | Lactic acid production, bacteriocin-like compounds production | <i>in vitro</i> inhibition of AFB | (41) |
| <i>L. kunkeei</i> | Gastrointestinal tract tolerance, antibiotic susceptibility, cell surface hydrophobicity, as well as auto- and co-aggregation capacities | <i>in vivo</i> inhibition of AFB | (35, 42) |
| <i>L. kunkeei</i> | Potential antibacterial activity | <i>in vitro</i> and <i>in vivo</i> inhibition of EFB | (28, 31) |
| <i>Fructobacillus fructosus</i> | Gastrointestinal tract tolerance, antibiotic susceptibility, cell surface hydrophobicity, as well as auto- and co-aggregation capacities | <i>in vitro</i> inhibition of AFB | (28, 31, 35) |
| <i>L. plantarum</i> KX519413 <i>L. plantarum</i> KX519414 | Gastrointestinal tract tolerance, exopolysaccharide production, biofilm formation, as well as auto-aggregation | NR | (36) |
| <i>L. crispatus</i> IG9 | Isolated from fermented foods | <i>in vitro</i> inhibition of AFB | (43) |
| Acja3 (<i>Bacillus</i> strain) | Antimicrobial compounds production, tolerance to gut environmental conditions | <i>in vitro</i> inhibition of EFB | (22) |
| <i>L. plantarum</i> Lp39 <i>L. rhamnosus</i> GR-1 <i>L. kunkeei</i> BR-1 | Upregulation of immune-related gene expression | <i>in vitro</i> inhibition of AFB | (2) |
| <i>L. plantarum</i> <i>L. apis</i> <i>L. apinorum</i> | Isolated from the digestive tract of adult honeybees in Košice, Slovakia. | <i>in vitro</i> inhibition of AFB | (44) |
| <i>L. helsingborgensis</i> <i>L. kimbladii</i> <i>L. kullabergensis</i> <i>Lmellifer</i> | Isolated from the digestive tract of honeybees <i>Apis mellifera</i> , <i>Apis andreniformis</i> , and <i>Melipona beechi</i> | <i>in vitro</i> and <i>in vivo</i> inhibition of AFB and EFB | (29, 31) |

| Bacteria | Features | Effects against AFB and EFB | Ref |
|---|---|---|------|
| <i>L.apinorum</i> <i>L. apis</i> | Isolated from stomach of <i>Apis mellifera</i> in Czech Republic | <i>in vitro</i> inhibition of AFB and EFB | (45) |
| <i>B. asteroides</i> | Isolated from the guts of honey bee workers | <i>in vitro</i> and <i>in vivo</i> inhibition of AFB | (46) |
| <i>L. plantarum</i> <i>L. brevis</i> | Production of lactic acid | <i>in vitro</i> inhibition of AFB | (44) |
| <i>B. longum</i> <i>B. indicum</i> | Analysis of communities in the midguts of <i>Apis mellifera</i> and <i>Apis cerana</i> honey bees in Thailand | <i>in vitro</i> and <i>in vivo</i> inhibition of AFB | (6) |
| <i>B. cereus</i> , <i>B. megaterium</i> , <i>B. licheniformis</i> , <i>B. pumilus</i> , <i>B. subtilis</i> , <i>B. laterosporus</i> | Not studied for probiotic properties | <i>in vitro</i> inhibition of different strains of <i>P. larvae</i> | (47) |
| <i>B. cereus</i> , <i>B. fusiformis</i> , <i>Brevibacillus formosus</i> | Isolated from honeybee larvae Not investigated for probiotic properties | <i>in vitro</i> inhibition of different strains of <i>P.larvae</i> | (10) |
| <i>L.kunkeei</i> , <i>Bifidobacterium asteroides</i> related phylotypes, and <i>Lactobacillus</i> phylotypes | Synergistic action of mixed cultures against <i>P. larvae</i> | <i>Invitro</i> and <i>in vivo</i> inhibition of AFB | (6) |
| Bacillus isolates (Acj 115, Acj 209, Acj 214, and Acj 219) | Isolated from the digestive tract of the Japanese honeybee, <i>Apis cerana japonica</i> | <i>In vitro</i> inhibition of AFB | (48) |
| <i>B.subtilis</i> and <i>B.leichniformis</i> | Isolated from honeybee gut and honey samples | NR | |

(NR= not reported; AFB: American foulbrood; EFB: European foulbrood)

3.5. Future Prospective

Owing to the possible benefits of gut residing LAB species to honey bee health, it is of utmost importance to find tools that could enhance the growth and population of these naturally occurring beneficial bacteria in the body of the host. The mechanism underlying the ability of these bacterial communities to live in harmony in the insect gut is yet not explored. Therefore, it is essential to identify and locate the factors that directly or indirectly influence the microbiota, using diverse approaches, including the latest biotechnological tools. Recent advances in the field of biotechnology have displayed that the resistance of honey bees to viral and bacterial pathogens could be enhanced by microbiome engineering, resulting in overall host health improvements in these insects (49, 50).

4. Conclusions

Population decline in honey bees causes high annual losses of managed honey bees. It is of great concern owing to the importance of bee pollinators for plant reproduction and crop production. *P. larvae* and *M. plutonius* are the primary bacterial pathogens of honey bees, as well as the causative agents of AFB and EFB

disease, respectively. The subsequent overuse of antibiotics for the treatment of AFB and EFB could also perturb the gut natural microbiota, leading to microbial flora imbalance and the emergence of resistant virulent strains. Evidence suggested that honey bee guts are a wide reservoir of various LAB species that evolve from a broad range of environmental sources. Among LAB, *Lactobacillus* species are the dominant species; moreover, *Bifidobacterium* and *Enterococcus* species are also found in adequate amounts. These bacteria are well studied for probiotic characteristics and have demonstrated their essential role in the proper functioning of the bee colony and their survival. It is a known fact that competition for adhesion receptors on the intestinal epithelium, nutrients, antibacterial substances production, and immune system stimulation are the major mechanisms of probiotic effects of several gut bacteria, underling their beneficiary effects on the host.

Authors' Contribution

Study concept and design: N. M. and M. M.

Acquisition of data: N. M.

Analysis and interpretation of data: N. M.

Drafting of the manuscript: N. M., S. T., and M. B.

Critical revision of the manuscript for important intellectual content: N. M. and M. B.

Intellectual content: N. M.

Administrative, technical, and material support: N. M., M. M., M. B., and S. T.

Conflict of Interest

The authors declare that they have no conflict of interest.

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