

Microbiota and Its Importance in Honey Bees

Ayşe Ebru BORUM^{1,*} 

¹Balıkesir University, Veterinary Medicine Faculty, Department of Microbiology, Balıkesir, Turkey

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*Corresponding Author

Tel.: +905436780123
E-mail: ebruborum@balikesir.edu.tr

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Abstract

In recent years, research on human and animal health has emerged as a very important microbiota and microbiome. Microbiota; has important functions in metabolism, immune system, growth and development. In recent years, it has been understood that the microbiota is effective in the protection of bee health in colony so prevent losses in honey bees. Season, flora, food sources, age of the individual, duties in the hive, chemicals used in the fight against parasites and pathogens, and many other factors can be effective on the microbiome of honey bees.

Introduction

A larger organism or microbial community living in an intensive environment is called the "microbiota". Animals that live as a community and have social relationships often use a characteristic microbiota (Pascale et al., 2018). Microbiota is defined as a mixed ecosystem of microorganisms that are critical in various metabolic functions such as regulation of glucose and lipid homeostasis, energy management and vitamin production, and regulation of satiety (Greiner & Bäckhed, 2011; LeBlanc et al., 2013). It is effective in the production of metabolites and other substances that regulate various biochemical and physiological mechanisms. It has important functions in the functioning of the immune system and in stimulating immune responses, producing anticarcinogenic and anti-inflammatory activities_ also regulates induction of protective responses of the host against pathogens and harmless antigen tolerance (Molloy, Bouladoux, & Belkaid, 2012; Belkaid & Harrison, 2017; Agus, Planchais, & Sokol, 2018). The composition of the

microbiota varies considerably, both between different species and within the same species. Microbiota diversity is affected by short-term changes in microbial communities and topographic differences. Specific microorganisms settle in specific environments during different growth and developmental stages of the host (Nicholson et al., 2012).

Characteristics

Insects are the most diverse animal species in terms of number of species, ecological habitats, and overall biomass. (Engel & Moran, 2013a). *Apis mellifera* has a high host adaptive microbial community. The honey bee microbiota has some similarities with mammals. But in reality, it is of a simpler composition (Zheng, Steele, Leonard, Motta, & Moran, 2018).

Young worker bees in a honeybee colony have few or no core gut microbiota (Dong et al., 2020). Microbial communities begin to colonize as they are fed orally by nurse bees (Martinson, Moy, & Moran,

2012; Motta, Raymann, & Moran, 2018). In the pupal stage, bacteria in the gut microbiome are excreted together with the intestinal epithelium. The next colonization is formed by contact with other bees in the hive and by trophallaxis. (Bleau, Bouslama, Giovenazzo, & Derome, 2020). Bacterial diversity in the gut microbiome reaches its maximum in 3-5 days after the bee matures into adulthood. Taxonomic changes occur after 3-8 days (Li et al., 2017). The rectal microbiome completes its development three days after the adult stage. The ileum is more variable and its final structure appears after 8 days. Prevalence of core species, associated alteration of ileum environment and host immune response are factors affecting this process (Anderson & Ricigliano, 2017).

Worker bees perform different tasks depending on age. New adult bees are generally responsible for hive cleaning and maintenance. Interactions between adult bees take place through social contact, cleaning and feeding behaviors. As a result, the microbiome of the species is formed (Powell, Martinson, Urban-Mead, & Moran, 2014; Li et al., 2017). The intestinal microbiota of *A. mellifera* worker bees were investigated within 0-40 days after hatching. It was determined that *Snodgrassella*, *Gilliamella* and *Frischella* species were colonized in the honey bee intestine from the 1st day. *Bifidobacterium*, *Commensalibacter*, and *Lactobacillus* colonize within 3 days, while *Gilliamella* is reduced simultaneously. *Lactobacillus kunkeei* and *Bartonella* sp., colonize significantly in 12 days. *Shigella* sp., *Escherichia* sp., *Bacteroides* sp., and *Porphyromonadaceae* 19 to 25 days, *Commensalibacter* sp. and *Bifidobacterium* sp. decreased at 25 days (Dong et al., 2020).

The microbiota of honey bees consists of microbial communities in different intestinal sections, also called the stomach, which is located between the esophagus and the ventriculus, which is used to store and transport nectar to the hive. Different microbial communities in the hindgut, ileum, lumen, and in the distal rectum form the honeybee microbiome (Vásquez et al., 2012; Engel & Moran, 2013a). *Parasaccharibacter* sp, which is located in the microbiome, is relatively abundant in worker hypopharyngeal glands (Corby-Harris et al. 2014). Adult worker bees are thought to have about nine bacterial species in their guts (Jones et al., 2017).

Similar to humans, the microbial communities of honey bees also contain anaerobic microorganisms. The shaping of bacterial species in the microbiota occurs through social interactions between colony individuals (Alberoni, Gaggia, Baffoni, & Di Gioia, 2016). All bacterial species that make up the microbiota in honey bees can be grown under laboratory conditions, unlike the mammalian gut microbiome (Kwong & Moran, 2016a). As a result of 16S rDNA research, nine different bacterial species that make up 95-99.9% of the microbiota have been identified in the intestines of almost all worker bees (Jones et al., 2017; Moran,

Hansen, Powell, & Sabree, 2012; Tola, Waweru, Hurst, Slippers, & Paredes, 2020). Two gram negative bacterial species that are members of the *Protobacteria* phylum have been identified. *Snodgrassella alvi*, a member of the *Neisseriaceae* family, is a type of bacteria that does not ferment sugars and forms films directly on the intestinal wall. The other, *Gilliamella apicola*, lives in the central areas of the lumen and has the ability to ferment sugar. It belongs to the *Orbaceae* family (Jones et al., 2017; Zheng, Powell, Steele, Dietrich, & Moran, 2017; Tola et al., 2020). Gram-positive bacteria in the *Firmicutes* phylum are abundant in many environments. *Lactobacillus* Firm-4 and Firm-5 living in the distal rectum are among these bacterial species. (Jones et al., 2017; Zheng et al., 2017). Most adult workers also have much smaller amounts of *Bifidobacterium asteroides*. (Kwong and Moran, 2016a; Bleau et al., 2020). These bacteria are the most important microorganisms in the honey bee gut and are called "core bacteria" (Kešnerová et al., 2020). Less dense species than *Proteobacteria* are; *Commensalibacter* sp. (Alpha 2.1) and *Bartonella apis* (Alpha 1); *Parasaccharibacter apium*, *Bombella apis*, *Bombella mellum*, *Bombella favorum* (*Acetobacteraceae* family, Alpha 2.2), *Apibacter adventoris*, *Apibacter mensalis* and *Frischella perrara* (*Orbaceae* family) (Kešnerová, Moritz, & Engel, 2016; Kwong & Moran, 2016b; Jones et al., 2017; Kwong, Steele, & Moran, 2018; Bleau et al., 2020; Dong et al., 2020; Tola et al., 2020; Hilgarth, Redwitz, Ehrmann, Vogel, & Jakob, 2021). Four *Lactobacillus* species, 2 *Gilliamella* species, 1 *Bifidobacterium* species and 1 *Snodgrassella* species, which constitute the intestinal core microbiome of honey bees, were determined. *Bartonella* sp. and *Frischella* sp. are non-nuclear members of the honeybee gut microbiome that may vary depending on the environment. *Parasaccharibacter apium* is a sporadic species in honey bees. This species is often isolated from both the worker's and queen's gut, and from environments such as honey and bee bread (Martinson et al., 2011; Anderson et al., 2013; Anderson, Rodrigues, Mott, Maes, & Corby-Harris, 2016). *Lactobacillus* Firm-4 and Firm-5 are actual symbionts of the gut and are rarely isolated outside of bee guts. Other *Lactobacillus* species, such as *Lactobacillus kunkeei*, can be found inside and in hive materials (Olofsson, Alsterfjord, Nilson, Butler, & Vásquez, 2014; Milani et al., 2018; Raymann et al., 2018a).

In ileum; *Lactobacillus* Firm-4 and Firm-5, *Snodgrassella alvi*, *Bifidobacterium* sp. *Gilliamella apicola*, typical core bacteria, while *Parasaccharibacter apium*, *Frischella perrara*, *Bombella apis*, *Bartonella apis*, *Apibacter adventoris* and *Apibacter mensalis* are less common. In the honey crop part, there are fewer environmental bacteria species with *Apilactobacillus kunkei* predominant. The midgut microbiome is unstable. In the rectum, *Bifidobacterium* sp., *Lactobacillus* Firm-4 and Firm-5 are the dominant

species (Subotic et al., 2019; Kešnerová et al., 2020).

Lactic acid bacteria (LAB) are an important part of the microbiom in honeybees as in other animals (Piccart, Vásquez, Piepers, De Vlieghe, & Olofsson, 2016). The microaerophilic environment of the honey bee digestive system is an ideal environment for sugars from nectar and lactic acid bacteria with a temperature of 35°C (Iorizzo et al., 2020a). LAB plays a role in many different functions that have positive effects on the host. LAB in the microbiota prevents the colonization and invasion of the intestine by competing with the pathogens for the food in the environment (Iorizzo et al., 2020b). Metabolism products such as carbon dioxide, organic acids, hydrogen peroxide or ethanol produced by the microbiota play an important role in defense against pathogens (Serna-Cock et al., 2019). LAB also produces bacteriocins. They can biosynthesize many different types of antagonistic molecules (Alvarez-Sieiro, Montalbán-López, Mu, D., & Kuipers 2016). As a component of the microbiota, LAB participates in important interactions in immunomodulation (Foligne et al., 2007). LAB increases anti-inflammatory and pro-inflammatory cytokines. LAB components can directly induce the immune system. It has been determined that LAB also affects lipid metabolism (Kishino et al., 2013). LAB effectively protects both human and animal intestinal epithelial cells from enteric viral infections. *Lactobacillus rhamnosus* and *Lactobacillus casei* Shirota are the most effective species for protection. It has significant antiviral effects on *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Enterococcus faecium* (Maragkoudakis, Chingwaru, Gradisnik, Tsakalidou, & Cencic, 2010).

Thirteen bacterial species belonging to *Lactobacillus* and *Bifidobacterium* genera were determined in honey bees. *Lactobacillus kunkei* was found to be the most dominant species (Vásquez et al. 2012). The honey bee microbiota and bacteria isolated under hive environmental conditions are presented in Table 1.

Functions

The gut microbiome is involved in the growth, development, and reproduction of insects. It has important contributions to their metabolism. These microorganisms enhance effective digestion, promote the absorption of food and synthesize essential nutritional compounds. (Pernice, Simpson, & Ponton, 2014). The insect gut microbiome is often limited due to the lack of interaction between individuals. However, in social insects such as honey bees, the microbiome is more various. Social interactions ensure the diversity of gut microorganisms. Thus, social insects such as honey bees have a gut microbiome that has important functions in nutrition and protection (Engel & Moran, 2013b).

Metabolic Functions

The gut microbiota of honey bees is as important as the mammalian microbiome. Intestinal microbiota has very important functions especially in nutrient biosynthesis and biomass degradation. In nutritional function, the insect microbiome has been proven to help produce nutrients such as vitamins and amino acids that are not found in food. In biomass degradation and digestion, it was determined that the release of cellulolytic enzymes responsible for hydrolysis and the activity of microorganisms increase (Shi, Syrenne, Sun, & Yuan, 2010; Anderson & Ricigliano, 2017; Belkaid & Harrison, 2017; Zheng et al., 2017).

The gut microbiota has important functions in the digestion of lipids and proteins, detoxification of secondary plant compounds. It also has positive effects on insect resistance to insecticides, while positively affecting survival, development and egg production (Jing, Qi, & Wang, 2020).

Compared to the gut microbiota of other animals, the honeybee microbiota has effective functions in specific adaptations to a sugar-rich diet. It provides sugar uptake pathways of various phosphotransferase systems to the honeybee. Most carriers are classified in the mannose family. Nectar contains traces of mannose, but becomes highly toxic when ingested at higher concentrations. Therefore, this feature of bacteria is very important (Engel & Moran, 2013b). The bee microbiome enriches the host with arabinose flow permeases. This family of carriers plays a role in the transport of different compounds such as sugars, antimicrobial proteins, and amino acids. Various carriers protect against various pesticides applied in agriculture (Engel & Moran, 2013a). In addition, the intestinal microbiome plays an important role in the conversion of nectar into honey and bee secretions into propolis with its fermentation properties. It is also responsible for the freshness of honey (Pachila, Ptaszynska, Wicha, Olenska, & Małek, 2017; Silva et al., 2017).

LAB, which constitutes an important group of bee microbiota, contributes to the nutrition of honey bees. It has been suggested that bacteria of the genus *Bifidobacterium*, *Simonsiella* and *Lactobacillus* can produce acetic acid, a waste product of carbohydrate metabolism. The microbiota supports the diet of bees by aiding the digestion of these compounds. After consumption, food is stored in the rectum for quite a long time. Thus, extra nutrients are obtained from rectal bacteria during wintering (Martinson et al., 2011).

This effect of the microbiota on body weight gain causes an increase in the level of vitellogenin, which is responsible for the regulation of nutrition in honey bees, by making insulin and insulin-like signal changes. This is associated with changes in gene and endocrine signal expression (Ihle, Baker, & Amdam, 2014; Zheng

et al., 2017). In a study, it was determined that *Bifidobacterium asteroides* stimulated the production of juvenile hormone and host-derived prostaglandin derivatives, which are effective in bee growth (Kešnerová et al., 2017).

Intestinal microorganisms can also prolong the lifespan of insects. It was determined that the life span of *Drosophila melanogaster* increased significantly after probiotic and symbiotic applications (Westfall, Lomis, & Prakash, 2018). The microbiome synthesizes enzymes such as glycosidases and proteases. These enzymes synthesize the vitamins necessary for the host, metabolize indigestible polysaccharides, and are responsible for xenobiotic metabolism. With these features, the microbiota contributes significantly to the biochemical activities of the host (Sommer & Bäckhed, 2013).

Carbohydrates and oligosaccharides that cannot be digested by the host are fermented by microbiome bacteria of the genus *Bifidobacterium*, *Bacteroides*, *Faecalibacterium*, and *Roseburia* to form short-chain fatty acids (SCFAs) such as propionate, acetate, and butyrate (Jandhyala et al., 2015; Wang et al., 2020). These SCFAs formed provide rich energy sources to the host. The resulting butyrate prevents the accumulation of toxic by-products for metabolism (Jandhyala et al., 2015). Pollen is an important bee food. It is the most important source of vitamins, minerals, amino acids, and fats. Most of these are absorbed by the host midgut. Compounds that are the most difficult to digest, such as cellulose, emicellulose, pectin, remain. These compounds are degraded by the hindgut microbiota (Mollet, Leroux, Dardelle, & Lehner, 2013).

Immune System Functions

Honeybee microbiota has important functions in regulating and stimulating the immune system of honey bees and in the formation of an effective immune response. Microbiota species are very effective in the development and morphogenesis of the immune system (Egert & Simmering, 2016; Schroeder & Bäckhed, 2016; Kwong, Mancenido, & Moran, 2017). Microorganisms alter the intestinal environment, limiting the growth of insect parasites and stimulating the host immune system. Additionally, species in the microbiota produce antimicrobial peptides that play an important role in control and defense against bacterial pathogens and parasites. The overall microbiome or *S. alvi* colonization alone increases the regulation of the antimicrobial peptides apidaesin and hymenoptaesin in intestinal epithelial cells (Azambuja, Garcia, & Ratcliffe, 2005; Kwong et al., 2017). Antimicrobial peptides are important innate immune components that play a role in the destruction of pathogenic microorganisms. A high increase in these antimicrobial peptides was detected in honey bees treated with probiotics (Kwong et al., 2017). *F. perrara* is a member of the microbiota in the majority of honey bees. It was determined that

the host immune response increased considerably by colonizing the pylorus, which is the midgut ileum transition region (Powell et al., 2014).

The largest surface area in contact with foreign antigens originating from the external environment is the intestinal microbiota. It therefore plays a primary role in mucosal immunity (Sekirov, Russell, Antunes, & Finlay, 2010). The microbiome controls the overgrowth and migration of microorganisms in mucosal immunity, while preventing the induction of harmful systemic immune response (Purchiaroni et al., 2013). It can affect the host by altering the composition of the gut microbiome. *Ascospaera apis*, *Nosema* sp., *Paenibacillus larvae*, *Melissococcus plutonius*, and *Serratia marcescens*, such pathogens cause significant infections in honey bees. LAB, an important member of the microbiota, is highly effective in protecting against these pathogens. (Wu et al., 2014; Arredondo et al., 2018; Iorizzi et al., 2020b; Peghaire et al., 2020).

Bacteria constituting the microbiome control intestinal homeostasis through various mechanisms including lipopolysaccharide, peptidoglycans, and flagellin. These structures interact with cell receptors in the toll like pathway. This mechanism activates intracellular signaling pathways associated with cell survival, inflammatory response, replication, and apoptosis (Evans et al., 2006; Valentini et al., 2014; Yiu, Dorweiler, & Woo, 2017).

The gut microbiome is involved in xenobiotic metabolism. This ability ensures the preservation of the microbiome, which is a necessary condition for drugs used in the treatment of various diseases to be effective (Bäckhed et al., 2004; Vergnolle, 2016).

Honey bees are frequently exposed to a wide variety of pesticides. The microbiota also plays an important role in the detoxification of xenobiotics, particularly neonicotinoid insecticides. Microbiota microorganisms stimulate the expression of detoxification enzymes. Thus, an effective endogenous detoxification occurs in the host, increasing thiacloprid and fluvalinate resistance (Wu et al., 2020).

Social and Individual Behavior Functions

It has been proven by various studies that gut microbiota and brain compatibility. This means that gut microorganisms cause changes in host neurophysiology and insect behavior (Westfall et al., 2018; Leger & McFrederick, 2020).

Microbiota species can alter both the volatile profiles and olfactory behaviors of the host. As a result, they regulate the way individuals make decisions regarding interacting, gathering in groups, foraging, and mating through chemical communication. In addition, intestinal microorganisms increase the host's memorization and learning capacity. Thus, it also affects the neurophysiological development of the host by supporting memory (Liberti & Engel, 2020). It has been determined that the gut microbiome of honey

bees influences the neurophysiology and behavior of their hosts. Levels of biogenic amines such as octopamine, dopamine, and serotonin can be altered by microbiota species, affecting host behavior. The levels of these compounds in the brain of worker bees change seasonally. Levels of the compounds increase during the summer months, when foraging activity is at its highest. The brain activity of newly developed bees, whose microbiome is not yet fully formed, is significantly lower than that of older adults (Harris & Woodring, 1992). Microbiota plays an important role in the regulation of social behavior in honey bees (Vernier et al., 2020).

Factors affecting honey bee microbiota

Many factors affect the microbiome, such as the host species, feeding preference, host habitat, and the host life stage. Especially the high diversity of plants causes changes in the honey bee microbiome, especially *Proteobacteria* and *Firmicutes* species (Jones et al., 2017).

Many pesticides such as imidacloprid, coumaphos and chlorothalonil have serious adverse effects on bee health. It also causes significant problems by negatively affecting the structure and function of the microbiome. It also increases susceptibility to opportunistic pathogens. Honey bees are exposed to pesticides through contaminated water, nectar and pollen. It was determined that the amount of *Lactobacillales* decreased significantly in honey bees exposed to chlorothalonil. Non-lethal doses of insecticides such as thiamethoxam, fipronil, imidacloprid and coumaphos caused *Bifidobacterium* sp. and *Lactobacillus* sp. has been found to greatly reduce the number of (Rouzé, Moné, Delbac, Belzunces, & Blot, 2019). The gut microbiome of honey bees exposed to glyphosate was adversely affected, affecting the total bacterial count in the gut and *Bifidobacterium* sp., *Lactobacillus* (Firm-4 and Firm-5), and decreased in *S. alvi* (Motta et al., 2018). Nitenpyram, a neonicotinoid insecticide, has caused significant changes in the microbiome community. Due to the insecticide effect, important metabolic changes have occurred in the host and the effectiveness of the immune system has decreased (Zhu, Qi, Xue, Niu, & Wu, 2020). Pesticides such as imidacloprid and thiacloprid, *Bifidobacterium* sp., *Frischella* sp. and *Lactobacillus* (Firm-4 and Firm-5), produce numerous adverse changes in various species, causing intestinal dysbiosis (Daisley et al., 2017; Diaz, Del-Val, Ayala, & Larsen, 2019).

Honeybee gut core microbiota is also affected by global seasonal changes (Rouzé et al., 2019). While the amount of Enterobacteriaceae decreased in autumn, Neisseriaceae increased (Bleau et al., 2020). The lowest diversity in the microbiome was detected in winter (Purchiaroni et al., 2013).

As a result of the high-fat honey bee diet containing palm oil, the amount of *Gilliamella* sp. in the

microbiome increased significantly, while the rate of *Bartonella* sp. decreased (Wang et al., 2021).

Antibiotics can affect the host by causing changes in the species that form the gut microbiota. (Daisley et al., 2020). Antibiotic applications cause a decrease in bacterial species in the honeybee microbiota. This weakens the immune system of bees and increases their susceptibility to infections (Raymann, Bobay, & Moran, 2018b). In one study, the microbiome was destroyed as a result of antibiotic administration. As a result, the expression of genes encoding antimicrobial peptides decreased and honey bees became more susceptible to *Nosema ceranae* infection (Li et al., 2017). In another study, disruption of the gut microbiota by tetracycline was caused by the opportunistic pathogen *Serratia* sp. increased infections and shortened the life span of bees (Bonilla-Rosso & Engel, 2018).

Air pollution, microplastics and heavy metals also negatively affect the honeybee gut microbiome composition (Mutlu et al., 2018; Costa et al., 2019; Rothman, Leger, Kirkwood, & McFrederick, 2019).

Conclusion

Honey bee microbiota and microbiome are very important for growth, development, metabolism and immune system. It is especially effective in inhibiting pathogens in bee health. Misfeeding, mistakes in colony management and beekeeping practices, unnecessary and continuous antibiotic applications affect the microbiota negatively. Feeding and practices that support and strengthen the microbiota are extremely important for healthy, strong and highly productive beekeeping.

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