

# Honeybee Gastrointestinal Bacteria for Novel and Sustainable Disease Control Strategies

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Agricultural economic development strongly relies on the health of pollinators including honeybees. Honeybee health is afflicted by multiple risk factors such as toxicity from pesticide application, shortage of floral resources, climate change, reduction in genetic diversity and diseases caused by various pathogenic bacteria, fungi and viruses. Controlling disease is necessary for maintaining honeybee health and this will benefit both agricultural and apicultural industries. As other insects and animals, honeybees possess a diverse set of individual or colony level disease defense mechanisms. One route by which honeybees combat diseases is through the shielding effects of gastrointestinal bacteria. Except for some transient bacteria, a set of consistent and distinctive phylotypes of bacteria colonize honeybee digestive tracts. The beneficial bacterial communities inhabiting honeybee guts play key roles in maintaining honeybee health not only by participating in nutrient processing but also by enhancing the immune system, and inhibiting the growth of pathogenic organisms with metabolic products or obstruction of pathogen colonization. Therefore, a symbiotic microbial balance in the honeybee digestive tract is critical for protecting honeybees from disease and other risks. Many researchers have emphasized the beneficial roles of gastrointestinal bacteria in sustaining honeybee health and suggest their contributions to development of novel and sustainable disease control strategies.

**Key words:** Honeybee health, Digestive tract, Gastrointestinal bacteria, Sustainable, Disease control

## 1. Introduction

Pollinators play vital roles in the maintenance of wild ecosystems and agricultural production. Honeybees are beneficial insects for humans as the most economically valuable pollinators. One-third of the food consumed in the world is linked to pollination by insects including honeybees and the worldwide total economic value of insect pollination amounted to €153 billion in 2005 (Gallai *et al.*, 2009). On the other hand, the value of bee pollination to natural plant biodiversity is not simply estimable (Moritz *et al.*, 2010). Recently, honeybees are facing serious health threats and widespread population declines, through problems such as colony collapse disorder (CCD), es-

pecially in commercial beekeeping industries in both the United States and Europe (Ellis *et al.*, 2010; Potts *et al.*, 2010). Although there has been an almost 50% decrease in world honeybee stocks over the last century, human demands for pollinator-dependent crops to maintain health are simultaneously increasing by >300% (Aizen and Harder, 2009). Such an importance of honeybees for human welfare causes great concern about the health of honeybees.

Honeybee diseases are considered major afflicting factors of honeybee health and huge colony losses are linked to these diseases (Cox-Foster *et al.*, 2007). Consequently, disease control is a most challenging task for protecting honeybee populations. At present, honeybee disease control mainly depends on antibi-

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otics such as tetracycline and fumagillin (Arbia and Babbay, 2011; Williams *et al.*, 2008). However, using antibiotics in apiculture is legally banned in many countries of the European Union (Mutinelli, 2003), because of risks they present for both human and honeybee health (Martel *et al.*, 2006; Pettis *et al.*, 2004; Thompson *et al.*, 2005). In addition, uses of antibiotics may also lead to the emergence of resistant bacterial strains (Miyagi *et al.*, 2000). Therefore, novel and sustainable disease control methods are urgently needed to improve honeybee health and provide benefits for agriculture by increasing yield and quality of crop production.

Honeybees harbor a diverse assemblage of microbes including bacteria in their gastrointestinal tract (Gilliam *et al.*, 1988; Gilliam and Prest, 1987). Previous studies well document the roles of beneficial gastrointestinal bacteria for food fermentation, preservation of food stores (Gilliam, 1997; Gilliam *et al.*, 1989) and inhibition of the growth of pathogenic bacteria (Evans and Armstrong, 2006). Gastrointestinal bacteria are receiving great interest due to their potentiality as an alternative for sustainable disease control in honeybees. To develop novel and sustainable disease control strategies for honeybees, better understanding of the associations between gastrointestinal bacteria and honeybees are needed. In this review, we briefly introduce honeybee diseases and discuss disease control methods for protecting honeybee health. Subsequently, we briefly discuss the gastrointestinal bacteria associated with honeybees and their potential roles for improving disease control strategies.

## 2. Honeybee Diseases and Controls

Population declines in honeybees and other wild bees in the United States, Europe and elsewhere has led to worldwide concerns about its impact on the yield of agriculture and biodiversity of natural plants (Biesmeijer *et al.*, 2006; Breeze *et al.*, 2011; Gallai *et al.*, 2009; Garibaldi *et al.*, 2011). Many researchers have studied and revealed some risk factors that threaten honeybee colonies; including pathogens, malnutrition, pesticides, beekeeping practices, climate change and genetic diversity (Stokstad, 2007; vanEngelsdorp *et al.*, 2008). Although multiple drivers and interactive effects may be responsible for the widespread declines of pollinator bees (Didham *et al.*, 2007; Neumann and Carreck, 2010; Oldroyd, 2007),

diseases caused by various pathogens are recognized as one significant factor afflicting honeybee health (Cox-Foster *et al.*, 2007).

Honeybees are vulnerable to various pathogens such as bacteria, viruses, protozoa, fungi, and parasitic mites and subjected to heavy damages every year. There are many types of diseases that threaten honeybee colonies, well known as bacterial diseases (American Foulbrood, European Foulbrood), fungal diseases (Chalkbrood, Stonebrood), viral diseases (Sacbrood Virus, Cloudy Wing Virus, Chronic Paralysis Virus, Black Queen Cell Virus, Kashmir Bee Virus) and protozoan diseases (Nosemosis) (Allen and Ball, 1996; vanEngelsdorp and Meixner, 2010; Wolfgang and Pongthep, 2006). These diseases cause large amounts of economic losses in apiculture and agriculture of the world by affecting survival of managed and wild honeybees. To control these diseases from afflicting honeybees, present treatments typically rely on chemicals such as antibiotics, acetic acid and other chemicals (Thompson and Brown, 2001; Wolfgang and Pongthep, 2006). However, the application of chemicals in apiculture is of great concern because of chemical residues in honeybee products for human consumption (Mutinelli, 2003), and toxicity to honeybee broods and honeybee beneficial intestinal microflora (Pettis *et al.*, 2004; Thompson *et al.*, 2005). Therefore, there is an urgent need for development of alternative novel and sustainable disease management strategies to protect honeybee health.

## 3. Honeybee Gastrointestinal Bacteria

As in all animals, the gastrointestinal tract of adult honeybees is a complex ecosystem that harbors diverse microbial communities including bacteria and these bacteria play a key role in maintaining host honeybee health (Gilliam, 1997; Servin, 2004). Except for some older larvae that acquire microorganisms through ingestion of contaminated food; eggs, larvae, pupae and newly emerged adult honeybees are usually free of internal microorganisms. However, because of pollen or beebread consumption, and food exchange with other honeybees in a colony, adult honeybees become inoculated with various microbes after emergence (Gilliam and Prest, 1987). With the exception of some constant microorganisms, species composition of gastrointestinal microbes varies with honeybee age, season and geographical differences (Gilliam *et al.*, 1988; Gilliam and Valentine, 1974). Early studies on iden-

tification of microbes associated with honeybees are restricted to only species that can be cultured and revealed that the gastrointestinal microbiota consist of Gram-positive bacteria such as *Bacillus*, *Lactobacillus*, *Bifidobacterium*, *Corynebacterium*, *Streptococcus*, *Clostridium*, and Gram-negative and Gram-variable bacteria such as *Achromobacter*, *Citrobacter*, *Enterobacter*, *Erwinia*, *Escherichia coli*, *Flavobacterium*, *Klebsiella*, *Proteus*, and *Pseudomonas* (Gilliam, 1997). However, recent culture-independent 16S rRNA sequencing and metagenomic surveys suggest that a consistent microbial community, contrary to the results of previous culture-dependent studies, dominate the honeybee digestive tract despite of different honeybee species, colonies and geographic locations (Babendreier *et al.*, 2007; Cox-Foster *et al.*, 2007; Jeyaprakash *et al.*, 2003; Mohr and Tebbe, 2006). Therefore, except for some constant bacterial phylotypes, bacterial communities in the honeybee digestive tract are affected to by life stage, geographic location and species (Ahn *et al.*, 2012; Disayathanoowat *et al.*, 2012). Variance in the composition of gut bacteria reported may also result from different methodologies and sampling strategies in independent studies. However, it is clear that honeybees harbor two different types of microorganisms, resident and transient bacteria, in their digestive tracts. Although honeybees are microorganism free at the beginning of their life cycle, transference between generations and maintenance of resident gut microbiota may result from the honeybee colony social behavior (Martinson *et al.*, 2011). Other recent research also indicates consistent microbial composition of honeybee guts in individuals, even though emergence frequencies may differ within individuals and some distant bacterial phylotypes may exist in some individuals (Moran *et al.*, 2012). This result together with discovery of lactic acid bacteria (LAB) of the genera *Lactobacillus* and *Bifidobacteria* originating from the honey stomach (Olofsson and Vásquez, 2008), give support to our conclusions that some bacteria associated with the honeybee digestive tract have evolved mutually with honeybees and some bacteria are acquired accidentally from the environment. Moreover, colonization of honeybee resident bacteria in the digestive tract favor different organs of the gut (Martinson *et al.*, 2012) and may serve particular functions for their host. Honeybees worldwide harbor a set of simple and distinctive gastrointestinal microbial community, whereas the genetic diversity within

the bacterial species dominating the honeybee gut leads to functional diversity in host interactions, bio-film formation and nutritional digestion (Engel, 2012). Such functional diversity at the bacterial strain level and differences in colonization patterns in the gut niche may diversify and enhance the beneficial roles of the simple set of resident gastrointestinal bacteria for maintaining honeybee health.

#### 4. Honeybee Health: Potential Role of Gastrointestinal Bacteria

The gastrointestinal tract of animals is a complex ecosystem that protects hosts from attack by pathogens through physical and chemical barriers created by the gastrointestinal epithelium (Bevins *et al.*, 1999; Kagnoff and Eckmann, 1997). In addition, microbiota colonizing the gastrointestinal tract environment, together with epithelial cells, play important roles in normal gut function and maintaining host health (Berg, 1996). Early studies on interactions between honeybee and gastrointestinal microbes have mainly focused on the nutritional activities of bacterial communities in honeybee guts, such as functions in food fermentation and food preservation (Gilliam *et al.*, 1989; Human and Nicolson, 2006). However, the advent of modern microbiology and methodologies have led to improvements in our understanding of many other roles for gastrointestinal microbiota such as maintaining honeybee health by combating disease pathogens (Evans and Spivak, 2010; Parker *et al.*, 2011).

Previous studies have confirmed that bacterial probiotics can induce immune responses (Evans and Lopez, 2004) and contribute to maintaining a healthy bee colony (Patruica and Mot, 2012). There is also increasing evidence that some species of honeybee endogenous intestinal bacteria or bacterial metabolic products show antagonistic activities to pathogens that threaten honeybee health such as *Ascospaera apis*, the causative agent of Chalkbrood disease; *Paenibacillus larvae*, the causative agent of American Foulbrood and *Nosema ceranae*, the causal microsporidian of Nosemosis (Evans and Armstrong, 2005; Forsgren *et al.*, 2010; Sabaté *et al.*, 2009; Yoshiyama and Kimura, 2009). Furthermore, our present studies also demonstrate that bacteria isolated from the gastrointestinal tract of the Japanese honeybee inhibit the growth of *Melissococcus plutonius*, the causative agent of European Foulbrood (EFB) (Wu *et al.*, 2013). These studies on antagonistic activity to various path-

ogens indicate that gastrointestinal bacteria function in the disease defense system of honeybees by yet unknown mechanisms.

To provide further insight into the disease preventative functions of gastrointestinal microbiota, numerous mechanisms by which gastrointestinal bacteria can defend against infection with pathogens have been postulated by intensive studies. These results suggest that in addition to the competitive inhibition of the epithelial and mucosal adherence of pathogens and inhibition of epithelial invasion by pathogens, these bacteria also enhance the immune system of the host (Bibiloni *et al.*, 2001; Evans and Lopez, 2004; Gopal *et al.*, 2001; Nicaise *et al.*, 1999). Moreover, gut bacteria may also show antagonistic activity against pathogens by producing various antimicrobial substances such as antimicrobial peptides/molecules, fatty acids and H<sub>2</sub>O<sub>2</sub> (Klaenhammer, 1993; Servin, 2004). Although little is known about how members of the intestinal microbiota interact with honeybees to establish mutually beneficial relationships and what mechanisms function in their defense system, gastrointestinal microbiota attract great attention because of their potential roles in the protection of honeybee health and development of honeybee disease management strategies.

## 5. Conclusion

In conclusion, honeybees harbor distinct bacterial communities in their digestive tracts and take advantage of these gastrointestinal bacteria despite some bacteria are disease causing. Besides functions related to nutritional consumption of plant pollen or nectar, recent findings reveal crucial roles of bacterial communities associated with honeybees in enhancing the immune system and disease prevention. This suggests that the gut bacterial community structure may become an indicator of honeybee health and maintaining the balance of these gut bacteria is critical for sustaining honeybee health and vigor. In spite that more research is needed to clearly understand the microbial defense activity mechanisms of honeybees, we can conclude that gut microbial communities provide an important novel tool to improve disease management strategies and contribute to the development of modern sustainable agriculture.

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