CHAPTER 2

Literature review

2.1 Honey bees

Honey bees are classified in the genus *Apis*, family Apidae, and order Hymenoptera. Genus *Apis* includes nine species: the cavity-nesting honey bees, *A. mellifera*, *A. cerana*, *A. koschevnikovi*, *A. nuluensis*, and *A. nigrocincta*; the giant honey bees, *A. dorsata* and *A. laboriosa*; and the dwarf honey bees, *A. florea* and *A. andreniformis* (Oldroyd and Wongsiri, 2006). Honey bees live in large societies which can be regarded as a superorganism. These societies have several groups of specialist individuals performing functions that are loosely analogous to the organs of metazoan organisms. They are eusocial species due to cooperation in the rearing of the young, and have a pronounced reproductive division of labor and overlapping generations.

There are three castes of adult honey bees: queens, drones, and workers, which are different in morphology, physiology, and their behaviors (Figure 2.1). Queens and drones have been particularly associated with reproduction. Workers have many responsibilities in the hive.

Queens begin life from a fertilized egg. They are diploid and mate with many haploid males (drones). They are reared in special cells and treated with special food from secretions of the hypopharangeal glands of the workers. They have a long and slender abdomen that is larger than other castes. Many of the task-related structures found in workers are reduced or absent in the queens. Queens have some well-developed glands for pheromone production. The queen'sovaries are enormous because of her egg-laying function. These ovarioles can produce an unlimited number of egg soften as many as one million or more during the queen's lifetime. Queens are the most long-lived of the three castes, generally surviving for 1-3 years (Seeley, 1978).

Drones are recognizable by their large eyes and short tongues. They arise parthenogenetically from unfertilized haploid eggs. Drones have only one significant function: mating. Since drones perform no work for the colony and are fed by workers, the drone's work-related structures are reduced or absent. In contrast, orientation, flight, and mating-related structures are highly developed. Drones mate only once and then die. Drones live an average of 21-32 days, although mean life spans have been observed as short as 14 days (Fukuda and Ohtani, 1977).

The majority of the population in the hive is workers, which are all female. Workers are small, have short abdomens, and have pollen baskets on their hind legs that are used to tote pollen back from the field. Workers perform brood rearing, comb building, and foraging. At the early stage of development, workers are house bees. As they get older, their duties are outside of the hive as foraging bees. Workers have mean longevities of 15-38 days depending on race, seasonal factors, food availability, and activities performed during their lifetime.

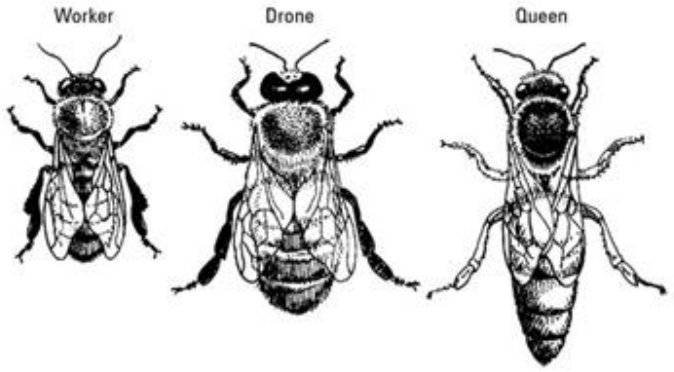

Figure 2.1 Morphology of three castes of honey bee (http://honeybeenet.gsfc.nasa.gov/Honeybees.htm).

2.1.1 Life-cycle of the honey bees

Development of all three castes involves a transition through four major stages: egg, larva, pupa, and adult (Figure 2.2). In this study, workers (day 1) are called emerged bees. The queen lays eggs in worker or drone cells. The fertilized eggs can develop into either workers or queens whereas unfertilized eggs develop into drones. The embryo grows inside the egg for 3 days, consuming the protein-rich egg yolk. Larvae are fed by the adult bees and grow tremendously in size. Their food is a mixture of honey, pollen, and brood food secreted from the hypopharyngeal glands of the adult nurse bees. A larval bee's feeding ceases when she has lived about 8 days, at which time the adult workers construct a wax capping that seals the larva in its cell. While cells are uncapped, the larvae spin their cocoons and change into pupae. Pupal development is construction process. Their nourishment comes from the large larval cells that are digested. When the transformation is complete, the teneral adult chews its way out of the cells and emerges as a soft young bee. All organs finish developing during the next few days. In the domestic honey bee, *A. mellifera*, the whole process from egg to adult can take as little as 16 days for queens or as long as 24 days for drones. The development times as well as the quality of the emerged adult are particularly dependent on temperature, nutrition, and the race of bee (Winston, 1987).

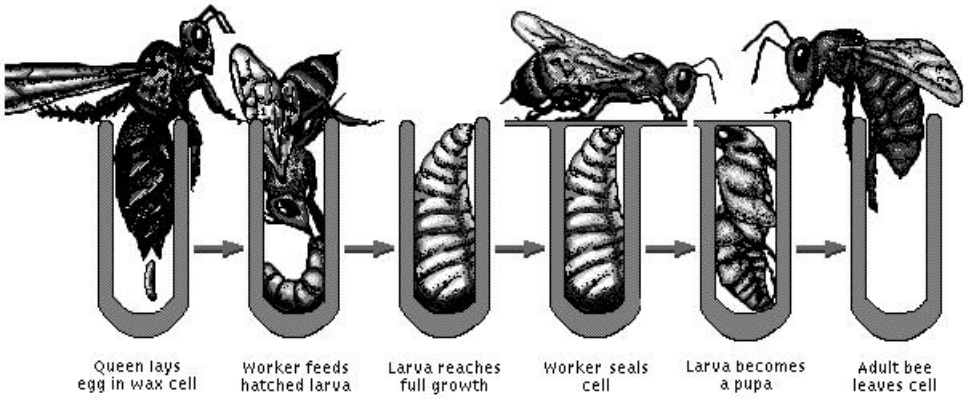

Figure 2.2 Honey bee life cycle from egg to adult (www.ebka.org).

2.1.2 Honey bee anatomy

As in most other insects, the body consists of external and internal structures. The external structure is comprised of three regions which mainly aid in protection. Internal structures are internal organs involved in circulation, digestion, and stinging.

2.1.2.1 External structure

The outer layer consists of a series of hardened plates covered by a dense pile of hairs. This external skeleton provides protection from predators, prevents water loss, serves as a framework for internal muscle attachment, and allows rapid but precise movements by a complex arrangement of internal ridges against which the muscles can contact. The exoskeleton and internal parts are arranged in three regions: head, thorax, and abdomen. The head functions largely to ingest and partially digest food through the mouthparts and associated glands, and also serves as the major sensory region of the body via the eyes, antennae, and sensory hairs. The thorax is made up of three segments, each bearing one pair of legs. The thorax is the principal locomotory region of a bee's body and contains powerful muscles for flight, walking, and specialized function, such as pollen collection. The abdomen consists of seven visible segments and contains the bulk of the internal organs and a sting. The sting apparatus lies tucked inside a special sting chamber in the last abdominal segment (Figure 2.3).

2.1.2.2 Internal structure

2.1.2.2.1 Circulatory, respiratory and nervous systems

The bee circulatory system is an open system with only a dorsal heart and aorta to assist in blood circulation (Figure 2.4).Bees use a system of tubes that carry oxygen and carbon dioxide away from cells. Tracheae are breathing tubes and have a series of holes in the cuticle called spiracles. The nervous system is simple, consisting of a brain and seven ganglia or nerve centers at various junctions throughout the body.

2.1.2.2.2 Glandular systems

The glands of worker honey bees are used for four basic functions: wax production, communication, defense, and food processing. Wax is produced by modified epidermal cells located ventrally on the fourth to seventh abdominal segments. The Nasonov gland, mandibular glands, setose sting glands, and tarsal Arnhart glands produce odors used in communication. The poison gland, a large sac associated with the sting, holds the venomand performs a defensive function. There are two types of food processing glands: those which partially digest food and those involved in brood food production. The digestive types are the labial or salivary glands, including a gland in the posterior part of the head, as well as the thoracic labial gland (Figure 2.5).

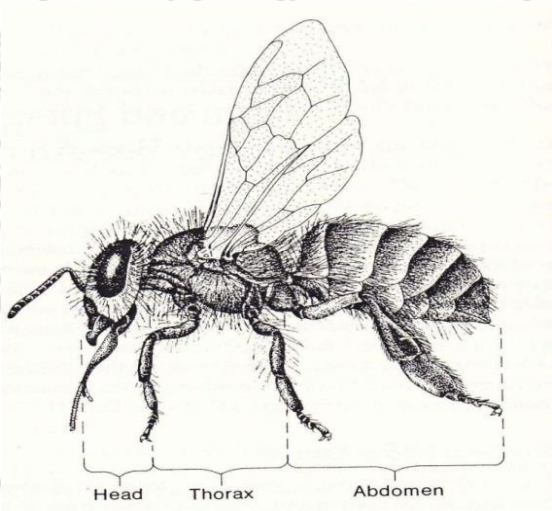

Figure 2.3 The three major body regions of honey bee worker (Winston,1987).

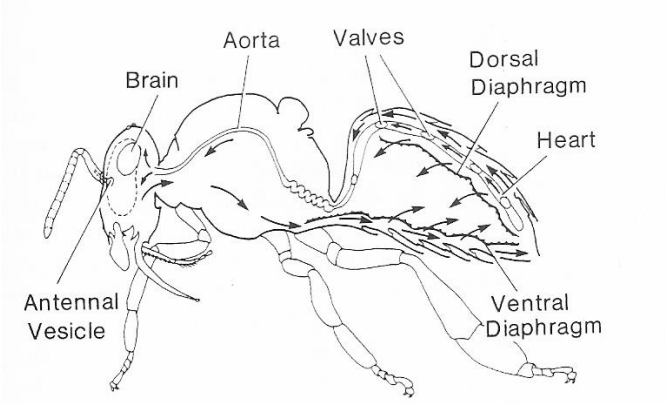

Figure 2.4 The honey bee worker circulatory system (Redrawn from Dade, 1977).

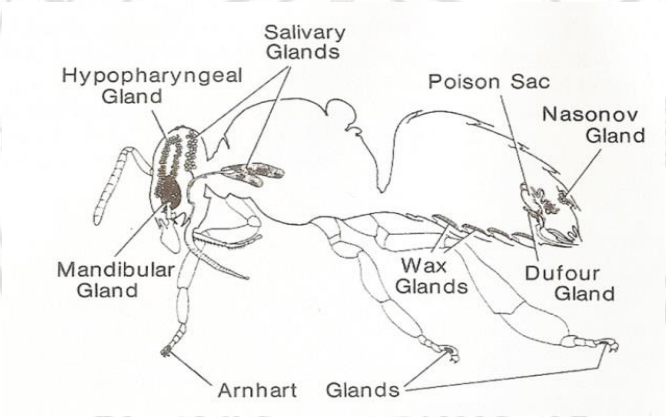

Figure 2.5 The worker glandular system (Redrawn from Michener, 1974).

2.1.2.2.3 Digestive and secretory system

Larvae have huge digestive system. Most of the body cavity is taken up by the midgut and hindgut with the enzyme-secreting salivary glands and the excretory tubules being the other principle internal structures (Figure 2.6). The larvae defecate early in cocoon construction; the secretory tubules and midgut are closed off until feeding is completed, when their contents are discharged into the base of the cell. In pupal stage, the internal organs undergo massive changes into their adult forms. The digestive system of adult honey bees is located primarily in the abdomen, connected to the mouth via the long esophagus (Figure 2.7). The posterior end of the esophagus opens into the crop or honey

stomach, an expandable bag that holds honey ingested in the hive and used for energy during flight, and nectar or water collected in the field by workers for transport back to the nest. A valve at the end of the crop, the proventricular value prevents most of the liquid crop contents from passing through to the ventriculus, or midgut. Pollen grains are filtered from the crop along with some of the liquid crop contents and passed onto the midgut, where most of the digestion and absorption take place. Solid wastes, consisting primarily of pollen husks' fat globules and dead midgut cells, are then passed through the intestine to the rectum for excretion. Liquid nitrogenous wastes are absorbed from the blood by the Mulpigian tubules and passed to the intestine for excretion. The rectum expands considerably to hold waste material and excrete outside the hive.

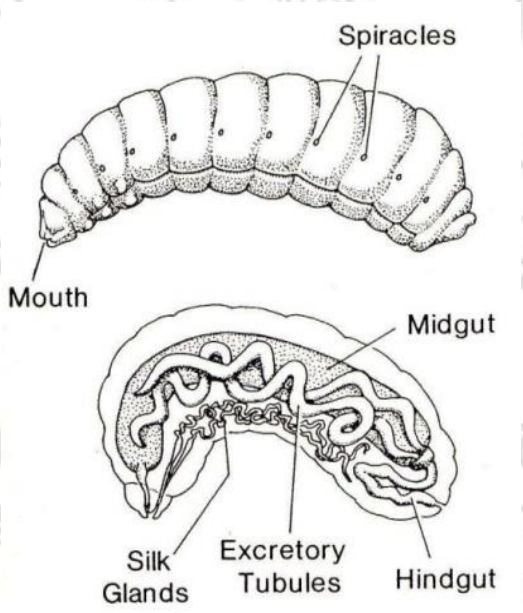

Figure 2.6 External and internal anatomy of a worker larva (Redrawn from Dade, 1977).

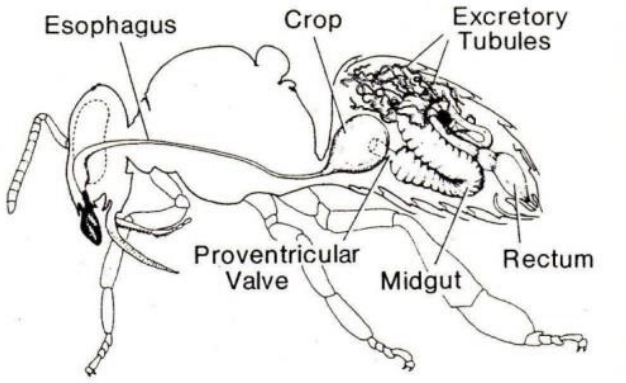

Figure 2.7 The digestive and secretory system of a worker bee (Redrawn from Michener, 1974 and Dade, 1977).

2.1.3 Asian honey bees

There are three subgenera of the genus *Apis*: *Megapis* (giant), *Micrapis* (dwarf), and *Apis* (cavity-nesting). In this study, two species (*A. dorsata* and *A. florea*) were investigated because they are wild honey bees and cannot be framed on an industrial scale. However, these two species have many differences between them (Table 2.1).

2.1.3.1 Giant Asian honey bee (*Apis dorsata*)

A. dorsata is found throughout South and Southeast Asia. It builds a large, single comb in the open and its behavior is ferocious. The combs of *A. dorsata* are always built on the undersurface of a stout branch or building. In large colonies, the combs are massive (1.5 m x 1 m) and the number of workers can be over 50,000 (Figure 2.8A).This species has a strong tendency to be highly aggregated onto a single water tower and tree. Individual workers are huge (17 mm long). Apart from the great size, the giant bees are distinguished from all other honey bees by their wings, which are fuscous and hairy. The size dimorphism between castes is much less pronounced than in other *Apis*. Queens (20 mm) are slightly longer than workers (17 mm), and drones slightly shorter (16 mm) (Figure 2.9). The honey-storage area is usually quite obvious because the cells are highly elongated up to

15 cm long. The amount of honey stored is modest only amounting to about 10 kg, and often much less or even none.

2.1.3.1 Dwarf Asian honey bee (*Apis florea*)

A. florea is distributed from the Middle East, east to peninsular Malaysia. It builds a small single comb in the open (Figure 2.8B). It is not defensive. The favored nest sites are racemes of coconut palm inflorescences and small shrubs. The top of the single comb is built around a small tree branch. The crown of the comb, where honey is stored, is a messy affair. *A. florea* has a generally reddish-brown (rufous) appearance and pale yellow to white hairs on the hind tibia and basitarsis. The average dimensions of *A. florea* combs are 12 cm. Queens and drones are much larger than workers. Workers are on average 25.5 mg (8 mm), whereas drones are about 80 mg, and queens are 86 mg (Figure 2.9).

(A) **(B)**

Figure 2.8 The nests of *A. dorsata* (A) and *A. florea* (B) (Photo by P. Saraithong and http://en.wikipedia.org/wiki/Apis_florea).

Table 2.1 Comparison of the two Asian honey bee species (Seeley, 1985).

Honey bee species	*A. dorsata*	*A. florea*
Worker body size (mg, partially loaded)	155	32
Nest site	Tree limb or cliff	Branch of shrub
Height (m)	High (>15)	Low (<5)
Visibility	Conspicuous	Hidden
Dispersion	Clustered	Widely spaced
Colony population	37,000	6,000
Aggressiveness	High	Low
Movements	Migratory	Local
Foraging area (km^2)	Large (300?)	Small (<3?)
Mass (kg)	6.0	0.2

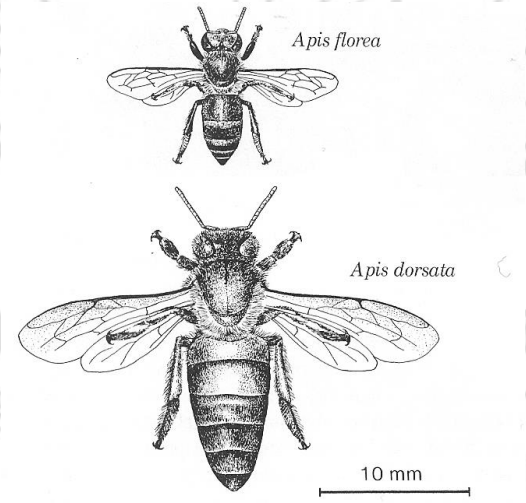

Figure 2.9 Workers of *A. dorsata* and *A. florea* (Seeley, 1985)

2.2 Honey bee and microorganism

Honey bees encounter with pathogenic and non-pathogenicmicrobes including viruses, bacteria, and fungi. Most commensal bacteria reside in the gut. Microbiota govern an immenserange of functions involved with the host's development, pathogen resistance, nutrition, and physiology (Engel and Moran, 2013). However, previous studies focus on bacterial communities in *A. mellifera* and *A. cerana* guts (Cox-Foster et al., 2007; Martinson et al., 2011; Disayathanoowat et al., 2012; Moran et al., 2012). Little is known about microorganisms related to wild honey bees.

2.2.1 Honey bee pathogens

2.2.1.1 Viruses

Nearly twenty positive-strand RNA viruses were detected in honey bees. They are in the family Dicistroviridae and Iflaviridae, with additional taxa that are not yet formally placed to family. Pathogen viruses belong to family Dicistroviridae, such as Israeli acute paralysis virus (IAPV), acute bee paralysis virus (ABPV), Kashmir bee virus (KBV), and black queen cell virus (BQCV). Family Iflaviridae is deformed wing virus/Kakugo virus (DWV/KV), *Varroa destructor* virus (VDV-1), sacbrood virus (SBV), and slow bee paralysis virus (SBPV). Unclassified RNA viruses are chronic bee paralysis virus (CBPV) and Lake Sinai virus (LSV-1, LSV-2) (Evans and Schwarz, 2011). Although RNA viruses predominate in honey bees, DNA viruses have occasionally been reported (Clark, 1978). Bee viruses affect the morphology, physiology, and behavior of bees and have been widely associated with weak and dying colonies. After *V. destructor* emerged, both DWV and ABPV became worrying issues. DWV is a benign virus mainly causing covert and symptomless infections (Hails et al., 2008), as long as it is transmitted vertically (through drones and queens) or horizontally (through larval food) (Yue and Genersch, 2005; Yue et al., 2006; 2007). *V. destructor* transmits DWV to pupae, causing deformed wings, shortened and bloated abdomen, and miscolouring (Yang and Cox-Foster, 2007; Yue and Genersch, 2005). ABPV also transmits by *V. destructor* but the virulence still remains

elusive. The same member of ABPV, IAPV was related to severe colony mortality (Maori et al. 2007). So far, it is less knowledge about the transmission and pathomechanisms of IAPV.BQCV and DWV were detected in *A. dorsata* and *A. florea* by coexisting or individual infection. The phylogenetic studies reveal the spread of virus from *A. mellifera* to wild bees. The genetic relatedness as well as the geographical proximity of host species has an important role in host range of the viruses (Zhang et al., 2012).

2.2.1.2 Bacteria

Two firmicute bacteria, *Melissococcus plutonius* and *Paenibacillus larvae* are the bacterial pathogens known for honey bees. *M. plutonius*, causes European foulbrood (EFB) (Bailey, 1956) and *P. larvae*, causes American foulbrood (AFB) (Genersch et al., 2006). These two bacteria only infect honey bee larvae. *M. plutonius* has previously been reported in *A. mellifera* and two Asian honey bees (*A. cerana* and *A. laboriosa*) (Bailey et al., 1983; Allen et al., 1990). It is a gram positive bacterium formerly known as *Bacillus pluton*. European foulbrood is widespread and has been of economic importance around the world (Matheson, 1993). It has typical disease symptoms: a pepperbox appearance with many uncapped and diseased cells mixed with normal capped cells. Another symptom is concave and punctured caps. Workers, drones, and queen larvae are susceptible to EFB. When larvae ingest food contaminated with *M. plutonius*, bacteria proliferate in the larval midguts. After larvae die from starvation, they are decomposed by secondary invaders, *Paenibacillus alvei* and *Enterococcus faecalis* (Bailey, 1983). These two saprophytic bacteria are frequently found in association with European foulbrood. *Paenibacillus larvae* is a gram positive, spore-forming bacterium. Larvae ingested spores pass through the foregut and germinate in the larval midgut, causing larvae to become a brownish, semi-fluid, and glue-like colloid (ropy stage). American foulbrood is easy to diagnose and control. *Spiroplas maapis* and *Spiroplas mamelliferum* are parasites in adult bees (Mouches et al., 1983; Clark et al., 1985). These bacteria break the gut barrier and invade the hemolymph, causing a systemic infection. However, *Spiroplasma* infections are much more difficult to diagnose.

2.2.1.1 Fungi

Two species of the phylum Microsporidia, *Nosemaapis* and *Nosemaceranae*, are pathogenic for adult honey bees (*A. mellifera* and *A. cerana*). *N. ceranae* were detected only in *A. dorsata* and *A. florea* (Chaimanee et al., 2010). However, both species have received extensive attention. They are obligate intracellular, fungal parasites. The microsporidia invade epithelial cells of the adult midgut and undergo repeated cell divisions to ultimately produce new infectious spores. *N. apis* infects only midgut epithelium (Fries, 1988), but *N. ceranae* infects other organs (Chen et al., 2009). After infection, the viral load increases dramatically as tens of millions of spores per bee cause the mortality of the bee host (Martin-Hernandez et al., 2011). Nosema infection is related to seasonal/climate conditions, nutritional status of the colony, the inoculum dose, coinfection with other pathogens, and host genetics. Another fungal pathogen in the honey bee is *Ascosphaera apis*, which causes chalkbrood disease in larvae. The dead larvae become hardened and desiccated by the overgrowing fungus. The virulence strains are involved with spore and enzyme production (Theantana and Chantawannakul, 2008).

2.2.3.2 Non-pathogenic microorganism associated with honey bees

Fungi such as genus *Penicillium* and *Aspergillus* and other groups (*Cladosporium* and *Alternaria*) can be found in honey bees. *Ascosphaera* species can grow on many substrates within a bee nest (Wynns et al., 2013).The gut of honey bee larvae revealed various molds and yeast (Gilliam and Prest, 1987; Evans and Armstrong, 2006). Yeast has been reported in honey bee workers (Gilliam et al., 1988). Most studies aimed to investigate bacteria in the gut compartments ranging from community diversity surveys to molecular studies on how gut bacteria interact with host immune systems (Dillon and Dillon, 2004; Engel and Moran, 2013). Studies by using culture-independent techniques reveal 7-12 core bacterial species in bee gut (Cox-Foster et al., 2007; Martinson et al., 2011; Moran et al., 2012; Martinson et al., 2012). The dominant recurring honey bee-associated clusters are Alpha-1 and Alpha-2 (Alphaproteobacteria), Beta (Betaproteobacteria), Gamma-1 and Gamma-2 (Gammaproteobacteria), Firm-4 and Firm-5 (Firmicutes), and

Bifido (Actinobacteria) (Martinson et al., 2012).Figure 2.10 shows phylogenetic framework generated by published sequences (Newton and Roeselers, 2012). In general, microbial communities in differenthoney bee species, life stages, and geographic locations show different groups of bacteria (Disayathanoowat et al., 2012; Ahn et al., 2012). Few studies reported bacterial community structure in wild Asian honey bees. Several data revealed consistent bacteria groups of the native honey bees and other honey bee species (Martinson et al., 2011; Disayathanoowat et al., 2012).

2.2.3.2.1 Non-pathogenic bacteria

(A) Alphaproteobacteria

The first and second larval *A. mellifera* instars contained Alpha 2.2, a core Acetobacteraceae (Vojvodic et al., 2013). Worker's guts contained Alpha-1 belonging to genus *Bartonella* within the *Rhizabiales*. Genus *Bartonella*, a group of intracellular pathogens can infect many insects (Minnick and Battiati, 2009). Alpha-2 phylotype sequences are divided into two distinct clades within Acetobacteraceae. However, the second clade clusters within genus *Gluconobacter* and contains only sequences originating from *A. mellifera* (Babendreier et al., 2007). *A. dorsata* gut microbiota revealed Alpha-1.A bacterium isolated from pollen, *Saccharibacterfloricola*, was detected in *A. dorsata* and other bee guts (Jojima et al., 2004; Martinson et al., 2011).

(B) Betaproteobacteria

This group of bacteria is a core bacteria in *A. mellifera* workers (Cox-Foster et al.,2007; Martinson et al., 2011; Moran et al., 2012; Martinson et al., 2012). A well-supported clade corresponding to this group fell within Neisseriaceae and grouped with the genera *Simonsiella* and *Alysiella*. *A. dorsata* and *A. andreniformis* had high infection rate of Betaproteobacteria (Martinson et al., 2011). *Snodgrassellaalvi* was detected in *A. dorsata* worker gut (Koch et al., 2013). A beneficial function of *Snodgrassella* is as a parasite defense in bumblebees (Koch and Schmid-Hempel, 2012).

(C) Gammaproteobacteria

This group of bacteria was common in all stages of *A. mellifera* and *A. cerana* guts (Disayathanoowat et al., 2012). Gamma-1 was found in *A. dorsata* worker's guts. Gamma-1 and Gamma-2 taxaform a highly supported clade branching between the Enterobacteriaceae and Pasteurellaceae. Members of this clade are widespread associates within insect guts (Martinson et al., 2011). Gammaproteobacteria carries genes encoding pectin-degrading enzymes involved in the breakdown of pollen walls (Engel et al., 2012).

(D)Firmicutes

The Firmicutes group presented in worker honey bees (*A. mellifera* and *A. cerana*) and later in instar larvae were dominated by one of two different *Lactobacillus spp*. (Disayathanoowat et al., 2012; Vojvodic et al., 2013). Firm-4 and Firm-5 belong to genus *Lactobacillus*. Firm-4 forms a clade sister to the acidophilus clade, but Firm-5 is within the acidophilus clade. The Firm-5 was cultured from Italian *A. mellifera* gut. Firm-4 was detected in *A. andreniformis* (Martinson et al., 2011). Lactic acid bacteria such as *Lactobacillus kunkeei* were isolated in *A. dorsata* and *A. florea* crops and showed potent antimicrobial properties (Vasquez et al., 2012).

(E) Actinobacteria

Genus *Bifidobacterium* distributed among the *A. mellifera* cultured from guts. While *B. coryneforme* and *B. indicum* cluster with the sister clade, *B. asteroids* sequences form a clade with one set of *A. mellifera* sequences. Although many studies demonstrate the presence of this group of bacteria in the honey bee gut, it was identified in their habitat (Cox-Foster et al., 2007; Jeyaprakash et al., 2003; Martinson et al., 2011; Olofsson and Vasquez, 2008; Promnuan et al., 2009).

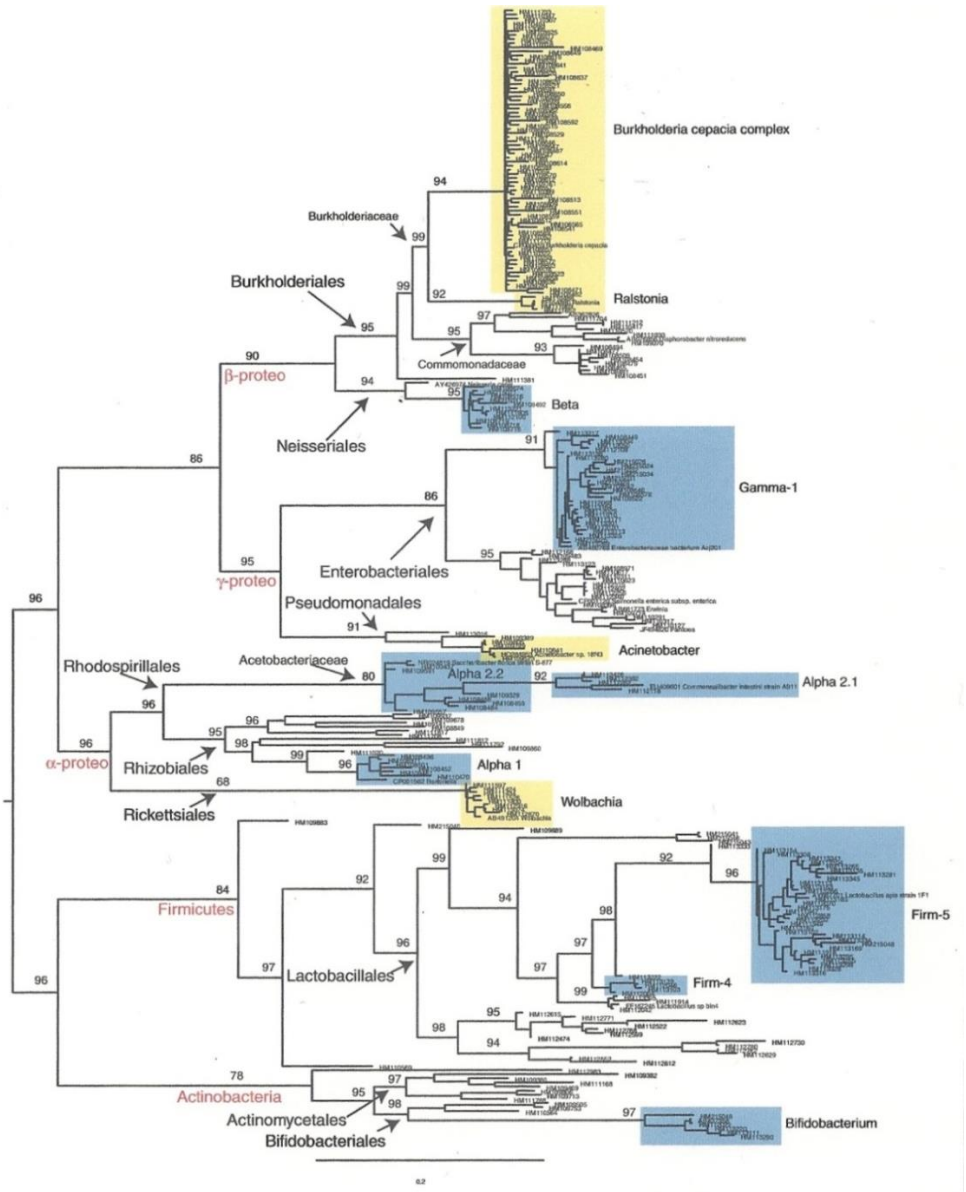

Figure 2.10 Phylogenetic relationships of the bacterial species from the honey bee database (with bootstrap support indicated above branches if >75%). Class level is indicated in red. Lower rank taxonomy is indicated by arrow. Specific clades identified previously in honey bees are highlighted in blue. The novel clades identified in this figure including cultured isolates and well-described genera (such as *Wolbachia*) are highlighted in yellow (Newton and Roeselers, 2012).

2.3 Microflora in bee nests and bee products

The honey bee nest environment contains a rich microbial community that differs according to niche. Recently, no documents report the microbial community in *A. dorsata* and *A. florea* nests and their products. In this topic, we referred to most studies involved with *A. mellifera*. These studies focused on the prevalence of bee pathogens in the reservoirs of *A. mellifera*. For example, two bacterial pathogens known from honey bees are *Paenibacillus larvae*, a bacterium that causes American foulbrood, and *Melissococcus pluton*, the causative agent of European foulbrood. *P. larvae* persisted in old combs and contaminated honey (Hansen and Rasmussen, 1986). *M. pluton* was secreted from infected larva faeces which were deposited on the walls of the brood cells (Bailey and Ball, 1991). Detection of *Clostridium botulinum* spores in honey has been reported (Nakano et al., 1990). *N. ceranae* spores have been found in corbicular pollen (Higes et al., 2008). Honey and royal jelly may also be a source of *N. ceranae* spores (Cox-Foster et al., 2007). Level of infection has been shown to have a seasonal cycle. *Aspergillus* sp., which causes stonebrood, was detected in hive substrates. *A. flavus*, *A. nominus*, and *A. phoenicis* of the ten species identified in hives were pathogenic to honey bee larvae (Foley et al., 2014). *Ascosphaera apis*, a fungus that causes chalkbrood, was detected in an old comb (Aronstein and Murray, 2010).

Honey bee nests are filled with stored pollen, honey, plant resins, and wax, all of which are the sources of microorganisms. Bee nests are a remarkably dynamic environment; however, recent studies show that the food stores are not dominated by the gut core microbiota. It appears some of the main microbial players in non-gut hive niches are those able to tolerate the sugary and acidic environments of bee bread, royal jelly, and honey, such as Acetobacteraceae Alpha 2.2 (Corby-Harris et al., 2014). These Alpha 2.2 bacteria formed a clade separate from the *Saccharibacter* sp. bacteria found in bees that provide their young with pollen and flora samples. Bee products are rich sources of fructophilic lactic acid bacteria, *Lactobacillus kunkeei*, *Lactobacillus brevis*, and *Fructobacillus fructosus* (Neveling et al., 2012; Endo and Salminen, 2013). *L. kunkeei*, which are widespread in the pollination environment and in honey bee nests, are more prevalent in

royal jelly and stored pollen or bee bread (Anderson et al., 2014; Corby-Harris et al., 2014). Bee bread was detected in a variety of bacteria (Acetobacteraceae, *Lactobacillus* sp., *Bifidobacterium* sp., *Pseudomonas* sp., *Bacillus* sp., *Enterococcus* sp.), including pathogens, food spoilage organisms, and beneficial bacteria (Anderson et al., 2013).

A newly identified *Parasaccharibacter apium* was found in high relative proportions in stored pollen. However, new pollen contained few bacteria which decreased significantly as pollen were stored over 96 hours. Some bacteria are from hive environment, but Bradyrhizobiaceae, Xanthomonadaceae, Enterobacteriaceae, Rhodobacterales, Pseudomonadales, Bacteriodetes, and many groups of Actinobacteria are of unknown origins. The core gut microbiota of honey bee, such as Alpha 2.1 phylotype, *Lactobacilus* sp. (Firm 4), *Lactobacilus* sp. (Firm 5), Frischellaperrara (Gamma 2 phylotype), *Gilliamella apicola* (Gamma 1 phylotype), *Snodgrassella alvi* (Beta phylotype), and a honey bee-associated *Bifidobacterium* sp. have been identified in almost all honey bee tissues and in hive environments (Anderson et al., 2013; Corby-Harris et al., 2014).*Stenotrophomonas* sp. and *Rhodanobacter* sp. were prevalent in royal jelly. The ratio of microbes to pollen surface areas was estimated to be approximately 1:1,000,000. It indicated a non-effect of microbial metabolism on hive-stored pollen (Anderson et al., 2014). In addition, many different groups of Actinobacteria were identified (Anderson et al., 2013). *Actinomaduraapis* was isolated from a bee hive (Promnuan et al., 2011). *Penicillium* sp., *Aspergillus* sp., *Candida* sp., *Saccharomyces* sp., and *Crytococcus* sp. were detected in beebread, nectar, and honey (Gilliam, 1997). *Mucor* sp. was detected in nectar and honey (Cox-Foster et al., 2007).

2.4 Roles of midgut microflora in insect and honey bee

Many insect species harborlarge, diverse microorganisms. Past studies using traditional culture-dependent methods defined the gut microbiota by phenotyping the isolates. It is known that most microbes in the environment cannot be cultivated. Culture-independent approaches based on 16S rRNA sequencing gene are enabling the definition of the microbial community of insects. By these studies, the diversity of gut microbe relates in part to the variety of specialized structures, redox conditions, digestive enzymes, and type

of food. Metagenome sequencing studied deeply the potential functions related to host interactions, digestion, and defense which are associated with bacterial species or with strains within species (Engel et al., 2012). Furthermore, metatranscriptome sequencing identified active microbial members and inferred community metabolic functions (Lee et al., 2014).

To summarize the main function roles of gut microorganisms of insects, Figure 2.11 gives an overview of six functions such as colonization resistance, intestinal cell renewal, promotion of systemic growth, diet breakdown, nutrient supplementation, and intra- and inter-specific communication. For example, bacteria in the gut of the bumble bee (*Bombus terrestris*), desert locust (*Schistocerca gregaris*), and various mosquito species show the defense mechanism against pathogens (Pumpuni et al., 1993; Dillon et al., 2005; Koch and Schmid-Hempel, 2011).The commensal gut microbiota of *Drosophila melanogaster* are involved in intestinal cell renewal and promotion of systemic growth (Buchon et al., 2009; Storelli et al., 2011).The hindgut of termites has microbes that can degrade cellulose (Warnecke et al., 2007). Gut bacteria can also degrade toxins from the diet (Kikuchi et al., 2012). The synthesis of vitamins and essential amino acids or the nitrogen fixation has been shown for gut symbionts of blood-feeding kissing bugs, stinkbugs, and termites, respectively (Hongoh et al., 2008; Nikoh et al., 2011). Bacteria in the termite gut can convert nitrogenous waste products to high-value nutrients (Hongoh et al., 2008).Moreover, gut microbes also produce molecules for intra- and inter-specific communication, such as pheromones and kairomones (Dillon et al. 2002, Leroy et al., 2011).

The honey bee (*A. mellifera*) acquires microbes during developmental stages by social interactions in their hives. An adult honey bee contains approximately 10^9 bacteria cells (Martinson et al., 2012). The core species of gram-negative bacteria, *Snodgrassella alvi*, *Gilliamella apicola,* and *Frischella perrara*, were dependent on the presence of nurses or hindgut material, whereas some gram-positive species were more often transferred through exposure to hive components (Powell et al., 2014). Honey bee guts contain only a few groups of bacteria as well as other *Apis* species and *Bombus* species (bumble bees) (Martinson et al., 2011; Koch et al., 2013).

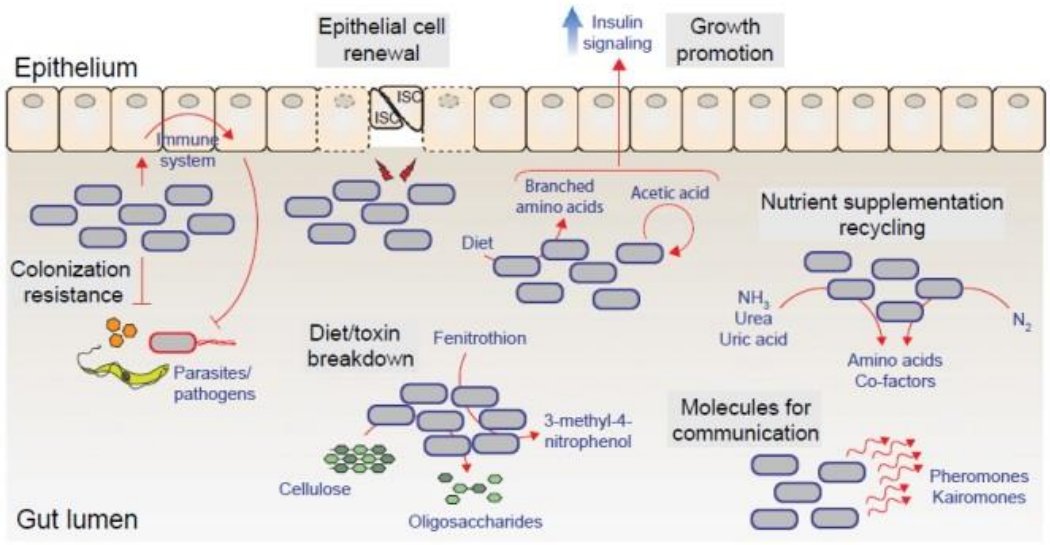

Figure 2.11 Beneficial functions of insect gut microbs (Engel and Moran, 2013)

Comparative analysis of gene contents from honey bee midgut bacteria suggests that different species perform distinct functions linked to host interaction, biofilm formation and carbohydrate breakdown. *Gilliamella apicola* (gammaproteobacteria) carries genes encoding pectin-degrading enzymes likely involved in the breakdown of pollen walls. Three major bacterial classes (Gammaproteobacteria, Bacilli, and Actinobacteria) studied by metatranscriptome sequencing are predicted to breakdown the complex macromolecules (polysaccharides and polypeptides), ferment the component parts, and generate various fermentation products such as short-chain fatty acids and alcohol. The community-level physiological profiling confirmed the metabolism of carbon-rich food sources (Lee et al., 2014).

The purpose of probiotics isolated from commensal bacteria in honey bee larvae is to defend against pathogens such as *Paenibacilus larvae* (Evans and Lopez, 2004). Probiotics could be used to enrich honey bee health. For example, colonies fed with lactic

acids (*Lactobacillus johnsonii*) not only increased the beehive population and the amount of fat bodies per bee, but also reduced the intensity of pathogens (Maggi et al., 2013). Lactic acid bacteria such as *Lactobacillus kunkeei* isolated from *A. dorsata*'s stomach showed potent antimicrobial properties and could be good candidates for potential application as probiotics and natural food preservatives (Tajabadi et al., 2011; Vásquez et al., 2012). *Bacillus* sp. may help to preserve bee bread, and fungi may continue to slowly digest the pollen, altering the nutritional quality (Gilliam, 1997). *Penicillium* and *Aspergillus* sp. have been shown to inhibit the growth of highly pathogenic hive fungi (Gilliam et al., 1988). These beneficial fungi could produce antibiotics that preserve beebread or converse process. Actinobacteria found in association with bees inhibited the growth of major honey bee pathogens (Promnuan et al., 2009).