

Gut-associated Lactic Acid Bacteria (LAB) in *Apis nigrocincta* (Smith)

C A Lombogia^{1,2}, M Tulung¹, J Posangi^{1,3} and T E Tallei^{1,4,*}

¹ Entomology Study Program, Postgraduate Program, Sam Ratulangi University, North Sulawesi, Indonesia

² Nursing Study Program, Halmahera College of Health Sciences, North Maluku, Indonesia

³ Public Health Study Program, Faculty of Public Health, Sam Ratulangi University, North Sulawesi, Indonesia

⁴ Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University

*Corresponding author. Email: trina_tallei@unsrat.ac.id

ABSTRACT

Honeybees are the world's most important pollinator of food crops. Besides, they also produce honey, which is very beneficial for human health. Therefore the health of honeybees is essential to note. One aspect that contributes to the health of honeybees is the balance of the gut microbiota. Lactic acid bacteria (LAB) are known to give importance to intestinal health in their hosts. This study aimed to study gut-associated LAB in *Apis nigrocincta* (Smith), a species of a cavity-nesting honeybee endemic to Sulawesi and Mindanao islands. The method used to obtain information about the presence of lactic acid bacteria was metagenomic profiling of the honeybee gut microbiome by targeting the V3-V4 region of 16S rRNA genes using the Illumina MiSeq. In total, 83,018 reads of the microbiome comprising 749 bacterial operational taxonomic units (OTUs). Some genera belong to lactic acid bacteria, among others are *Bifidobacterium*, *Enterococcus*, *Fructobacillus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Vagococcus*. These LAB may pose beneficial properties, which are essential not only for the honeybee itself, but also the food and health industries.

Keywords: Honeybee, *Apis nigrocincta*, Metagenomic profile, Lactic acid bacteria, Gut.

1. INTRODUCTION

Honeybees are one of the vital insects for the pollination of most of the crop plants in the world. However, their existence is declining due to habitat degradation, anthropogenic disturbance, and other factors such as pathogens, pesticides, lack of flowers, and climate change [1, 2]. Like other insects, honeybees have symbiotic and pathogenic interactions with microbes in the digestive tract. It is assumed that the microbes in their digestive tract are influenced by their environment where they forage for food. Moreover, these gut symbionts were proved to affect the foraging behavior of insects [3].

To explore host-microbe interactions, high-throughput sequencing techniques have been developed. This technique has opened up the opportunities to study the impact of the microbiome on insect health. Several studies have shown that lactic acid (LAB) bacteria play vital roles in the health of the host, as well as providing nutritional benefits such as the improved nutritional

value of food and gut infection control [4]. LAB have been shown to have a symbiotic relationship with several *Apis* species [5]. Three different species of *Lactobacillus* was found to be associated with *A. florea* in different regions of Iran [5]. *Lactobacillus kunkeei* was indicated as the dominant LAB member in the gut of bees [6].

One of the endemic *Apis* species recorded in Mindanao island (the Philippines), Sangihe islands (North Sulawesi, Indonesia), and the mainland of Sulawesi (Indonesia) was *Apis nigrocincta* [7]. This species is a generalist and nests in cavities such as holes in trunks and caves [8]. Considering the importance of knowing what lactic acid bacteria are present in *A. nigrocincta*, this study was carried out.

2. MATERIAL AND METHODS

2.1. Extraction and Amplification of Gut Bacterial gDNA of *A. nigrocincta*

The gut was dissected out from surface-sterile honeybee [9] and homogenized using a FastPrep-24 instrument at 4 m/s for 25 sec. ZymoBiomics DNA Mini Kit (Zymo Research) was used to extract the gut

bacterial gDNA (genomic DNA). MyTaq™ HS Red Mix (Bioline, BIO-25044) was used to amplify hypervariable V3-V4 regions of the 16S rRNA gene. The following reaction condition was performed in Agilent SureCycler 8800 Thermal Cycler: initial denaturation at 95oC for 3 min, followed by 35 cycles of denaturation at 95oC for 15 sec, annealing at 52oC for 30 sec, and extension at 72oC for 45 sec, then followed by a final extension at 72oC for 3 min.

2.2. 16S rRNA Library Preparation and Bioinformatics Analysis

The preparation of the 16S rRNA library and analysis of bioinformatics was conducted following previous research [10, 11, 12].

3. RESULT AND DISCUSSION

Using the Illumina sequence technique, 83,018 reads of V3-V4 regions of 16S rRNA were produced after removing chimera and singleton. Some genera associated with LAB were found (see Figure 1), among others are from phylum Actinobacteria such as Bifidobacterium (7.96%), and Firmicutes such as Lactococcus (13.45%), Lactobacillus (8.19%), Enterococcus (4.47%), Vagococcus (3.76%), Leuconostoc (0.12%), and Fructobacillus (0.06%). LAB are Gram-positive bacteria that are capable of fermentation in anaerobic conditions. These bacteria are unable to produce catalase and spores [13]. They have been used in food/drink fermentation, as biocontrol agents, or as probiotics [14].

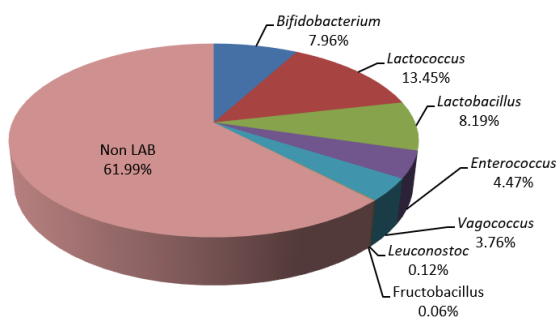


Figure 1. The composition of LAB genera in the gut of *Apis nigrocincta*

Table 1 shows the summary of LAB genera found in some *Apis* species. There are three phylotypes of the honeybee microbiota. Two of them belong to the genus *Lactobacillus* in the phylum Firmicutes (Firm-4 and Firm-5) [15]. These bacteria are considered as the core gut of *A. mellifera* [16]. The third phylotype was called Bifido and belonged to the genus *Bifidobacterium* in the phylum Actinobacteria [15]. Hence, *Lactobacilli* and *Bifidobacteria* are the two dominant phylotypes in the honeybee gut. One of the species of *Lactobacillus*, which is commonly isolated from the honeybees is *L. kunkeei*. This bacterium is a Gram-positive fructophilic LAB (FLAB) with a weak catalase activity [17], and produces antibacterial compound type II bacteriocins [18].

Bifidobacterium was found in *A. nigrocincta* and *A. mellifera*. This bacterium was found in this research as the third predominant genus in the gut of *A. nigrocincta* after Lactococcus and Lactobacillus. This genus commonly inhabits the gastrointestinal tract of various animals and insects [28, 29], and are known as anaerobic fermentative bacteria. It produces antimicrobial peptide (AMP), which is able to inhibit the growth of both antibiotic sensitive and resistant *Helicobacter pylori* strains [30]. The AMP is known as the potential alternative of the safe antimicrobial agents [31].

Lactococcus is a common inhabitant of the gut and associated with food [22]. It has been found in the stomach of *A. cerana indica*. It produces an AMP called nisin [32] with activity against Gram-negative pathogenic *Escherichia coli* and *Salmonella* [33]. It also produces another AMP called lactococcin [34]. Apart from being a potential alternative for antibiotics, nisin also shows a pharmacological effect, including cytotoxic and anti-tumor activity [35].

Enterococcus was detected in *A. nigrocincta* and *A. mellifera*. This genus produces enterocin, which shows antibacterial activity against a wide range of Gram-positive bacteria of enterococcal strains. This AMP was presumed to have amphiphilic α -helices, like in lactococcin G [35]. This genus has many benefits to health, including cholesterol assimilation ability, improve the immune system, and produce many kinds of AMPs [36]. *Leuconostoc* was found in *A. nigrocincta* and *A. cerana indica*. *Fructobacillus* was isolated from *A. nigrocincta* and *A. mellifera*. A probiotic *Leuconostoc mesenteroides* has a capability to remove Pb(II) toxicity [37] and produces leucocyclicin, a cyclic bacteriocin [38].

This research shows that several LAB associated with the gut of *A. nigrocincta* are also commonly found in other *Apis* species. The insignificant amount of

Leuconostoc and Fructobacillus was detected using high-throughput sequencing methods. This discovery needs to be studied further to obtain LAB, which have the potential to be developed as the next generation probiotics. Some strain of LAB has been shown to have antibacterial compounds as well as the capability of lowering cholesterol levels in vitro.

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Table 1. The LAB Found in Honeybees

Honeybee Species	Lactic Acid Bacteria	References
<i>Apis nigrocincta</i>	<i>Bifidobacterium</i> , <i>Enterococcus</i> , <i>Fructobacillus</i> , <i>Lactococcus</i> , <i>Lactobacillus</i> , <i>Leuconostoc</i> , <i>Vagacoccus</i>	This article
<i>A. mellifera</i>	<i>Bifidobacterium asteroides</i> , <i>Enterococcus faecium</i> , <i>Fructobacillus fructosus</i> , <i>Lactobacillus</i> , <i>L. kunkeei</i> , <i>L. johnsonii</i>	[19, 20, 21, 22]
<i>A. cerana</i>	<i>Lactobacillus</i> , <i>L. apis</i> , <i>L. bombicola</i> , <i>L. helsingborgensis</i> , <i>L. panisapium</i>	[23, 24]
<i>A. cerana indica</i>	<i>Lactococcus</i> , <i>Lactobacillus</i> , <i>L. plantarum</i> , <i>Enterococcus</i> , <i>Leuconostoc</i> , <i>Streptococcus</i> , <i>Pediococcus</i>	[22, 25]
<i>A. dorsata</i>	<i>Lactobacillus</i> , <i>L. kunkeei</i> , <i>L. vermiform</i> ,	[22, 26]
<i>A. florea</i>	<i>L. kunkeei</i> , <i>L. plantarum</i> , <i>L. apis</i>	[22, 27]
<i>A. koschevnikovi</i>	<i>Lactobacillus</i>	[24]
<i>A. nuluensis</i>	<i>Lactobacillus</i>	[24]

4. CONCLUSION

It can be concluded from this research that there are several LAB found in *A. nigrocincta*, which also commonly found in other *Apis* species. *Leuconostoc*, *Lactobacillus*, and *Bifidobacterium* are proposed here as the core gut LAB of *A. nigrocincta*.

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