

Lactic Acid Bacteria from Honey Bees Digestive Tract and Their Potential as Probiotics

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ABSTRACT

Honey bees (*Apis* spp.) are social insects that possess unique gut microbiota community. There are three main phyla that dominate the honey bees gut microbiota; Proteobacteria, Firmicutes, and Actinobacteria in which several species among them belong to the lactic acid bacteria (LAB). In general, some species of LAB can be developed as potential probiotics due to their capability to produce bioactive compounds such as organic acid (lactic acid, acetate acid and formic acid), hydrogen peroxide (H₂O₂), ethanol, enzymes, benzoate, antimicrobial peptides (AMPs), free fatty acid, and volatile compounds—that in a synergistic action work as broad-spectrum antimicrobials toward several pathogens. Several strains of LAB that living in symbiosis within the digestive tract of honey bees have been isolated, among them are quite promising to be developed as probiotics. Multiple studies have shown the inhibitory effect of LAB from honey bees against pathogenic microorganisms. Formic acid and lactic acid produced by symbiotic LAB could decrease the environmental pH of wounds, therefore prevent the growth of pathogenic microbes. Furthermore, the volatile compound produced by LAB is toxic, while H₂O₂ in small numbers is needed for optimal wound recovery. LAB capable of producing broad-spectrum antibacterial compounds such as *Serratia marcescens*, *Eschericia coli*, methicillin-resistant *Staphylococcus aureus*, *Klebsiella aerogenes*, *Salmonella typhi*, *Pseudomonas* spp., *Klebsiella* spp., *Proteus* spp., *S. aureus*, and *Bacillus subtilis*. Additionally, LAB possess antioxidant activity and to adhere the epithelial cells. This paper aimed to discuss varieties of LAB from the digestive tract of honey bees and their potential as probiotics that could benefit healthcare.

Keywords: honey bees, lactic acid bacteria, gut, probiotic

1. INTRODUCTION

Honey bees (*Apis* spp.) are honey-producing insects that classified into subfamily *Apinae* of the family *Apidae* [1]. There are nine species from the genus *Apis* that are recognized worldwide [2], whereas in Indonesia there has been discovered at least five honey bees species namely *Apis andreniformis*, *A. nigrocincta*, *A. dorsata*, *A. koschevnikovi*, and *A. cerana* [3].

As social insects, honey bees possess unique gut microbiota community. There are three main phyla that dominate the honey bees gut microbiota: Proteobacteria (*Gilliamella*, *Parasaccharibacter* and *Frischella*), Actinobacteria (*Bifidobacterium*) and Firmicutes (*Lactobacillus* sp. Firm-4 and Firm-5), in which several species among them belong to the lactic acid bacteria (LAB) [4]. LAB belong to gram-positive bacteria, they are non-spore forming cocci or coccobacilli, anaerob or

facultative aerobic rods that produce organic acids, such as lactic acid which is the main fermentation result of carbohydrate metabolism [5]. LAB synergistically within the gastrointestinal tract of humans, insects, and other animals [6, 7]. Some species of LAB can be developed as potential probiotics. This is due to their capability to produce bioactive compounds such as lactic acid, acetate acid, and formic acid, hydrogen peroxide (H₂O₂), ethanol, enzymes, benzoate, antimicrobial peptides (AMPs), free fatty acid, and volatile compounds that in a synergistic action work as broad-spectrum antimicrobials toward several pathogens [8]. Among several isolated symbiotic LAB strains of the honey bees digestive tract, it is found that some of them showed potential results to be developed as probiotics [8, 9]. The objective of this review was to discuss the potential of various LAB found in the honey bees gut as probiotics that beneficial for healthcare.

1.1. Materials and Methods

This paper was incorporated by using a library research method. The data were collected by analysing several books, both printed and electronic documents, and other sources or information related to the research. In this paper we assembled premises from several literatures that discuss the lactic acid bacteria from honey bees digestive tract. After that we examined several studies regarding the potential probiotic of LAB from honey bees digestive tract. The conclusion of assumptions was then taken subjectively.

1.2. Our Contribution

This paper presents varieties of LAB from the gut of honeybees and their potential as probiotics that could benefit healthcare. By referring to this study which is supported by various references regarding the probiotic potential of LAB from honeybees gut, further research on this topic can be developed.

1.3. Paper Structure

The rest of the paper is organized as follows. Section 2 explains about the microbiota community in the honeybees gut; LAB that are specific to honeybees that have been isolated from honeybee guts of various species; and potential probiotic of LAB from honeybees gut. Finally, Section 4 concludes the paper and it is believed that further studies to assess the health-promoting activity of probiotic bacteria isolated from honeybee's digestive tract are required.

2. RESULTS AND DISCUSSION

2.1. The Honey Bees Gut Microbiota

Among the microbiota community in the honey bees gut, there are nine dominating bacterial species clusters. These clusters are specific to bees and can be transmitted by social interactions between bees [10]. There are five main bacteria groups in the honey bees gut community: Gram-negative bacteria group (*Snodgrassella alvi* and *Gilliamella apicola*), members of the phylum Proteobacteria [10, 11] to Gram-positive bacteria, Firmicutes (*Lactobacillus* Firm-4 and *Lactobacillus* Firm-5 groups) [10], phylum Actinobacteria (*Bifidobacterium asteroids*) [10, 12], a small number of Proteobacteria species (*Frischella perrara*, *Bartonella apis*, *Parasaccharibacter apiu*, and a *Gluconobacter*-related species group designated Alpha2.1). Over millions of years, these bacteria together formed a specialized microbial community that have co-evolved and diversified with their bee hosts [10]. This microbiota community is very dynamic and adaptive since its existence is influenced by several factors such as nutrition, hive environment, social interaction, and the age of honey bees, whereas its composition follows seasonal patterns [4].

2.2. Lactic Acid Bacteria Specific to Honey Bees

The LAB that are specific to honey bees that have been isolated from various species of the honey bees gut can be seen in Table 1. The LAB species of the honey bees are all Gram-positive, facultative anaerobic bacteria that do not form spores, immotile, and produce lactic acid. The abundance of LAB species in honey bees varies depending on the season, their health, floral resources, and their contact with older bees of the colony [4, 15]. As the most dominant species among the sub-species *Apis* [19], *L.*

Table 1 The lactic acid bacteria honey bees gut

Honey bee species	Lactic acid bacteria	References
<i>Apis mellifera</i>	<i>Lactobacillus kunkeei</i> , <i>Fructobacillus fructosus</i> , <i>L. johnsonii</i> , <i>Enterococcus faecium</i> , <i>Micrococcus</i>	[13 –16]
<i>A. cerana indica</i>	<i>Lactobacillus</i> , <i>L. plantarum</i> , <i>Lactococcus</i> , <i>Enterococcus</i> , <i>Micrococcus</i> , <i>Streptococcus</i> , <i>Pediococcus</i> , <i>Leuconostoc</i>	[15, 17]
<i>A. dorsata</i>	<i>Lactobacillus</i> , <i>L. kunkeei</i> , <i>L. vermiorm</i> , <i>Lactococcus lactis</i>	[6, 15]
<i>A. florea</i>	<i>L. kunkeei</i> , <i>L. plantarum</i> , <i>L. apis</i> , <i>Micrococcus</i>	[7,15]
<i>A. nigrocincta</i>	<i>Bifidobacterium</i> , <i>Enterococcus</i> , <i>Fructobacillus</i> , <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Leuconostoc</i> , <i>Vagococcus</i>	[18]

kunkeei was abundant during spring and summer but almost absent during winter [4]. A healthy *A. mellifera* adults were collected from five geographically different locations of Apulia, Italy and *Lactobacillus kunkeei* and *F. fructosus* were successfully isolated from its digestive tract with the range of 69% and 31%, respectively. Almost all of the isolates were fructophilic lactic acid bacteria (FLAB) which consumed fructose as the main source of carbohydrate [14]. Other studies of the worker bee *A. mellifera* gut showed eight strains of *Lactobacillus* spp. and five strains of *Enterococcus* spp., which according to 16S rRNA sequencing AJ5, IG9, A15, and CRL1647 strain had 99% similarity with *L. johnsonii*, whereas SM21 showed 99% similarity with *Enterococcus faecium* [13]. From the abdomen of *A. dorsata* bee in Malaysia, it was discovered that there were 34 strains that produced three different rRNA sequences from 15 different strains of *Lactobacillus*, consisting of *L. kunkeei* (56%), other sequence related to other *Lactobacillus* sp. (38%) and *L. vermiform* (6%) [6].

L. kunkeei, *L. plantarum*, and *L. apis* were found from the gastrointestinal tract of the Asian dwarf honey bee (*A. florea*) which was distributed from different regions of Iran [7]. From the *A. Nigrocincta* gut in the urban areas of Manado, Indonesia, *Bifidobacterium*, *Enterococcus*, *Fructobacillus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Vagococcus* were found [18]. *Bifidobacterium* along with other rare species namely *Leuconostoc* and *Vagococcus* were only found in Lombogia's study.

2.3. Probiotic Potential of Lactic Acid Bacteria from Honey Bees Gut

According to the Food and Agriculture Organization (FAO) and World Health Organization (WHO), probiotics are living microorganisms, which can provide health benefit on the host when it was given in adequate amount [20]. Probiotic microorganisms are commonly obtained from conventional sources such as dairy foods. On the other hand, the use of probiotic from unconventional sources such as non-intestinal sources, non-dairy fermented food products, and various parts of digestive tract from animals are potentially to increase [21].

Several LAB strains from the honey bee gut have the potential as probiotics that beneficial for health [19]. Various microbiota LAB in honey bees are thought to be the first line of defense toward potential pathogens against its host. One of the bioactive components in the LAB that is isolated from the honey bee gut is antimicrobial peptide (AMP), a very important part of the immune system which is also a promising alternative to the current antibiotic treatment or prevention of microbial infection [22,23]. AMPs belong to a diverse group of LAB from digestive tract of honey bees that could be seen in Table 2. Helveticin J, bacteriolysin, alivaricin, enterolysin A, and thermophilin A are AMPs that can be found in several strains of *Lactobacillus* isolated from *A. mellifera* [24, 25]. However, bacteriolysin is also found in *Bifidobacterium strain* [24]. Meanwhile bacteriocin and bacteriocin-like compounds are found in strains of *Enterococcus* [13, 26]. These various AMPs enable LAB from honey bees to act as probiotics to inhibit pathogenic bacteria. The inhibitory effect of LAB towards bacterial pathogens have been proved in several studies [8, 13, 16, 27–30] as shown in Table 3.

Lactobacillus johnsonii CRL164, AJ5 and IG9 produced a significant amount of lactic acid ranged from 177 to 275 mM. *In vitro*, these strains are capable of inhibiting various human food-borne pathogens and the American foulbrood causal agent, *Paenibacillus* larvae. Furthermore, *E. faecium* is thought to have an anti-lysteria effect [13]. *Lactobacillus apis* sp. nov., which was isolated from the abdomen of *A. mellifera*, could inhibit *Paenibacillus larvae subsp. Larvae* and *Melissococcus plutonius* (causal agent of European foulbrood) in *in vitro* manner [28]. A study of thirteen *Lactobacillus* strains from *A. mellifera* in severe wounds by methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and *Pseudomonas aeruginosa* showed result of strong and synergic antimicrobial activities for each symbiont against the pathogens. Its mode of action is through elucidating the production of active compounds such as organic acid, phosphate acid and, lactic acid as well as other volatile compounds and hydrogen peroxide (H₂O₂). Organic acid reduces the pH and will create an unfavorable environment to promote wound development. Formic and lactic acid produced by LAB symbiont

Table 2 The lactic acid bacteria from honey bees's digestive tract and their antimicrobial peptides

Honey bee species	Antimicrobial peptides	Lactic acid bacteria	References
<i>Aphis mellifera</i>	Helveticin J	<i>Lactobacillus Bma5N</i>	[24]
<i>A. mellifera</i>	Bacteriolysin	<i>L. Fhon13N, Hon2N, L. kunkeeiFhon2N, Bifidobacterium Hma3N</i>	[24]
<i>A. mellifera</i>	Salivaricin, enterolysin A, helveticin J, thermophilin A	<i>L. salivarius A3iob, L. johnsonii CRL1647</i>	[25]
<i>A. mellifera</i>	Bacteriocin	<i>Enterococcus avium</i>	[26]
<i>A. mellifera</i>	Bacteriocin-like compounds	<i>E. faecium</i>	[13]

Table 3 *In vitro* studies of antimicrobial activities against pathogens by LAB isolated from honey bees gut

Lactic acid bacteria species	Target pathogen/effect	References
<i>Lactobacillus johnsonii</i>	<i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Bacillus cereus</i> , <i>Paenibacillus larvae</i> , <i>Listeria monocytogenes</i>	[13]
<i>Enterococcus faecium</i>	<i>L. monocytogenes</i>	[13]
<i>L. kunkeei</i> Fhon2, <i>L. kunkeei</i> Yubipro, <i>L. kunkeei</i> Lahm	<i>Serratia marcescens</i> , <i>E.coli</i> , <i>Klebsiella aerogenes</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , MRSA	[27]
<i>Lactobacillus</i>	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA), <i>Pseudomonas aeruginosa</i> , vancomycin-resistant <i>Enterococcus</i> (VRE)	[8]
<i>L. apis</i> sp. nov	<i>Paenibacillus larvae</i> subsp. larvae, <i>Melissococcus plutonius</i>	[28]
<i>L. gasseri</i> , <i>L. kunkeei</i> , <i>L. amylovorus</i> , <i>L. fructivorans</i>	<i>Paenibacillus</i>	[29]
<i>F. fructosus</i>	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA), <i>Pseudomonas aeruginosa</i>	[16]

decrease the pH of the wound environment. Moreover, the volatile compounds produced by the LAB are toxic. Small amounts of H₂O₂ are needed for optimal wound healing [8]. A review that was made based on several studies revealed that *L. kunkeei* Fhon2, *L. kunkeei* Lahm, and *L. kunkeei* Yubipro that were isolated from the digestive tract of honey bees, are the 3 types of LAB that could potentially produce bioactive compounds with antibiotic-like activity.

Lactic acid bacteria are capable of producing broad-spectrum antibacterial compounds against various pathogenic microbes such as *S. marcescens*, *P. aeruginosa*, *K. aerogenes*, *E.coli*, *S. aureus* and MRSA [27]. The antimicrobial evaluation activity of *Lactobacillus* spp. isolated from *A. mellifera* showed that the best antimicrobial effect of *Lactobacillus* spp. (*L. fructivorans*, *L. amylovorus*, *L. gasseri*, *L. kunkeei*) was against *Paenibacillus* larvae that present in the digestive tract of *L. gasseri* supernatant. A lower level of antimicrobial activity was detected in *L. kunkeei* supernatant. The strongest antimicrobial activity against larva *Paenibacillus* CCM 4438 was detected in *L. gasseri* and *L. amylovorus*, while the lowest was detected in *L. fructivorans* [29]. A study of LAB isolates from the gut of *A. mellifera* showed that *F. fructosus* (SHGH-1, SHGH-4, and SHGH-14) have the highest inhibition zone against *Pseudomonas aeruginosa* then *F. fructosus* (SHGH-11) against *Staphylococcus aureus* (MRSA) [16]. There are 6 isolates from the gut of *A. cerana indica* that have antimicrobial activity against pathogen such as *Salmonella typhi*, *S. paratyphi* A, *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *S. aureus*, and *Bacillus subtilis* [30]. All of these studies revealed that LAB have the potential sources of antibacterial substances that are effective against pathogenic Gram negative and Gram positive bacteria. Besides having antimicrobial potential, *Lactobacillus* has the best antioxidant effect as proven by

the discovery of *L. plantarum* H28, and *L. plantarum* H24 from the digestive tract of honey bees [31].

3. CONCLUSION

There are various studies found that honey bees have a unique source of microbiota in their digestive systems. They consist of three main phylum namely Proteobacteria, Firmicutes and Actinobacteria, which some of its species are classified as LAB. LAB microbiota isolated from the digestive tract of honey bees potential as probiotics, have the antioxidant activity as well as the ability to inhibit pathogens. This ability by bioactive compounds such as AMPs and organic acid that contained in LAB. Further studies are required to assess the health-promoting activity of probiotic bacteria isolated from honey bee digestive tract.

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