ACADEMIA JOURNAL OF BIOLOGY 2025, 47(1): 43–53
DOI: 10.15625/2615-9023/22384

GUT MICROBIOTA ANALYSIS OF HEALTHY AND SACBROOD VIRUS-INFECTED *Apis mellifera* REVEALS POTENTIAL PROBIOTIC BACTERIA FOR HONEYBEE HEALTH AND DISEASE RESISTANCE

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Received 7 February 2025; accepted 20 March 2025

ABSTRACT

This study aimed to determine the gut microbiota composition of adult *Apis mellifera* honeybees from bee farms in Bac Giang province, including both healthy colonies and those infected with Sacbrood virus (SBV). The gut microbiota of healthy and SBV-infected bees was assessed using next-generation sequencing (NGS) of the V3-V4 region in the 16S rRNA gene on the Illumina MiSeq system. As a result, NGS analysis identified 1,659 operational taxonomic units (OTUs) with a coverage of 99% and an average read length of 430 bp. The results revealed that SBV-infected bees harbored four microbial phyla: Proteobacteria (48.44%), Firmicutes (38.65%), Actinobacteria (1.57%), and Bacteria_uc (10.95%). In contrast, the healthy bee group consisted of three phyla: Proteobacteria (40.61%), Firmicutes (45.55%), and Bacteria_uc (13.37%). The species composition analysis showed that both healthy and SBV-infected bees shared common core bacterial species. However, *Bifidobacterium_uc* and *Commensalibacter AY370188_s* were more prevalent in SBV-infected bees and significantly reduced in healthy bees. Conversely, *Fructobacillus fructosus* and *Lactobacillus kunkeei* were found exclusively in healthy bees. These lactic acid bacteria (LAB) have been shown to inhibit the growth of pathogenic bacteria. Our findings provide a valuable scientific foundation for developing biological products to improve honeybee health and disease resistance.

Keywords: *Apis mellifera*, gut microbiota, honeybees, metagenomics, Next-generation sequencing, Sacbrood virus.

Citation: Dong Van Quyen, Bui Thi Thuy Duong, Pham Thi Lanh, Ha Thi Thu, 2025. Gut microbiota analysis of healthy and sacbrood virus-infected *Apis mellifera* reveals potential probiotic bacteria for honeybee health and disease resistance. *Academia Journal of Biology*, 47(1): 43–53. https://doi.org/10.15625/2615-9023/22384

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INTRODUCTION

Honeybees play a crucial role in agriculture and biodiversity by pollinating crops and flowering plants worldwide. However, in recent decades, honeybee populations have been declining in many countries. Among the various factors threatening bee colony health, viruses-particularly Sacbrood virus (SBV), which causes sacbrood disease (SD) is among the most significant. SBV is considered the first viral pathogen identified in honeybees and remains one of the most frequently detected viruses in bee hives (Chen & Siede, 2007).

Enhancing bee resistance to pathogens is essential for the survival and development of colonies, and one promising approach is the supplementation of beneficial bacteria through probiotics (Wei et al., 2022). Previous studies have shown that the gut microbiota of *Apis mellifera* honeybees is primarily composed of *Gilliamella apicola, Frischella perrara, Snodgrassella alvi, Bartonella apis, Bifidobacterium asteroides, Lactobacillus Firm-4,* and *Lactobacillus Firm-5*. Bees acquire these microbes through contact with food sources, nesting materials, and by contacting other bees (Dosch et al., 2021).

The beneficial bacteria in the honeybee gut perform critical functions, including digestion, metabolism, development, reproduction, behavioral regulation, and immune defense (Kwong et al., 2017). Research has shown that gut bacteria play a key role in breaking down pollen, the primary protein source for honeybees (Kwong et al., 2017; Engel et al., 2016). Moreover, the gut microbiota contributes to the synthesis of vitamins, fatty acids, and amino acids and promotes intestinal cell renewal (Tlak Gajger et al., 2023). Furthermore, this bacterial community plays a crucial role in regulating, stimulating, and shaping immune responses. It can protect the host by directly stimulating the honeybee immune system, enhancing the expression levels of antimicrobial peptides (AMPs) such as abaecin, hymenoptaecin, apidaecin, defensin-1, defensin-2, and apisimin (Iorizzo et al., 2022; Kwong et al., 2017). In addition, several bacteria in the gut microbiota produce various antimicrobial compounds, including bacteriocins, hydrogen peroxide, siderophores, lysozyme, and proteases, which help control and defend against pathogenic bacteria and parasites (Kwong et al., 2017). Specifically, certain bacteria generate organic acids and volatile fatty acids (e.g., lactic, acetic, butyric, and propionic acids), which lower the pH in the gastrointestinal tract, produce pheromones, and form protective biofilms, thereby inhibiting the growth of opportunistic pathogenic microorganisms (Tlak Gajger et al., 2023; Iorizzo et al., 2022).

An imbalance in the gut microbiota, known as dysbiosis, could impair bee development, reduce body mass, and shorten worker bee lifespan (Maes et al., 2016). Such microbial imbalances weaken bees' resistance to pathogens, including SBV. To investigate the impact of SBV infection on gut microbiota and bee health, this study compared the diversity and composition of gut bacteria in healthy and SBV-infected *A. mellifera* honeybees. The findings provide a scientific foundation for developing biological products aimed at enhancing disease resistance and protecting honeybee populations.

MATERIALS AND METHODS

Sample collection and detection of SBV-infected honeybees

Adult *A. mellifera* samples including both SBV-infected and healthy colonies were collected from honeybee farms in Bac Giang province (21^{o}29'39"N, 106^{o}38'16"E). The samples were stored at 4 ^{o}C and transported to the laboratory for analysis. SBV-infected colonies were identified by PCR method with specific primers: SBV-F (5'-ACCAACGATT CCTCAGTAG-3') and SBV-R (5'-CCTTGGA ACTCTGCTGTGTA-3'), as described in our previous study (Lanh et al., 2024). From the collected samples, three healthy and three SBV-infected colonies were selected for total DNA extraction and further analysis.

Total DNA extraction

For each selected colony, 10 grams of adult bee gut tissue were randomly sampled. The

entire gut was collected and homogenized in Falcon tubes containing 5 mL of PBS buffer (100 mM Tris-HCl, 50 mM EDTA, 50 mM NaCl, 1% SDS, pH 7.0). The homogenate was divided into 1.5 mL Eppendorf tubes and centrifuged at 2,000 rpm for 5 minutes. DNA was extracted from the supernatant using the Thermo Scientific GeneJET Genomic DNA Purification Kit following the manufacturer's protocol.

To verify the presence of bacterial DNA in the extracted samples, PCR amplification was performed using the universal 16S rRNA primer pair: 27F (5'-GAGAGTTTGATCCTG GCTCAG-3') and 1495R (5'-CTACGGCTA CCTTGTTACGA-3') (Weisburg et al., 1991). The PCR composition contained 12.5 µL of master mix buffer, 1 µL of 27F primer (10 pmol), 1 µL of 1495R primer (10 pmol), 1 µL of template DNA (50 ng), and 9.5 µL of nuclease-free water. PCR conditions were as follows: initial denaturation at 94 ^{o}C for 3 minutes, followed by 35 cycles of 94 ^{o}C for 45 seconds, 48 ^{o}C for 30 seconds, 72 ^{o}C for 30 seconds, and a final extension at 72 ^{o}C for 8 minutes. PCR products were visualized via electrophoresis on a 1% (w/v) agarose gel stained with ethidium bromide and observed under UV light.

Illumina MiSeq sequencing

The V3-V4 region of the bacterial 16S rRNA gene was amplified using the primer pair 341F (5'-TCGTCGGCAGCGTC-AGAT GTGTATAAGAGACAG-CCTACGGGNGG CWGCAG-3') and 805R (5'-GTCTCGTGGG CTCGG-AGATGTGTATAAGAGACAGGA CTACHVGGGTATCTAATCC-3'). The PCR conditions included an initial denaturation at 95 ^{o}C for 3 minutes, followed by 25 cycles of 95 ^{o}C for 30 seconds, 55 ^{o}C for 30 seconds, 72 ^{o}C for 30 seconds, and a final extension at 72 ^{o}C for 5 minutes.

The second amplification was performed to attach Illumina NexTera barcodes, following the same thermal cycling conditions as the first PCR, except with only 8 cycles. The PCR products were then run on a 1% agarose gel, visualized using a Gel Doc system (BioRad, Hercules, CA, USA) and purified using the Clean PCR Kit (CleanNA, Waddinxveen, The Netherlands). The quality and size of fragments were assessed using the Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA) with DNA 7500 chips. The sequencing process was conducted by Chunlab, Inc. (Seoul, Korea) on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) following the manufacturer's instructions.

16S rRNA sequencing analysis

Adapters, primers, and low-quality reads (< Q25) in sequencing data were removed using Trimmomatic v0.32 (Bolger et al., 2014). High-quality reads were assembled into paired-end sequences using PANDAseq (Masella et al., 2012), followed by primer trimming with a similarity threshold of 0.8 in ChunLab's pipeline. Non-specific 16S rRNA amplicons were identified using HMMER's program (Eddy, 2011). Noisy sequences were filtered out with DUDE-Seq (Lee et al., 2017). Unique sequences were clustered using UCLUST (Edgar, 2010).

Taxonomic classification was performed using USEARCH with the EzBioCloud database, and sequences were aligned pairwise based on previously established methods (Myers & Miller, 1988). Chimera detection was conducted using UCHIME (Edgar et al., 2011) in conjunction with the EzBioCloud non-chimeric 16S rRNA database. Operational taxonomic units (OTUs) were assigned at a 97% similarity threshold. Sequence clustering was performed using CD-HIT and UCLUST5 (Fu et al., 2012). Relative abundance plots were generated using bacterial species with a minimum cutoff of 1%.

RESULTS

Illumina MiSeq sequencing

Purified metagenomic DNA extracted from the gut of SBV-infected and healthy bees was used to sequence the V3-V4 region of the 16S rRNA gene on the Illumina MiSeq platform. The sequencing generated a total of 481.590 reads, with an average read length of approximately 430 bp and a GC content ranging from 51% to 54%. A total of 1.659

operational taxonomic units (OTUs) were identified at a 97% similarity threshold, with an average sequencing coverage of 99% (Table 1).

Table 1. NGS sequencing results for the V3-V4 region of the 16S rRNA gene in the gut microbiota of SBV-infected and healthy *Apis mellifera*

No.	Sample name	Group	Total valid read	OTUs	Coverage (%)
1	T1	Group 1: SBV-infected bees	67.490	297	99.8
2	T2		81.903	270	99.9
3	T3		84.564	275	99.9
4	T4	Group 2: Healthy bees	83.385	288	99.9
5	T5		78.322	277	99.9
6	T6		85.926	252	99.9
Total	6		481.590	1.659	

Gut microbial diversity in SBV-infected and healthy bees

Alpha diversity analysis was used to evaluate species diversity within samples based on indices such as observed species, Chao1, ACE, Shannon, and Simpson (Liu et al., 2020). Microbial diversity in the samples was positively correlated with the first four indices and inversely correlated with the Simpson index. The observed species count, Chao1, and ACE indices reflect species richness within the microbial community (Chao & Chiu, 2016). In contrast, the Shannon and Simpson indices represent species diversity, which considers both species richness and evenness (Qian et al., 2020).

The OTU results presented in Table 1, along with the Chao1, ACE, Shannon, and Simpson indices, indicate no significant differences in species diversity between the SBV-infected and healthy bee groups (Fig. 1).

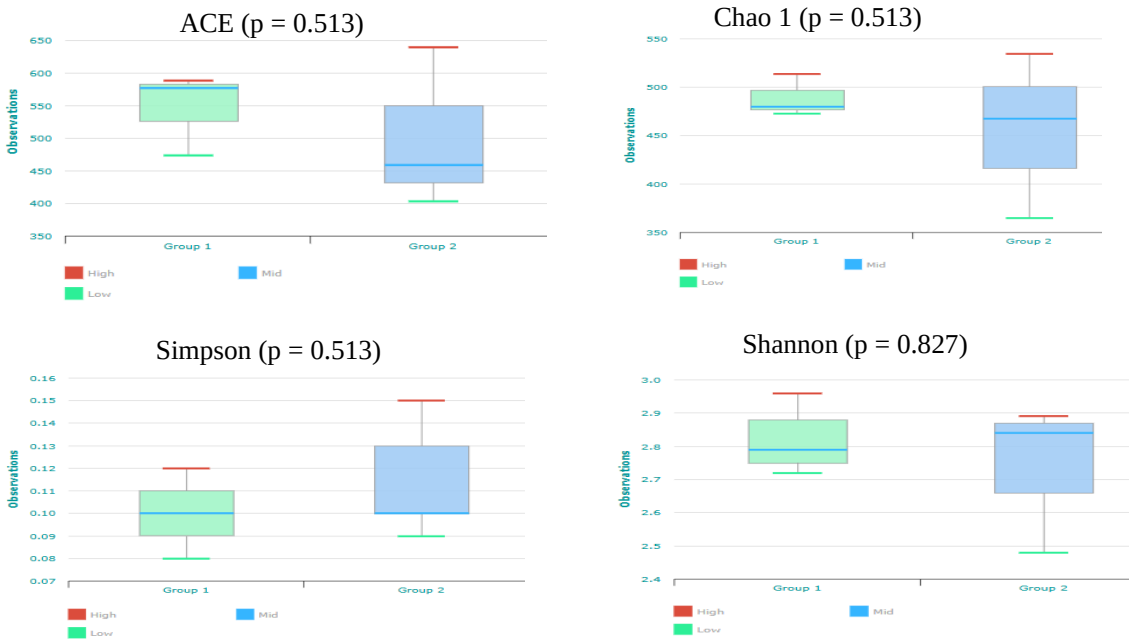

Figure 1. Comparison of gut microbial diversity between SBV-infected bees (Group 1) and healthy bees (Group 2) based on alpha diversity indices (ACE, Chao 1, Simpson and Shannon)

Bacterial composition in SBV-infected and healthy bees

The gut microbiota of SBV-infected bees comprised four bacterial phyla: Proteobacteria (48.44%), Firmicutes (38.65%), Bacteria_uc (10.95%) (*uc* - unclassified species) and Actinobacteria (1.57%). However, the healthy bee group contained three phyla including Proteobacteria (40.61%), Firmicutes (45.55%), and Bacteria_uc (13.37%).

At the order level, the SBV-infected bee group harbored bacterial taxa including Lactobacillales (43.23%), Orbales (29.25%), and Neisseriales (19.54%). In the healthy bee group, these bacterial orders were presented at 52.45%, 21.67%, and 20.67%, respectively. Additionally, Rhodospirillales (1.78%), Rhizobiales (1.25%), and Bifidobacteriales (1.76%) were detected exclusively in the SBV-infected bees, whereas Enterobacterales was unique to the healthy bee group, with a relative abundance of 2.41% (Fig. 2).

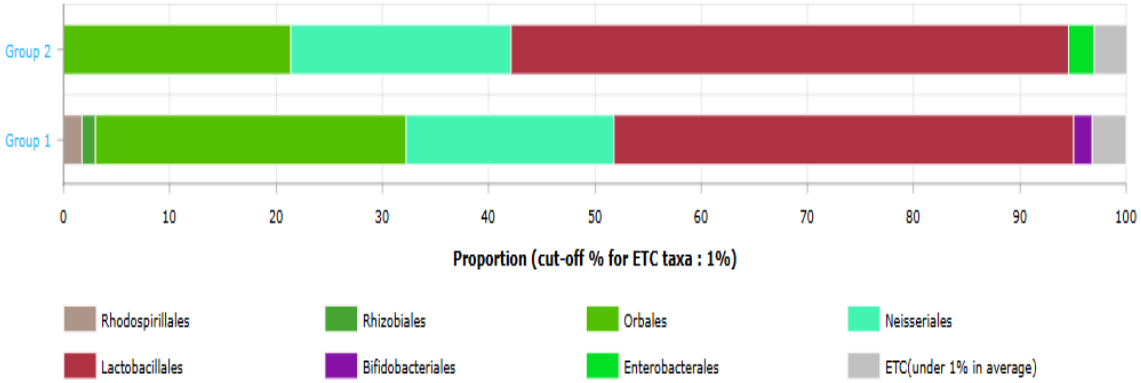

Figure 2. Bacterial composition at the order level in SBV-infected bees (Group 1) and healthy bees (Group 2)

At the genus level, a total of eight bacterial genera (with a relative abundance > 1%) were identified in both SBV-infected and healthy bee groups (Fig. 3). In the SBV-infected bee group, the dominant genera included *Lactobacillus* (43.15%), *Gilliamella* (19.93%), *Snodgrassella* (11.92%), *Frischella* (6.67%), *Neisseriaceae_uc* (6.67%), and *Orbaceae_uc* (2.83%). In the healthy bee group, these genera were presented at 51.53%, 16.2%, 12.19%, 3.1%, 7.79%, and 2.27%, respectively.

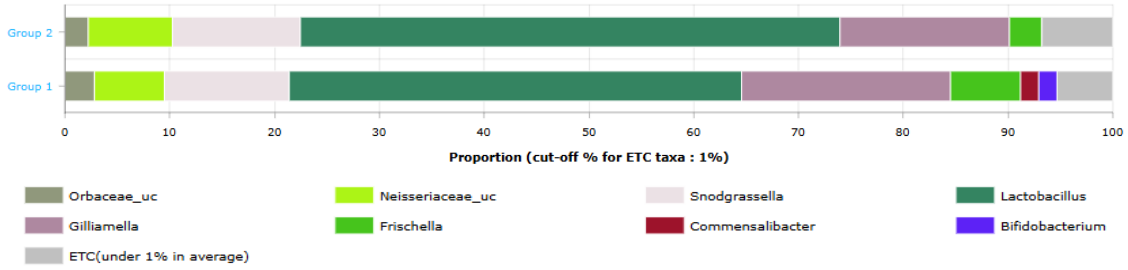

Figure 3. Bacterial composition at the genus level in SBV-infected bees (Group 1) and healthy bees (Group 2)

Notably, the genus Commensalibacter (1.71%) and Bifidobacterium (1.75%) were exclusively detected in the SBV-infected bee group. Additionally, bacterial genera with a

relative abundance of less than 1%, which were not listed individually, accounted for 5.3% of SBV-infected bees and 6.73% of healthy bees.

At species level with a cut-off value of 1%, a total of 15 bacterial species were identified in both the SBV-infected and healthy bee groups (Fig. 4). In the SBV-infected bee group, the following species were detected: *Lactobacillus_uc* (23.89%), *Lactobacillus mellis* (14.43%), *Lactobacillus apis* (2.8%), *Lactobacillus melliventris* (3.57%), *Lactobacillus helsingborgensis* (2.19%), *Gilliamella_uc* (13.37%), *Gilliamella picola* (3.86%), *Gilliamella LZGQ_s* (3.21%), *Gilliamella mensalis* group (1.12%), *Snodgrassella alvi* (10.02%), *Snodgrassella JFZW_s* (1.41%), and *Frischella perrara* (7.23%). In the healthy bee group, the following species were detected: *Lactobacillus_uc* (34.04%), *L. mellis* (10.55%), *L. apis* (4.40%), *L. melliventris* (3.85%), *L. helsingborgensis* (3.4%), *Gilliamella_uc* (9.94%), *G. picola* (3.86%), *G. mensalis* group (2.12%), *Gilliamella LZGQ_s* (2.02%), *S. alvi* (9.9%), *Snodgrassella JFZW_s* (1.73%), *Snodgrassella_uc* (2.31%), and *F. perrara* (3.44%).

At a lower cut-off of 0.1% or higher, additional species were detected in the SBV-infected bee group, including *Bifidobacterium_uc* (1.62%) and *Commensalibacter AY370188_s* (1.41%). In contrast, these bacterial species were presented at much lower proportions in the healthy bee group, with *Bifidobacterium_uc* at 0.34% and *Commensalibacter AY370188_s* at 0.66%.

Overall, the species composition between the SBV-infected and healthy bee groups was similar, except for the primary differences observed in the relative proportions of core bacterial species between the two groups.

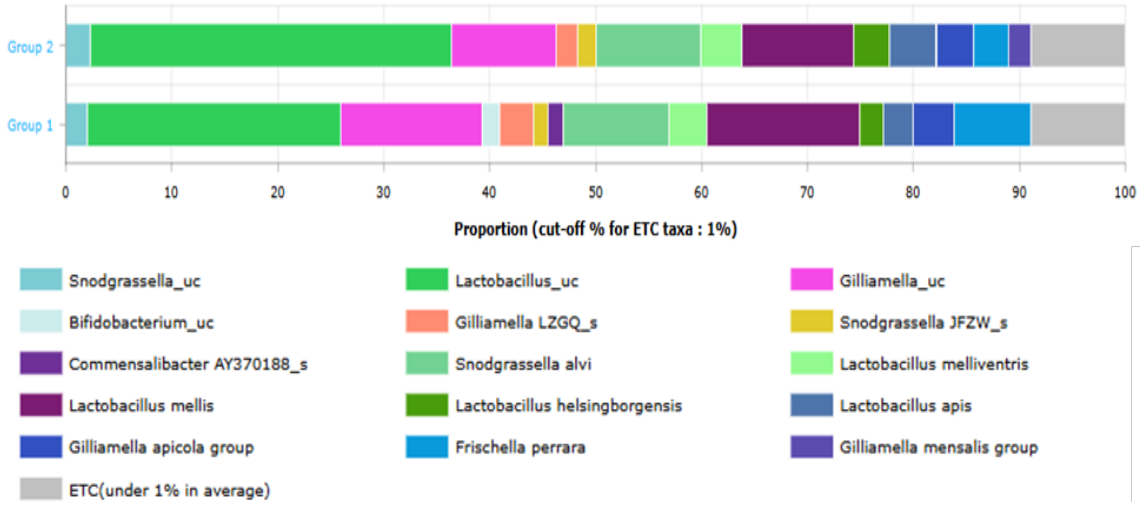

Figure 4. Bacterial species composition of SBV-infected bees (Group 1) and healthy bees (Group 2) to SBV (cut-off 1%). "JFON_s" and "JFZW_s" represent uncultured species, and "uc" denotes unclassified species

In addition to the main bacterial groups with proportions above 1%, lactic acid bacteria (LAB) were detected in both bee groups. At a cut-off ratio of 0.1%, the species *Lactobacillus mellifer*, *Lactobacillus kimbladii*, *L. HM534796_s*, and *L. HM534806_s* were found in both groups, with relative abundances ranging from 0.17% to 0.68%. The species *Frischella fructosus* and *Lactobacillus kunkeei* were only detected in the healthy bee group, with proportions of 0.16% and 0.5%, respectively (Table 2).

Table 2. Total lactic acid bacteria in healthy bees and SBV-infected bees

Bacterial species	SBV-infected bees (%)	Healthy bees (%)
Lactobacillus_uc	23.89	34.04
Lactobacillus mellis	14.43	10.55
Lactobacillus melliventris	3.57	3.85
Lactobacillus helsingborgensis	2.19	3.4
Bifidobacterium_uc	1.62	0.36
Lactobacillus mellifer	0.68	0.3
Lactobacillus kimbladii	0.46	0.63
Lactobacillus HM534796_s	0.19	0.42
Lactobacillus HM534806_s	0.17	0.35
Frischella fructosus	0	0.16
Lactobacillus kunkeei group	0	0.5

DISCUSSION

When analyzing the composition and proportions of intestinal bacteria in healthy and SBV-infected *A. mellifera* bees, we observed significant changes in the relative abundance of the *Firmicutes* and *Proteobacteria* phyla. The observation that SBV-infected bees generally exhibit higher microbial diversity compared to healthy bees can be explained by the fact that viral infections suppress the bee immune system, making them more susceptible to colonization by opportunistic microbes, including bacteria, fungi, and other viruses. This weakened immune response allows a broader range of microorganisms to thrive, leading to increased microbial diversity. Specifically, the *Proteobacteria* phylum accounted for 48.44% of the bacteria in the SBV-infected bee group, compared to 40.61% in the healthy bee group. In contrast, the *Firmicutes* phylum accounted for 38.65% in the SBV-infected bees, but increased to 45.55% in the healthy bees. These findings are consistent with previous studies (Daisley et al., 2020), which identified an increase in *Proteobacteria* and a decrease in lactic acid bacteria (LAB) in honeybees with disrupted beneficial bacteria, leading to bee populations that often suffer from health issues. Two stages of dysbiosis in honeybees, during which the gut microbiota becomes unbalanced, have been observed. Both stages exhibit a decrease in the abundance of *Firmicutes* and an increase in *Proteobacteria*, with the second stage also showing a reduction in *Actinobacteria* and an increase in non-core bacteria (Daisley et al., 2020).

In this study, we also detected bacteria belonging to different orders, including Rhodospirillales (1.78%), Rhizobiales (1.25%), and Bifidobacteriales (1.76%) in the SBV-infected bees. Among these, *Bartonella apis* from the order Rhizobiales accounted for 0.6% in the SBV-infected bees but were absent in the healthy bee group ($p < 0.05$). Hubert et al. (2017) reported an increase in *B. apis* and a decrease in *L. apis* in the bee gut following *Varroa destructor* invasion or SBV infection (Hubert et al., 2017). Our analysis also revealed a significant difference in the abundance of the *Bifidobacterium_uc* group, which belongs to the order Bifidobacteriales and the phylum Actinobacteria. The SBV-infected bee group had a higher proportion of this group (1.62%) compared to the healthy bee group (0.34%) ($p < 0.05$). Previous studies indicated that organic acids produced by Bifidobacterium, such as lactic acid and formic acid, help lower the pH of the digestive tract, creating an unfavorable environment for pathogenic microorganisms (Royan, 2019; Botero et al., 2023). Our finding further supports the role of Bifidobacterium in protecting bee colonies from pathogens.

Notably, in the healthy bee group, we detected *Lactobacillus kunkeei* at a proportion of 0.5%, while this bacterium was absent in

the SBV-infected bee samples. Additionally, other lactic acid bacteria (LAB) species were found in higher proportions in the healthy bee group, including *Lactobacillus_uc* (34.04%), *L. mellis* (10.55%), and *L. melliventris* (3.85%). In addition to these dominant species, the healthy bee group also harbored LAB species in smaller proportions, such as *L. apis*, *L. helsingborgensis*, *L. mellifer*, *L. kimbladii*, *L. HM534796_s*, and *L. HM534806_s* (Table 1). The increased abundance of LAB bacteria in the healthy bee samples further supports previous studies that have suggested LAB bacteria contribute to enhanced resistance, reducing the rate of infection or impact of bee-borne viruses (Iorizzo et al., 2022; Killer et al., 2014; Olofsson et al., 2016). These LAB strains hold potential as probiotics for developing biological products aimed at improving the health and disease resistance of honeybees.

Several studies have demonstrated that *Lactobacillus* strains can inhibit the growth of pathogenic bacteria. For example, *L. helsingborgensis* and *L. melliventris* can inhibit *Chalkbrood* infections, while *L. apis*, *L. helsingborgensis*, and *L. melliventris* can inhibit *Paenibacillus larvae*, the causative agent of American foulbrood disease (Iorizzo et al., 2022). *L. apis* has also been shown to combat bacteria responsible for both American foulbrood (*P. larvae*) and European foulbrood (*M. plutonius*) diseases (Killer et al., 2014). Furthermore, *L. kunkeei* strains are known to form biofilms that block pathogen attachment sites and secrete biologically active substances to combat pathogens such as *Serratia marcescens* NJ19 5c, *Klebsiella aerogenes* Clmp R, *Staphylococcus aureus* 74022 PR, and *E. coli* V5 (Olofsson et al., 2016). A study by Yun et al. (2022) on the intestinal microflora of *A. cerana* bees infected with SBV in Korea observed the disappearance of *L. kunkeei*, this finding is similar to our results. However, unlike the decrease in *L. mellis* reported by Yun et al., we found an increase in *L. mellis* in the SBV-infected bee group in our study. In contrast, the healthy bee group had a higher proportion of species from the genus *Lactobacillus*, while the SBV-infected bee group showed a greater presence of bacteria from the genera *Gilliamella* and *Snodgrassella*. Specifically, the *Gilliamella* genus included species such as *Gilliamella_uc*, *Gilliamella LZGQ_s*, the *G. apicola* group, and the *G. mensalis* group. The *Snodgrassella* genus included species like *S. alvi*, *Snodgrassella JFZW_s*, and *Snodgrassella_uc*. These findings suggest that gut microbiota may influence the antiviral capacity of honeybees. The genus *Lactobacillus*, a group of lactic acid bacteria, is known for its ability to enhance host health through the production of antibacterial compounds, immunomodulation, and maintenance of intestinal microbiota balance (Shehata et al., 2024). The high prevalence of *Lactobacillus* in healthy bees may be linked to several protective mechanisms, such as immune enhancement, stimulation of the innate immune system, resistance to viral invasion, and the inhibition of competing bacteria. *Lactobacillus* produces lactic acid and antibacterial compounds that can suppress the growth of harmful microorganisms, including those that may promote viral growth. Moreover, *Lactobacillus* helps maintain a balanced gut microbiota, and a stable gut ecosystem dominated by *Lactobacillus* may support better overall bee health and reduce the risk of SBV infection (Evans & Lopez, 2004).

Conversely, the high presence of bacteria from the genus *Gilliamella* and *Snodgrassella* in SBV-infected bees raises important questions about their relationship to viral susceptibility. Species of *Gilliamella*, such as *Gilliamella_uc*, *Gilliamella LZGQ_s*, *G. apicola* group, and *G. mensalis* group, are known to play crucial roles in the degradation of complex carbohydrates from nectar and pollen. However, in some cases, *Gilliamella* may contribute to the production of harmful by-products or decrease the antiviral capacity of bees. One hypothesis is that an abnormal increase in *Gilliamella* could disrupt the microbiome, facilitating SBV spread (Kwong & Moran, 2016).

The genus *Snodgrassella*, including species such as *S. alvi*, *Snodgrassella JFZW_s*, and *Snodgrassella_uc*, is commonly found in the bee gut and is involved in digestion. However, previous studies have suggested that some *Snodgrassella* species might act as a "transit station" for pathogenic viruses or bacteria, enabling their entry into bee cells. The increased presence of *Snodgrassella* in SBV-infected bees may be associated with a suppressed immune response, making these bees more susceptible to SBV infection (Kešnerová et al., 2017; Kwong & Moran, 2016).

The gut microbiota of bees represents a complex ecosystem, where various bacteria interact with one another and the host in ways that are not yet fully understood. Changes in the proportions of bacterial groups may result from SBV infection, as the virus could alter the gut environment, leading to an imbalance in microbiota and an increase in bacteria such as *Gilliamella* and *Snodgrassella*. Alternatively, environmental and nutritional factors may also play a role. Variations in diet or living conditions could influence the gut microbiota, thereby affecting the bees' ability to resist viruses. Thus, further studies are required to verify these hypotheses. However, this study does not identify the specific mechanisms through which bacteria like *Gilliamella* and *Snodgrassella* influence honeybee susceptibility to SBV. Future research should focus on analyzing the specific metabolites produced by these bacteria and exploring the role of interactions between the microbiota and the bee's immune response.

CONCLUSIONS

In this study, we identified several bacterial groups characteristic of healthy and SBV-infected *A. mellifera* bees. The findings provide valuable insights to develop biological products targeted to protect *A. mellifera* colonies from SBV infection. Additionally, the study enhances our understanding of the relationship between gut microbiota and SBV resistance, and it opens up potential applications in bee health management. These include the use of *Lactobacillus*-based probiotics to boost virus resistance, as well as monitoring gut microbiota as an early indicator to assess bee health and the risk of SBV infection, along with other bee-pathogenic viruses.

Acknowledgements: This study was supported by the Vietnam Academy of Science and Technology for its senior researchers (Grant No. NCVCC08.01/25-25).

REFERENCES

Bolger A. M., Lohse M. & Usadel B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15): 2114–2120.

Botero J., Sombolestani A. S., Cnockaert M., Peeters C., Borremans W., De Vuyst L., Vandamme P., 2023. A phylogenomic and comparative genomic analysis of Commensalibacter, a versatile insect symbiont. *Animal Microbiome*, 5(1): 25.

Chao A., Chiu C. H., 2016. Species richness: estimation and comparison. *Wiley StatsRef: statistics reference online*, 1: 26.

Chen Y. P. & Siede R., 2007. Honey bee viruses. *Advances in virus research*, 70: 33−80.

Daisley B. A., Chmiel J. A., Pitek A. P., Thompson G. J., Reid G., 2020. Missing microbes in bees: how systematic depletion of key symbionts erodes immunity. Trends in Microbiology, 28: 1010−1021.

Dosch C., Manigk A., Streicher T., Tehel A., Paxton R. J., Tragust S., 2021. The gut microbiota can provide viral tolerance in the honey bee. *Microorganisms*, 9(4): 871.

Eddy S. R., 2011. Accelerated profile HMM searches. PLoS computational biology, 7(10), e1002195.

Edgar R. C., Haas B. J., Clemente J. C., Quince C., Knight R., 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, 27(16): 2194−2200.

Edgar R. C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26(19): 2460−2461.

Engel P., Kwong W. K., McFrederick Q., Anderson K. E., Barribeau S. M., Chandler J. A., Cornman R. S., Dainat J., De Miranda J. R., & Doublet V., 2016. The bee microbiome: impact on bee health and model for evolution and ecology of host–microbe interactions. *MBio*, 7(2): 10–1128.

Evans J. D., Lopez D. L., 2004. Bacterial probiotics induce an immune response in the honey bee (Hymenoptera: Apidae). *Journal of economic entomology*, 97(3): 752−756.

Fu L., Niu B., Zhu Z., Wu S., Li W., 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics*, 28(23): 3150−3152.

Hubert J., Bicianova M., Ledvinka O., Kamler M., Lester P. J., Nesvorna M., Erban T., 2017. Changes in the bacteriome of honey bees associated with the parasite Varroa destructor, and pathogens Nosema and Lotmaria passim. *Microbial ecology*, 73: 685−698.

Iorizzo M., Letizia F., Ganassi S., Testa B., Petrarca S., Albanese G., De Cristofaro A., 2022. Functional properties and antimicrobial activity from lactic acid bacteria as resources to improve the health and welfare of honey bees. *Insects*, 13(3): 308.

Kešnerová L., Mars R. A., Ellegaard K. M., Troilo M., Sauer U., Engel P., 2017. Disentangling metabolic functions of bacteria in the honey bee gut. *PLoS biology*, 15(12): e2003467.

Killer J., Dubná S., Sedláček I., Švec P., 2014. Lactobacillus apis sp. nov., from the stomach of honeybees (*Apis mellifera*), having an in vitro inhibitory effect on the causative agents of American and European foulbrood. *International journal of systematic and evolutionary microbiology*, 64(Pt_1): 152−157.

Kwong W. K., Medina L. A., Koch H., Sing K. W., Soh E. J. Y., Ascher J. S., Moran N. A., 2017. Dynamic microbiome evolution in social bees. *Science advances*, 3(3): e1600513.

Kwong W. K., Moran N. A., 2016. Gut microbial communities of social bees. *Nature reviews microbiology*, 14(6): 374−384.

Lanh P. T., Duong B. T., Thu H. T., Hoa N. T., Van Quyen D., 2024. Comprehensive analysis of the microbiome in *Apis cerana* honey highlights honey as a potential source for the isolation of beneficial bacterial strains. *PeerJ*, 12: e17157.

Lee B., Moon T., Yoon S., Weissman T., 2017. DUDE-Seq: fast, flexible, and robust denoising for targeted amplicon sequencing. *PloS one*, 12(7): e0181463.

Liu W., Zhang R., Shu R., Yu J., Li H., Long H., Wu Q., 2020. Study of the relationship between microbiome and colorectal cancer susceptibility using 16SrRNA sequencing. *BioMed research international*, doi: 10.1155/2020/7828392

Maes P. W., Rodrigues P. A., Oliver R., Mott B. M., Anderson K. E., 2016. Diet-related gut bacterial dysbiosis correlates with impaired development, increased mortality and Nosema disease in the honeybee (Apis mellifera). *Molecular Ecology*, 25(21): 5439−5450.

Masella A. P., Bartram A. K., Truszkowski J. M., Brown D. G., Neufeld J. D., 2012. PANDAseq: paired-end assembler for illumina sequences. *BMC bioinformatics*, 13: 1−7.

Myers E. W., Miller W., 1988. Optimal alignments in linear space. *Bioinformatics*, 4(1): 11−17.

Olofsson T. C., Butler È., Markowicz P., Lindholm C., Larsson L., Vásquez A., 2016. Lactic acid bacterial symbionts in honeybees–an unknown key to honey's antimicrobial and therapeutic activities. *International Wound Journal*, 13(5): 668−679.

Qian X. B., Chen T., Xu Y. P., Chen L., Sun F. X., Lu M. P., Liu Y. X., 2020. A guide to human microbiome research: study

design, sample collection, and bioinformatics analysis. *Chinese Medical Journal*, 133(15): 1844−1855.

Royan M. (2019). Mechanisms of probiotic action in the honeybee. *Critical Reviews™ in Eukaryotic Gene Expression*, 29(2).

Shehata M. G., Masry S. H., Abd El-Aziz N. M., Ridouane F. L., Mirza S. B., El-Sohaimy S. A. 2024. Probiotic potential of lactic acid bacteria isolated from honeybees stomach: Functional and technological insights. *Annals of Agricultural Sciences*, 69(1): 11−18.

Tlak Gajger I., Nejedli S., Cvetnić L., 2023. Influence of probiotic feed supplement on Nosema spp. infection level and the gut microbiota of adult honeybees (Apis mellifera L.). *Microorganisms*, 11(3): 610.

Wei R., Cao L., Feng Y., Chen Y., Chen G., Zheng H., 2022. Sacbrood virus: a growing threat to honeybees and wild pollinators. *Viruses*, 14(9): 1871.

Weisburg W. G., Barns S. M., Pelletier D. A., Lane D. J., 1991. 16S ribosomal DNA amplification for phylogenetic study. *Journal of bacteriology*, 173(2): 697−703.

Yun B. R., Truong A. T., Choi Y. S., Lee M. Y., Kim B. Y., Seo M., Cho Y. S., 2022. Comparison of the gut microbiome of sacbrood virus-resistant and-susceptible Apis cerana from South Korea. *Scientific Reports*, 12(1): 10010.