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THE GUT MICROBIOME IN DEFORMED WING VIRUS (DWV)-UNINFECTED AND -INFECTED *Apis cerana* HONEYBEES SUGGESTS THE ROLE OF GUT MICROBIOTA IN COMBATING VIRAL INFECTIONS

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ABSTRACT

Honeybees harbor a distinct core microbiota that plays a crucial role in stress tolerance and disease resistance. However, infections can significantly alter the composition and diversity of their gut microbiota, impacting overall bee health. This study investigates the effects of Deformed Wing Virus (DWV) infection on the gut microbiota of *Apis cerana* honeybee. The samples were collected from six colonies in Hanoi, Vietnam, and analyzed using high-throughput sequencing of the 16S rRNA gene. Our results show that while the overall diversity of gut microbiota in DWV-infected and uninfected bees did not significantly differ, notable changes were observed in the relative abundances of specific bacterial taxa. In DWV-infected bees, the relative abundance of Proteobacteria significantly decreased, whereas Firmicutes increased compared to uninfected bees. At the genus level, a significant decrease in *Gilliamella* and an increase in *Lactobacillus* were observed in infected bees. Functional gene predictions indicated that pathways related to carbohydrate, fatty acid, and lipid metabolism, as well as the biosynthesis of cofactors, vitamins, and amino acids, were upregulated in DWV-infected bees. These findings highlight the impact of DWV infection on the gut microbiota of *A. cerana* and suggest potential avenues for using probiotics to restore gut microbial balance and improve honeybee health. This research provides a foundation for developing strategies to enhance the resilience of honeybee colonies against viral infections.

Keywords: 16S rRNA, *Apis cerana*, Deformed Wing Virus, gut microbiome, high-throughput sequencing, honeybees.

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INTRODUCTION

Honeybees possess a distinct core microbiota that contributes significantly to host stress tolerance and disease resistance (Daisley et al., 2020; Dosch et al., 2021; Li et al., 2019). However, diseases affecting honeybees, such as viral, bacterial, and fungal infections, have been found to influence the composition and diversity of the gut microbiota, potentially affecting overall bee health (Panjad et al., 2021). Several studies indicated the association between pathogen infections and alterations in the gut microbial community. For instance, the SBV infection resulted in loss of bacteria in the gut microbiome that could affect host nutrients and inhibit honey bee pathogens, such as *Gilliamella JFON_s, Gilliamella_uc, Pseudomonas putida,* and *Lactobacillus kunkeei* in *Apis cerana* larvae and *Frischella_uc, Pantoea agglomerans, Snodgrassella_uc,* and *Bifidobacterium asteroides* in adult bees (Yun et al., 2022). Moreover, analysis of the gut microbiota in honeybee colonies affected by colony collapse disorder (CCD) has revealed changes in certain microbial phyla compared to healthy colonies, with the abundance of Firmicutes and Alphaproteobacteria decreases in diseased colonies compared to control colonies (Cox-Foster et al., 2007). In addition, Li et al. (2020) investigated the shifts in gut microbial diversity and disruption in the core microbiome of honeybees as a consequence of *Nosema ceranae* infection. These results suggested that a lower presence of beneficial bacterial species may weaken the host's immune system, thereby influencing honeybee health and colony survival (Cox-Foster et al., 2007).

Among honeybee viral pathogens, DWV is a major pathogenic factor threatening honeybee populations. However, the effects of DWV on the gut microbiome remain understudied. DWV exists in all stages of the honeybee, typically as a latent non-pathogenic infection. Meanwhile, DWV can replicate quickly under some stresses, which shortens honeybee lifespan and ultimately leads to whole colony losses (Deitch et al., 1991; Genersch & Aubert, 2010; Tantillo et al., 2015). DWV amount is also used as a metric to predict colony strength and is a negative marker of honeybee fitness (Budge et al., 2015). Recent studies about the relationship between gut bacteria disruption and DWV replication showed that DWV replication is affected by honeybees' nutrition status and immune response (Dosch et al., 2021; Li et al., 2019). These results provide evidence for a positive role of the gut microbiota in honey bee nutrition, metabolism, and immunity, which confer the ability to inhibit DWV replication, extend honey bee lifespan, and improve overall health (Dosch et al., 2021; Li et al., 2019). Therefore, understanding the influence of diseases on the gut microbiota of honeybees is crucial for comprehending the intricate interplay between pathogens and the resident microbial community, and if any changes occur in the gut microbiota, supplementation with selected strains can restore the dysbiosis, reducing honeybee mortality and improving honeybee health (Cox-Foster et al., 2007).

The present study aims to discern differences in gut microbial communities between *A. cerana* healthy and DWV-infected honeybees, shedding light on the impact of DWV infection on the gut microbiome through high-throughput sequencing of the 16S rRNA gene. The findings provide scientific information for the development or selection of probiotics to improve *A. cerana* honeybee's health.

MATERIALS AND METHODS

Sample collection

A. cerana samples were collected from 6 colonies at honeybee farms in Ha Noi, Vietnam (20.04^{o}N 105.5560^{o}E). The samples were collected, kept on ice, and immediately brought to the Lab of Molecular Microbiology, Institute of Biotechnology, Vietnam Academy of Science and Technology.

All the collected colonies were confirmed to be free of common honeybee pathogens by Polymerase Chain Reaction (PCR) using specific pairs of *Nosema* spp and Reverse Transcription Polymerase Chain Reaction (RT-PCR) using specific pairs of primer of seven common honeybee viruses, including Sacbrood

virus (SBV), Acute bee paralysis virus (ABPV), Deformed wing virus (DWV), Black queen cell virus (BQCV), Kashmir bee virus (KBV), Cloudy wing virus (CWV), Israeli acute paralysis virus (IAPV) as reported in our previous publication (Lanh et al., 2024).

DWV-infected colonies were identified by clinical signs, which were pupal death and, in newly emerged bees, deformed wings, and bloated, discoloured, and shortened abdomens. The presence of DWV in adult bees was confirmed by RT-PCR using DWV specific primers.

For gut sample preparation, we humanely dispatched honeybees by pinching off their heads. The guts were carefully extracted with alcohol-sterilized forceps by clamping the tip of the last abdominal segment and slowly pulling the entire gut (hindgut, midgut, and usually the honey stomach) from their bodies. The gut samples were stored at -20 ºC for subsequent analysis.

DNA extraction

The DNA of the gut microbiota was extracted using the DNA extraction method described in our previous study (Duong et al., 2020). Briefly, 10 g of each bee gut sample were homogenized in sterile DNA extraction buffer (100 mM Tris HCl, 50 mM EDTA, 50 mM NaCl, 1% SDS, pH 7.0) using a sterile elastic pestle and centrifuged at 2,000 rpm in 5 min. The aqueous upper phase was transferred into a new microcentrifuge tube with 50 µL of protease K (20 mg/mL) and 15 µL of lysozyme (100 mg/mL), incubated at 65 ^{o}C for an hour and then centrifuged at 5,000 rpm in 5 min. The aqueous upper phase was collected, and phenol/chloroform/isoamyl (25:24:1) was added with a 1:1 (v/v) ratio, mixed, and centrifuged at 12,000 rpm for 20 min. The aqueous upper phase was transferred into a new microcentrifuge tube. The DNA was precipitated using isopropanol with a 1:1 (v/v) ratio for 30 minutes at room temperature and centrifuged at 12,000 rpm for 20 minutes at 4 ºC. The DNA pellet was washed twice with 70% ethanol and then dried by Speedvac (Thermo Scientific, USA) for 10 min. The DNA was re-suspended in nuclease-free water. DNA quality was checked by 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light. The DNA concentration and purity were determined by a NanoDrop Lite (Thermo Scientific, USA). The qualified DNA samples were then sequenced by NGS.

Illumina sequencing, 16S rRNA sequence analysis, and statistical analysis

The 16S rRNA library preparation was performed using xGen 16S Amplicon Panel v2 Kit (IDT, USA) according to the manufacturer's instructions and then sequenced by NGS (2x150 PE) with the Illumina MiniSeq Sequencing system (Illumina, USA).

Adapters, primers, and low-quality sequences were removed using Trimmomatic version 0.39 and Cutadapt version 2.10. The qualified sequences were grouped and processed to create ASV (amplicon sequence variant) using the q2-DADA2 plugin and denoised method in the QIIME2 tool (Callahan et al., 2016). Classification of ASVs was performed with QIIME2 on the SILVA version 138 SSURef Nr99 database (Quast et al., 2012), with the q2-feature-classifier plugin and the classify-consensus-blast method.

Composition representation, heatmap, alpha diversity, and beta diversity analyses were performed with QIIME2. Alpha diversity indices were used to compare gut microbial diversities between the group of collected honey bees, and the *p*-value was calculated with the Kruskal-Wallis-pairwise test.

Functional gene prediction was conducted with PICRUSt2 version 2.3.0-b (Douglas et al., 2020) and the MetaCyc database. The pairwise T-test was used for difference analysis of each gene expression.

RESULTS

DNA extraction and 16S rRNA amplification

The DNA from the collected samples was extracted and the quality and quantity of the extracted DNA samples were confirmed by agarose gel electrophoresis and NanoDrop Lite, respectively. The DNA concentration of

the samples ranged from 678.5 ng/μL to 1,1218.7 ng/μL, with A260/280 values between 1.75 to 1.95. The results show that the DNA meets the standard requirements for 16S rRNA amplification by NGS (next-generation sequencing).

The 16S rRNA gene in all samples was amplified and sequenced by Illumina platforms. The results are shown in Table 1. The observed taxa in the sample were identified at the 97% sequence similarity cut-off.

Table 1. Summary of NGS-sequencing data

No.	Sample name	Group	Number of raw reads	Number of valid reads	%Q30	Observed taxa
1	Adults 1	DWV-uninfected	359,394	270,927	93.65	368
2	Adults 2		278,200	207,006	93.70	338
3	Adults 3		360,652	258,128	93.95	514
4	Adults 4	DWV-uninfected	361,394	265,184	93.45	334
5	Adults 5		400,000	288,547	93.35	347
6	Adults 6		345,024	252,206	93.70	410

Diversity analysis of gut microbiota in DWV-uninfected and -infected honeybees

The biodiversity of the gut microbiome of DWV-uninfected and -infected honeybees was shown by alpha diversity with Observed taxa, Evenness, Chao1, and Shannon parameters (Fig. 1). The results showed that the difference between the two groups was insignificant ($p > 0.05$) in all compared indices.

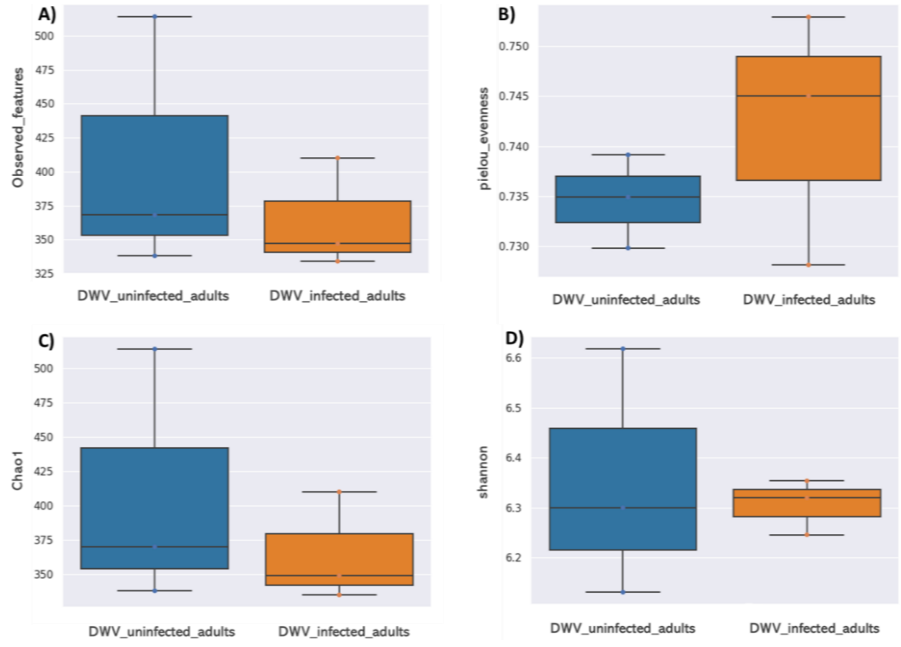

Figure 1. Alpha diversity boxplots designed with Observed taxa (A), Evenness (B), Chao1 (C), and Shannon (D) indices were used to compare the gut microbiome between DWV-uninfected and -infected honeybees

Furthermore, the principal coordinates analysis (PCoA) was used to investigate changes in β-diversity. The PCoA plot in Figure 2 shows the two principal coordinate

axes (PCoA1 and PCoA2), which respectively explain 43.29% and 28.65% of the variation at the species level. There were no differences in the bacterial communities between DWV-uninfected and -infected honey bees along the two PCoA axes (p = 0.293, PERMANOVA using the Bray-Curtis distance matrices).

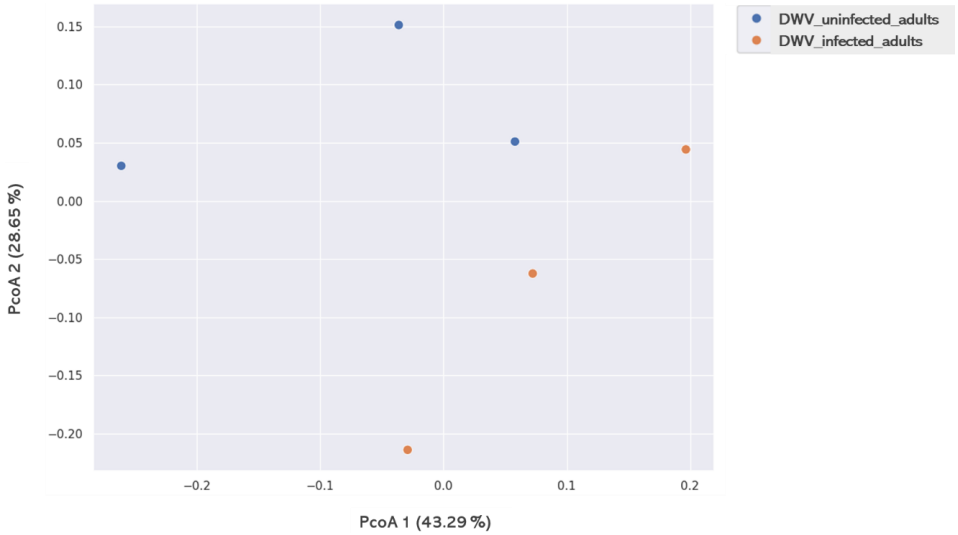

Figure 2. Gut bacterial communities of DWV-uninfected honeybees compared to DWV-infected were analyzed by the principal coordinate analysis (PCoA)

Gut microbiome of DWV-uninfected and DWV-infected *A. cerana*

The gut microbiota prevalent in DWV-uninfected *A. cerana* included the phyla Proteobacteria (81.0%), Firmicutes (9.22%), Bacteroidetes (8.41%), and Actinobacteria (0.5%) (Fig. 3), genera *Gilliamella* (61.34%), *Lactobacillus* (9.17%), *Snodgrassella* (8.94%), *Apibacter* (5.20%) and *Enterobacter* (2.44%) (Fig. 4).

The gut microbiota prevalent in DWV-infected *A. cerana* comprised the same phyla: Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria. However, the changes were identified by a significant decrease in the relative abundance of Proteobacteria (64.04%) (t = -3.655, p = 0.022, df = 4) and a considerable increase in the abundance of Firmicutes (26.45%) (t = 3.655, p = 0.024, df = 4) compared to those in the DWV-infected *A. cerana.*

Further, the genus composition of the gut microbiota in diseased *A. cerana* adults showed notable increases in the abundance of *Lactobacillus* (t = 3.531, p = 0.024, df = 4) and remarkable decreases in the abundance of *Gilliamella* (t = -3.52832, p = 0.024, df = 4). The other genera, such as *Snodgrassella*, *Apibacter*, and *Enterobacter*, were identified in lower percentages, and *Bifidobacterium*, *Pantoea*, *Frischella*, and *Serratia*, were found in inconsiderable percentages in both groups, all without significant differences (Fig. 4).

The LAB (Lactic acid bacteria) species in DWV-infected *A. cerana* was significantly increased compared to those in DWV-uninfected *A. cerana* (t = 3.576, p = 0.023, df = 4) (9.67% and 27.27% respectively). Further, *Lactobacilus* sp. was predominant in the gut of both groups. However, its relative abundance was distinct (t = 3.886, p = 0.018, df = 4) between DWV-infected *A. cerana* (16.18%) and only 4.62% in the other, followed by unclassified_*Lactobacillus* (t = 3.462, p = 0.01826. df = 4) (8.4% in DWV-infected and 3.2% in DWV-uninfected *A.cerana*). There was also an insignificant increase in the percentage of unclassified_

Bifidobacterium in DWV-infected *A. cerana* (1.31%) compared to DWV-infected *A. cerana* (0.78%) Additionally, *L. kunkeei*, *Lactobacillus oligofermentans*, *Lactobacillus*_uc (uncultured), *Lactobacillus sakei*, *B. asteroides*, unclassified_ *Bifidobacterium* were identified in minute percentages in both DWV-infected and uninfected *A. cerana* without notable difference between two groups.

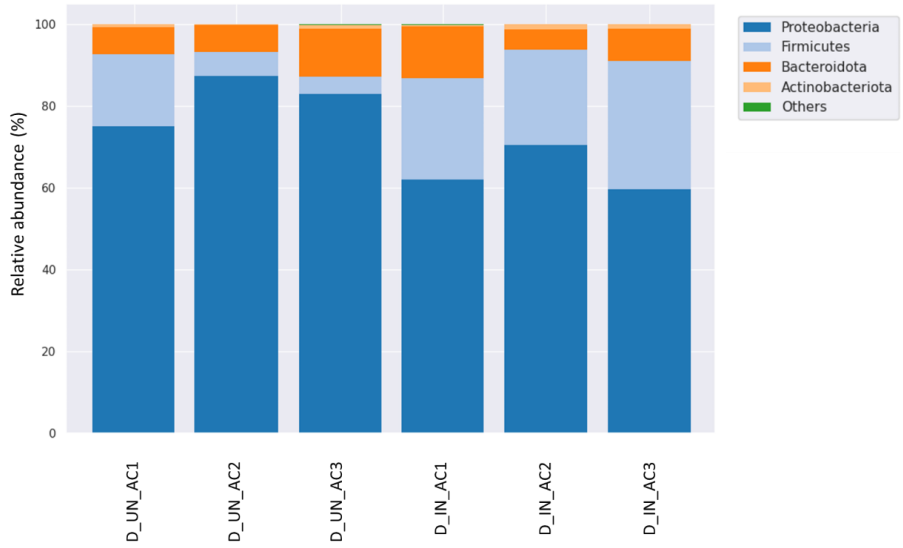

Figure 3. Gut microbiome of DWV-uninfected and -infected Apis cerana at the phylum level

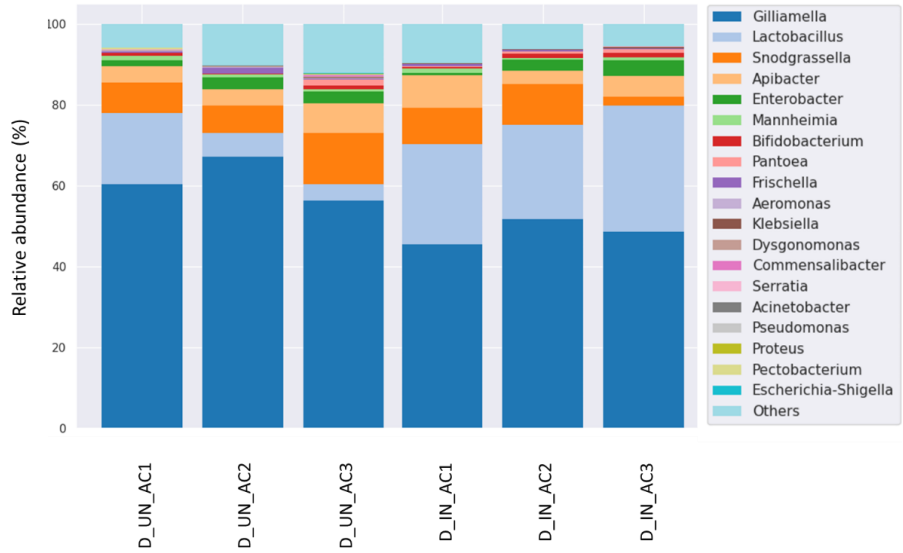

Figure 4. The gut microbiome of DWV-uninfected and -infected Apis cerana at the genus level

Functional gene prediction

Furthermore, the result of functional gene prediction showed several MetaCyc pathways that differed from DWV-uninfected *A. cerana* compared to DWV-infected *A. cerana*. In DWV-infected *A. cerana*, the pathways with the highest increase mainly were related to carbonhydrates, fatty acid and lipid

metabolisms; the cofactors, electron carriers, vitamins biosynthesis, and amino acid metabolism pathways, whereas the pathways with the strongest decrease were mainly related to core bacterial functions such as degradation/utilization/assimilation and nucleoside and nucleotide biosynthesis, cell structure biosynthesis (Fig. 5).

Figure 5. Heat map of functional prediction with PICRUSt2 and the MetaCyc database

DISCUSSION

In this study, we investigated the microbiota of DWV-infected and -uninfected *A. cerana* in Vietnam for the first time. As a result, the diversity of gut microbiota of *A. cerana* adults was not significantly different between DWV-infected and -uninfected adults. However, the changes were identified in the relative abundance of identified taxa. Our results demonstrated that the gut microbiota of DWV-infected and -uninfected *A. cerana* adults were composed of four major phyla: Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria, especially Proteobacteria was the most dominant. Among them, the relative abundance of Proteobacteria considerably decreased in diseased colonies compared to healthy colonies. The decline of this phylum was previously reported in the gut microbiota in honeybee colonies undergoing colony collapse disorder (CCD) (Cox-Foster et al., 2007). In contrast, the relative abundance of Firmicutes was significantly increased in DWV-infected *A. cerana* compared to normal flora in the adult gut of honeybees. A decrease in the relative abundance of Proteobacteria and increased abundances of Firmicutes were also observed in the midgut and hindgut of honeybees (*Apis mellifera*) with jujube flower disease (Ma et al., 2020).

At the genus level, we found that the gut microbiota of DWV-infected and -uninfected bees was dominated by a few core bacterial species, *Gilliamella*, *Lactobacillus*, and *Snodgrassella*. A comparison of the gut microbiota of healthy and DWV-infected *A. cerana* revealed that DWD progression resulted in a significant decrease in the abundance of *Gilliamella*. They are seasonal and nutritional-independent bacteria and vary due to the sources of nectar and the presence of other bacteria in the gut microbiota of the honeybee (Silva et al., 2017). Some of these bacterial species have been identified to have essential functions in the gut of honey bees. For instance, *Gilliamella* spp. are endosymbionts and play a role in degrading polysaccharides that could affect the absorption of host nutrients, immune function, and overall health of the honeybee (Ellegaard & Engel, 2019; Zhang et al., 2022; Zheng et al., 2019). This suggests a lower presence of beneficial bacterial species may weaken the host's immune system (Cox-Foster et al., 2007).

In addition, we observed a considered increase in the relative abundance of *Lactobacillus* (*Lactobacillus* sp.) in DWV-uninfected *A. cerana*. *Lactobacillus* is an important bacterial group of the honeybee gut microbiota known for their synthesis of antibacterial compounds, biofilm formation, and a central function in carbohydrate catabolism and, thus, in the pathogen resistance and the nutrition of their hosts (Kwong & Moran, 2016; Vásquez et al., 2012). Several studies have shown that *Lactobacillus* and other acid-lactic bacteria helped increase colony size by enhancing the egg-laying capacity of the queen and resistance to honey bee diseases such as nosemosis and varroosis (Audisio, 2017; Baffoni et al., 2016). This suggested that the increased amount of *Lactobacillus* was beneficial in improving the host immunity against DWV disease and honeybee injury. However, further studies on the usefulness of the *Lactobacillus* species unique to DWV-uninfected *A. cerana* might be essential to understand whether they provide practical maintenance of honeybee health.

Other LABs were found in both groups of *A. cerana* in this study without noticeable, including *B. asteroides*, *Lactobacillus helsingborgensis*, *L. kunkeei*, *Lactobacillus*_uc (uncultured), *B. asteroides*, unclassified_ *Bifidobacterium* that were also identified from honeybees (*A. mellifera* and *A. cerana*) and were isolated and screened for probiotic potential, especially *Lactobacillus* sp., *L. kunkeei B. asteroides*, *L. helsingborgensis*. They showed the ability to produce acid from d-glucose, d-fructose, d-mannose, N-Acetylglucosamine, arbutin, salicin, and d-tagatose (Engel et al., 2012). These bacteria are thought to be a beneficial gut symbiont in many organisms, including honeybees and humans. These microbes have adapted to various microenvironments in honeybees and play protective roles against environmental

microbial invasion, indicating their potential as probiotics for honeybees (Anderson et al., 2013; Endo & Salminen, 2013; Hubert et al., 2017).

Additionally, there were insignificant changes in the percentage of *Snodgrassella alvi* and *Gilliamella apicola*, core bacteria in honey bees. These bacteria affect the host's nutrient metabolisms and immune function (Kwong & Moran, 2016). Previous studies have found that the relative abundances of *S. alvi* showed increases in the gut microbiome of honeybees from colonies suffering CCD and with high *Varroa* infestation levels (Cox-Foster et al., 2007) and in colonies with jujube flower disease (Ma et al., 2020). Meanwhile, antibiotic exposure decreased the abundance of *S. alvi* but did not alter the abundance of *G. apicola* (Raymann et al., 2017). This could be because honeybee response to different stress was not the same (Ma et al., 2020).

Ecological imbalance in gut bacteria can lead to function loss, negatively affecting colony health (Cox-Foster et al., 2007; Ma et al., 2020). Our results showed that the metabolism function of the diseased honeybees was affected. In this study, the significant alterations in carbohydrate metabolism pathways may reflect the need for abundant energy for the host and gut microbiota to endure adverse environments (Ma et al., 2020). Symbiotic bacteria in the honeybee intestine, such as *Gilliamella*, *Lactobacillus*, and *Bifidobacterium*, can produce enzymes like pectin-degrading enzymes, glycoside hydrolases, and polysaccharide lyases that break down various carbohydrates (Engel et al., 2012; Engel & Moran, 2013), *Lactobacillus* and *Bifidobacterium* can metabolize a wide range of compounds in fermentative processes in the honeybee gut and are responsible for the greatest proportion of the metabolic output (Kešnerová et al., 2016; Saraiva et al., 2015) and are involved in nectar processing, carbohydrate metabolism, immunomodulation, and pathogen interference (Engel & Moran, 2013; Koch & Schmid-Hempel, 2011; Raymann et al., 2017; Spivak & Reuter, 2001; Vásquez et al., 2012; Wu et al., 2013). Therefore, the relatively high abundance of *Bifidobacteria* and *Lactobacillus* may be helpful for carbohydrate metabolism to provide more energy. Additionally, interspecies interactions mediate carbonhydrate metabolism and amino acid synthesis, thus benifiting the host (Zheng et al., 2019). Zhang et al. (2022) suggested that *Lactobacillus* spp. may altered amino acid metabolism pathways, especially, because they can upregulated the expression of genes related to olfactory functions that may result in more sensitive to sugars of the host, and labor division (Zhang et al., 2022). Similarly, in *Drosophila*, oral infection with *Erwinia carotovora* can stimulate the production of gut-derived specific proteins, which further promote lipid production and accumulation in neurons and modulate olfaction in aging flies (Cai et al., 2021). Moreover, *Bifidobacterium* can synthesize a variety of vitamins B to supplement host vitamin needs or be involved in biological response as coenzyme (Eichler & Schaub, 2002; Engel et al., 2012). Pathways for the metabolism of cofactors and vitamins were significantly up-regulated in diseased samples, suggesting their important functional consequences for the host physiology.

CONCLUSION

Our results revealed that the gut microbiota considerably changed in DWV-infected honeybees compared to the healthy honeybees in the relative abundance of identified taxa. In the diseased honeybees, a significant selective enrichment of *Lactobacillus* (*Lactobacillus* sp.) and a decrease in the abundance of *Gilliamella*. Similarly, there were shifts in the community functional potential that carbohydrate, fatty acid and amino acid metabolism, cofactor, prosthetic group, electron carrier, and vitamin biosynthesis were increased, while energy metabolism, nucleoside and nucleotide biosynthesis and cell structure biosynthesis were decreased in the diseased honeybees. The findings underscore the importance of further investigations to understand the role of the gut microbiome in honeybee health and disease resilience and to develop strain-based probiotics that could be formulated, including

the essential common bacterial group, specific species in healthy strains, and the important common LAB group.

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REFERENCES

Anderson K. E., Sheehan T. H., Mott B. M., Maes P., Snyder L., Schwan M. R., . . . Corby-Harris V., 2013. Microbial ecology of the hive and pollination landscape: bacterial associates from floral nectar, the alimentary tract and stored food of honey bees (Apis mellifera). *PLoS One*, 8(12): e83125.

Audisio M. C., 2017. Gram-positive bacteria with probiotic potential for the Apis mellifera L. honey bee: the experience in the northwest of Argentina. *Probiotics and Antimicrobial Proteins*, 9(1): 22−31.

Baffoni L., Gaggìa F., Alberoni D., Cabbri R., Nanetti A., Biavati B., & Di Gioia D., 2016. Effect of dietary supplementation of Bifidobacterium and Lactobacillus strains in Apis mellifera L. against Nosema ceranae. *Beneficial Microbes*, 7(1): 45−51.

Budge G. E., Pietravalle S., Brown M., Laurenson L., Jones B., Tomkies V., & Delaplane K. S., 2015. Pathogens as predictors of honey bee colony strength in England and Wales. *PLoS One*, 10(7): e0133228.

Cai X. T., Li H., Borch Jensen M., Maksoud E., Borneo J., Liang Y., . . . Jasper H., 2021. Gut cytokines modulate olfaction through metabolic reprogramming of glia. *Nature*, 596(7870): 97−102.

Callahan B. J., McMurdie P. J., Rosen M. J., Han A. W., Johnson A. J. A., & Holmes S. P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature methods*, 13(7): 581−583.

Cox-Foster D. L., Conlan S., Holmes E. C., Palacios G., Evans J. D., Moran N. A., . . . Geiser D. M., 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. *Science*, 318(5848): 283−287.

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(12): 1010−1021.

Deitch E. A., Xu D., Qi L., & Berg R. D., 1991. Bacterial translocation from the gut impairs systemic immunity. *Surgery*, 109(3 Pt 1): 269−276.

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.

Douglas G. M., Maffei V. J., Zaneveld J. R., Yurgel S. N., Brown J. R., Taylor C. M., . . . Langille M. G., 2020. PICRUSt2 for prediction of metagenome functions. *Nature biotechnology*, 38(6): 685−688.

Duong B. T. T., Lien N. T. K., Thu H. T., Hoa N. T., Lanh P. T., Yun B.-R., . . . Van Quyen D., 2020. Investigation of the gut microbiome of Apis cerana honeybees from Vietnam. *Biotechnology Letters*, 42(11): 2309−2317.

Eichler S., & Schaub G., 2002. Development of symbionts in triatomine bugs and the effects of infections with trypanosomatids. *Experimental parasitology*, 100(1): 17−27.

Ellegaard K. M., & Engel P., 2019. Genomic diversity landscape of the honey bee gut microbiota. *Nature communications*, 10(1): 446.

Endo A., & Salminen S., 2013. Honeybees and beehives are rich sources for fructophilic lactic acid bacteria. *Systematic and Applied Microbiology*, 36(6): 444−448.

Engel P., Martinson V. G., & Moran N. A., 2012. Functional diversity within the simple gut microbiota of the honey bee. *Proceedings of the National Academy of Sciences*, 109(27): 11002−11007.

Engel P., & Moran N. A., 2013. The gut microbiota of insects–diversity in

structure and function. *FEMS microbiology reviews*, 37(5): 699−735.

Engel P., Stepanauskas R., & Moran N. A., 2014. Hidden diversity in honey bee gut symbionts detected by single-cell genomics. *PLoS genetics*, 10(9): e1004596.

Genersch E., & Aubert M., 2010. Emerging and re-emerging viruses of the honey bee (Apis mellifera L.). *Veterinary research*, 41(6).

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.

Kešnerová L., Moritz R., & Engel P., 2016. Bartonella apis sp. nov., a honey bee gut symbiont of the class Alphaproteobacteria. *International journal of systematic and evolutionary microbiology*, 66(1): 414−421.

Koch H., & Schmid-Hempel P., 2011. Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proceedings of the National Academy of Sciences*, 108(48): 19288−19292.

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.

Li J., Heerman M. C., Evans J. D., Rose R., Li W., Rodríguez-García C., . . . Li Z., 2019. Pollen reverses decreased lifespan, altered nutritional metabolism and suppressed immunity in honey bees (Apis mellifera) treated with antibiotics. *Journal of Experimental Biology*, 222(7): jeb202077.

Ma W., Zheng X., Li L., Shen J., Li W., & Gao Y., 2020. Changes in the gut microbiota of honey bees associated with jujube flower disease. *Ecotoxicology and Environmental Safety*, *198*: 110616.

Panjad P., Yongsawas R., Sinpoo C., Pakwan C., Subta P., Krongdang S., Disayathanoowat T., 2021. Impact of Nosema disease and American foulbrood on gut bacterial communities of honeybees Apis mellifera. *Insects*, 12(6): 525.

Quast C., Pruesse E., Yilmaz P., Gerken J., Schweer T., Yarza P., . . . Glöckner F. O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, 41(D1): D590-D596.

Raymann K., & Moran N. A., 2018. The role of the gut microbiome in health and disease of adult honey bee workers. *Current opinion in insect science*, 26: 97−104.

Raymann K., Shaffer Z., & Moran N. A., 2017. Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees. *PLoS biology*, 15(3): e2001861.

Saraiva M. A., Zemolin A. P. P., Franco J. L., Boldo J. T., Stefenon V. M., Triplett E. W., Roesch L. F. W., 2015. Relationship between honeybee nutrition and their microbial communities. *Antonie Van Leeuwenhoek*, 107: 921−933.

Silva M. S., Rabadzhiev Y., Eller M. R., Iliev I., Ivanova I., & Santana W. C., 2017. Microorganisms in honey. *Honey analysis*, 500: 233−257.

Spivak M., & Reuter G. S., 2001. Resistance to American foulbrood disease by honey bee colonies Apis mellifera bred for hygienic behavior. *Apidologie*, 32(6): 555−565.

Tantillo G., Bottaro M., Di Pinto A., Martella V., Di Pinto P., & Terio V., 2015. Virus infections of honeybees Apis mellifera. *Italian journal of food safety*, 4(3).

Vásquez A., Forsgren E., Fries I., Paxton R. J., Flaberg E., Szekely L., & Olofsson T. C., 2012. Symbionts as major modulators of insect health: lactic acid bacteria and honeybees. *PLoS one*, 7(3): e33188.

Wu M., Sugimura Y., Taylor D., & Yoshiyama M., 2013. Honeybee gastrointestinal bacteria for novel and sustainable disease control strategies. *Journal of Developments in Sustainable Agriculture*, 8(2): 85−90.

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.

Zhang Z., Mu X., Shi Y., & Zheng H., 2022. Distinct roles of honeybee gut bacteria on host metabolism and neurological processes. *Microbiology Spectrum*, 10(2): e02438-02421.

Zheng H., Perreau J., Powell J. E., Han B., Zhang Z., Kwong W. K., Moran N. A., 2019. Division of labor in honey bee gut microbiota for plant polysaccharide digestion. *Proceedings of the National Academy of Sciences*, 116(51): 25909−25916.