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Research Article

Bacterial Community in *Apis cerana* and *Heterotrigona itama* Honey Using a Metabarcoding Approach

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ABSTRACT

Honey is a healthy, natural product with high nutritional value that is converted from sugar in nectar by bacteria in the honey stomach of the bees. Several beneficial bacteria in honey produce bioactive compounds, such as *Lactobacillus* in *Apis mellifera* honey, which synthesizes lactic acid, bacteriocins, and enzymes. Here, we employed the metabarcoding technique using the 16S rRNA gene to identify the bacterial community in honey from *A. cerana* and *Heterotrigona itama* collected in Sukabumi Regency, West Java, Indonesia. Genomic DNA from both honey samples was isolated using the ZymoBIOMICSTM DNA miniprep kit before sequencing with the Oxford Nanopore Technologies (ONT) platform. Our studies showed that the most dominant bacteria in the honey of *A. cerana* and *H. itama* were *Paenibacillus glucanolyticus* and *Limosilactobacillus*, respectively. In both types of honey, Gram-positive and Gram-negative bacteria were also detected, as well as lactic acid bacteria, including *Acetilactobacillus jinshanensis* and *Limosilactobacillus*. We also found Actinobacteria in *A. cerana* and *H. itama* honey. This genomic data showed that *A. cerana* honey has a higher bacterial diversity than *H. itama*. Our finding is the first genomic study of bacterial diversity found in the honey of *A. cerana* and *H. itama* that live sympatrically in a bee farm.

1. Introduction

Indonesia has a diverse range of bee species spread across every region. Two of these are the *Apis cerana* (honey bee) and *Heterotrigona itama* (stingless bee) species (Kahono *et al.* 2018). The nests of these two bee species differ, particularly in terms of shape. *A. cerana* is a honey bee that lives in colonies and has comb-shaped nests (Hadisoesilo 2001), while *H. itama* is a small to medium-sized stingless bee with a non-functional sting that lives in colonies and has pot-shaped nest structures (Mohammad *et al.* 2021; Trianto *et al.* 2024). Honey bees and stingless bees play a crucial role in maintaining the health of both plants and humans. Both assist in the pollination process and produce honey as a high-nutrient food source that has health benefits in treating diseases (Gairola *et al.* 2013).

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Honey is a natural product produced by bees by collecting nectar from various plant sources, which is converted into honey through digestion and fermentation by microbes in the stomach of the bees (Manyi-Loh *et al.* 2011). This process yields honey with distinctive physicochemical characteristics, including pH, moisture content, and varying sugar profiles, which impact the taste and quality of the honey (Melia *et al.* 2023). This procedure is influenced by the interaction of microorganisms living within the honey. Microbial interactions can influence the taste of honey. Honey from *H. itama* has unique characteristics, including a higher moisture content and a more acidic taste compared to honey from *A. cerana* (Ngalimat *et al.* 2019).

Microbes in honey can originate from the bee digestive tract, the environment around the hive, as well as the nectar and pollen collected by the bees (Engel *et al.* 2012). Interactions between these microbes can influence the quality of honey, including its antibacterial, antioxidant, and probiotic properties (Kwong and

Moran 2016). The gut microbiota of *A. mellifera* bees includes five bacterial species: *Gilliamella apicola, Snodgrassella alvi, Lactobacillus, Bifidobacterium*, and *Frischella perrara* (Castillo *et al.* 2025). These bacteria play important roles in breaking down pollen cell walls, producing energy through the tricarboxylic acid (TCA) cycle and gluconeogenesis, lactate production, carbohydrate fermentation, and regulating the immune system (Bonilla-Rosso and Engel 2018). They can also stimulate the expression of antimicrobial peptides such as apidaecin, which were involved in the bees immune defense (Forsgren *et al.* 2010). Furthermore, metagenomic analysis of *A. mellifera* honey has revealed significant microbial diversity, contributing to honey quality and characteristics (Castillo *et al.* 2025). The main bacterial communities in honey comprise the phyla Firmicutes, Proteobacteria, and Actinobacteria, with dominant species including *Lactobacillus, Bifidobacterium*, and *Gilliamella* (Olofsson and Vásquez 2008; Kwong and Moran 2016; Zheng *et al.* 2019).

The presence of these microorganisms not only influences the taste and aroma of honey but also has potential as natural probiotics that support human health (Kwong and Moran 2016; Zheng *et al.* 2019). Based on these studies, the bacterial community in *A. cerana* and *H. itama* honey remains unknown, particularly using the metabarcoding approach. Therefore, this study aimed to explore the bacterial community in *A. cerana* and *H. itama* honey using the DNA metabarcoding approach.

2. Materials and Methods

2.1. Sample Collection of Honey

Apis cerana and *H. itama* honey were sourced from a honey bee farm in Cijangkar Village, Nyalindung District, Sukabumi Regency. We used a strile knife to cut the honey part of the *A.cerana*. *A. cerana* nests were cut into ½ of the comb, placed in Ziplock bags, and stored in an icebox to decrease microbial metabolism in the honey. In the laboratory, *A. cerana* honey was slided using sterile gauze and sterile containers (Raffiudin *et al.* 2024), whereas *H. itama* honey was collected using a sterile syringe and placed in a 15 mL tube, which was stored in an icebox.

2.2. Extraction of the DNA of Honey

DNA was extracted from honey using the ZymoBIOMICSTM DNA miniprep kit (Zymo Research) (Pathiraja *et al.* 2023), with the following steps: the ZR BashingBeadTM lysis tube was prepared, and 750 µL of ZymoBIOMICSTM lysis solution was added. The 250 µL of honey was added, vortexed for 20 minutes, and centrifuged at 10,000 g for 1 minute. The 400 µL supernatant from the BashingBeads was transferred to a Zymo Spin III-F filter in a collection tube. It was centrifuged at 8,000 g for one minute before being disposed of. The filtrate collection tube was filled with 1,200 µL of ZymoBIOMICSTM DNA Binding Buffer; 800 µL of the collection tube was transferred to the Zymo Spin II-CR column with the collection tube, and the column was centrifuged at 10,000 g for one minute, after which the collection tube was discarded. 400 µL of ZymoBIOMICSTM DNA wash buffer 1 was added to the Zymo-Spin IICR column with a new collection tube, and the filtrate was discarded in the collection tube. The Zymo-Spin IICR column was placed into a collection tube, and 700 µL of ZymoBIOMICS DNA Wash Buffer 2 was added, and the mixture was centrifuged for 1 minute at 10,000 g. The Zymo-Spin IICR column was transferred to a 1.5 mL microtube, and 50 µL of ZymoBIOMICSTM DNase/RNase-free water was added, and it was centrifuged at 10,000 g for one minute in order to elute the DNA. The Zymo Spin III-HRC filter was centrifuged at 8,000 g for three minutes subsequently 600 µL of ZymoBIOMICSTM HRC prep solution was introduced to a fresh collection tube. The eluted DNA from the preceding stage was placed in a 1.5 mL microtube on a Zymo spin III-HRC filter and centrifuged for three minutes at 16,000 g.

2.3. Metabarcoding Analysis of the Bacterial Community

The DNA concentration was verified using the NanoDrop spectrophotometer and Qubit fluorometer. Amplification of gDNA was conducted using primer 16S 27F 5'AGAGTTTGATCMTGGCTCAG 3' and 1492R 5'GGTTACCTTGTTACGACTT 3' (Frank *et al.* 2008). A photograph of the agarose gel electrophoresis of the PCR product was used to verify the presence of DNA bands. A kit from Oxford Nanopore Technologies was used to prepare the library and extract full-length 16S rRNA gene sequences using the top strand adapter: 5'-TTTTTTTTCCTGTACTTCGTTCAGTTACGTATTGCT-3' and bottom strand adapter: 5'-ACGTAACTGAACGAAGTACAGG-3'. Nanopore sequencing was conducted using MinKNOW software version 24.02.16. The basecalling process utilized Dorado version 7.3.11, applying a high-accuracy model described by Wick *et al.* (2019). NanoFilt and NanoPlot were used for data visualization and quality control to

assess the quality of the FASTQ files (De Coster *et al.* 2018). The filtered sequencing reads were categorized using the Centrifuge classification tool (Kim *et al.* 2016). Which were built using the NCBI 16S RefSeq database (https://ftp.ncbi.nlm.nih.gov/refseq/TargetedLoci/) of the bacteria and Archaea index. Downstream analyses of alpha diversity, evenness, abundance, and bacterial distribution were performed using Pavian (https://github.com/fbreitwieser/pavian), Krona Tools (https://github.com/marbl/Krona), and RStudio using R version 4.3.3 (https://www.R-project.org/).

3. Results

3.1. The Fragments of the Bacterial 16S rRNA Gene Amplified from Honey DNA Samples

The results of electrophoresis for bacterial DNA in honey are shown in samples 1 (*A. cerana*) and 2 (*H. itama*), along with the (M) marker and a control without template (NTC), as seen in Figure 1. The 16S rRNA bacterial gene fragments in the honey DNA samples of *A. cerana* and *H. itama* show clear DNA bands at 1,465 bp for both samples, with non-specific bands in other areas, while no band was found in the NTC. This indicates that the PCR successfully amplified the target gene of 1,465 bp from both samples.

3.2. Bacterial Diversity

Metabarcoding analysis showed a moderate amount of Operational Taxonomy Unit (OTU) diversity and richness. Differences in bacterial diversity and abundance between *A. cerana* and *H. itama* honey samples were compared using observed, Chao1, ACE, Shannon, Simpson, and inverse Simpson and Fisher indices (Figure 2). OTUs were determined and identified to analyze the bacterial community composition in both honey samples. The alpha diversity index showed that the number of observed bacteria in *A. cerana* and *H. itama* honey were 641 and 476 OTUs, respectively. The estimated total number of species in *A. cerana* and *H. itama* honey was about 1,425.52 and 1,043.90 OTUs (Chao1 Index). The total estimated bacterial species were more in *A. cerana* honey than in *H. itama* honey. The Chao1 index value is higher than the observed value in both types of honey. This indicates that many bacteria in the honey samples remain undetected.

The ACE index is similar to the Chao1 calculation, but it focuses more on estimating the abundance of rare species. The ACE index values for *A. cerana* and *H. itama* honey were 1,488.21 and 1,128.98 OTUs, respectively. The evenness and abundance of species in the medium category honey, as seen from the Shannon index in both *A. cerana* and *H. itama* honey, were 2.8613

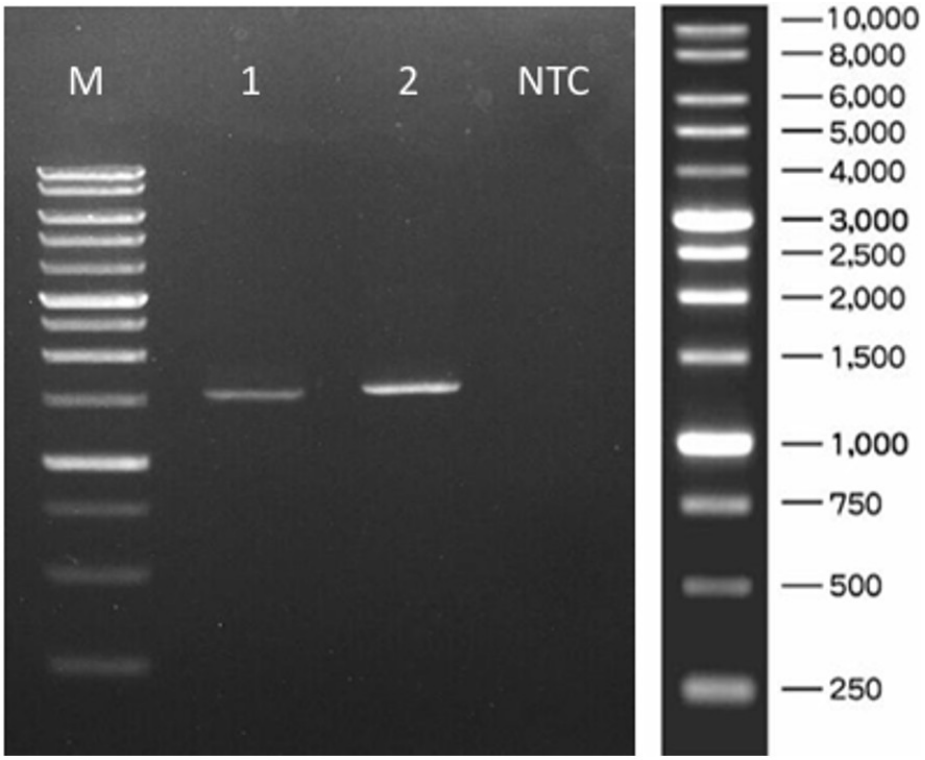

Figure 1. The 16S rRNA gene amplicons on gel electrophoresis with primers 27F and 1492R; marker (M), samples 1 and 2 are *A. cerana* and *H. itama* honey, and the control without template (NTC)

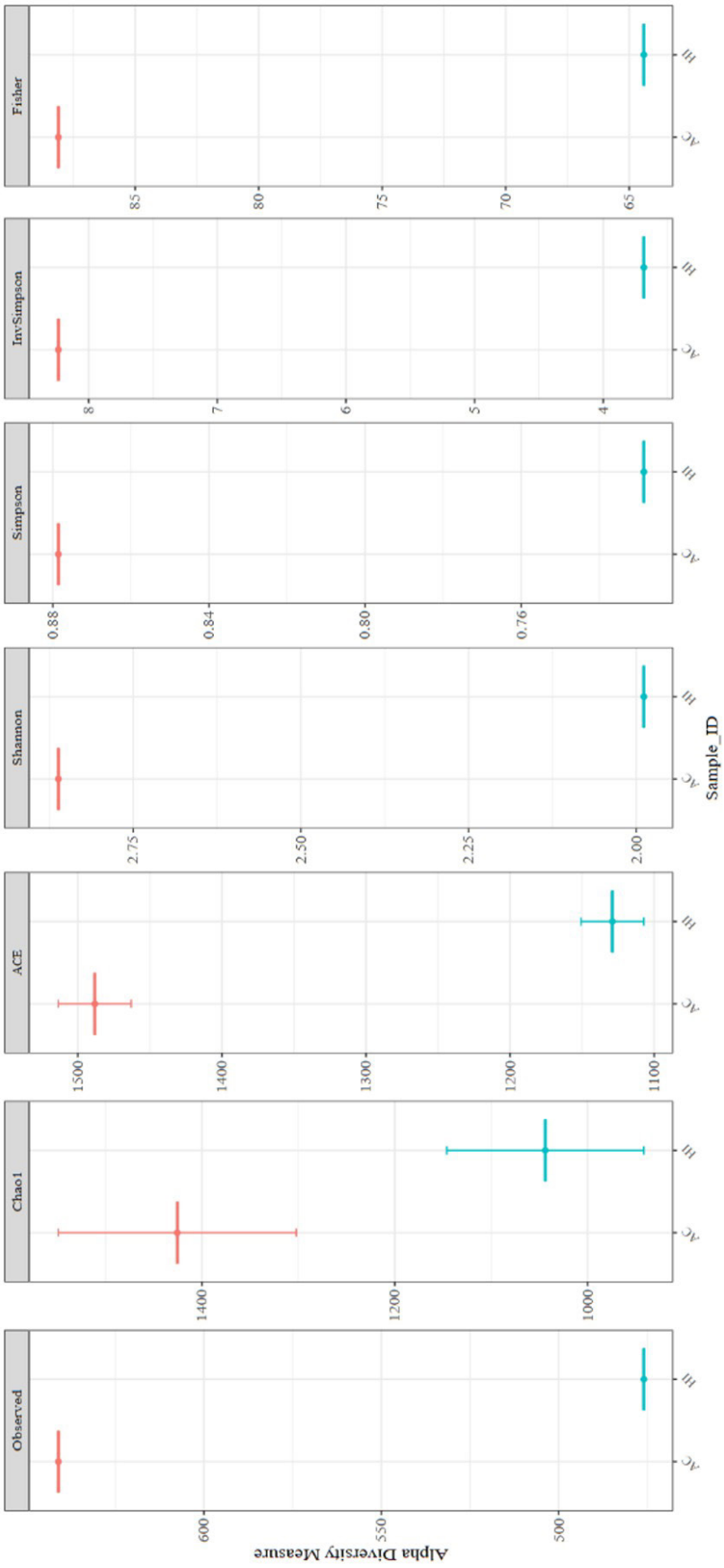

Figure 2. Alpha diversity measures of *A. cerana* (AC) and *H. itama* (HI) honey based on Observed, Chao1, ACE, Shannon, Simpson, Inverse Simpson, and Fisher indices

and 1.9887. These values indicate that *A. cerana* honey falls into the medium category for bacterial abundance, whereas the bacterial community of *H. itama* honey exhibits low abundance. The probability of two random individuals being of the same species was calculated using Simpson's index. The values of 0.7885 and 0.7286 (*A. cerana* and *H. itama*), respectively, indicate that both types of honey have very low diversity when viewed from the Simpson index. However, according to the inverse Simpson index, the diversity of *A. cerana* honey is high (8.2349), and that of *H. itama* honey is moderate (3.6846). Meanwhile, the distribution of species in both honeys was viewed from Fisher's alpha, and the distribution of bacteria in *A.cerana* honey was detected at around (88.1067) and in *H.itama* honey (64.4083). *A.cerana* honey has a more even abundance, diversity, and distribution of bacterial species than *H. itama* honey in terms of the alpha diversity index.

The number of species from both *A. cerana* and *H. itama* honey samples can be seen in Figure 3, which displays OTU 457, which is the number of species present only in *A. cerana* honey, and 292 only in *H. itama* honey, while 184 is the number of species present in both honey samples. Although *A. cerana* and *H. itama* honey come from the same farm environment, the bacterial communities in the two honeys differ, possibly due to different floral resources and honey collection techniques.

3.3. Bacterial Community in Honey

Abundance analysis showed that *Paenibacillus glucanolyticus* was the dominant species in *A. cerana* honey with relative abundance 46.57% (Figure 4). At the same time, *Paenibacillus* was the dominant genus, while in *Heterotrigona itama* honey, the dominant genus found was *Limosilactobacillus* (overall relative abundance was 57.48%). Meanwhile, the dominant families found were Paenibacillaceae (with an overall relative abundance of 31.63%) in *A. cerana* honey and Lactobacillaceae (with an overall relative abundance of 83.85%) in *H. itama* honey. *A. cerana* honey had dominant phyla of Pseudomonadota, Bacillota, and Actinomycota, with overall relative abundances of 41.95%, 31.4%, and 25.59%, respectively. In contrast, the dominant phyla in *H. itama* honey were Bacillota, Pseudomonadota, and Actinomycetota, with overall relative abundances of 95.67%, 3.37%, and 0.85%, respectively.

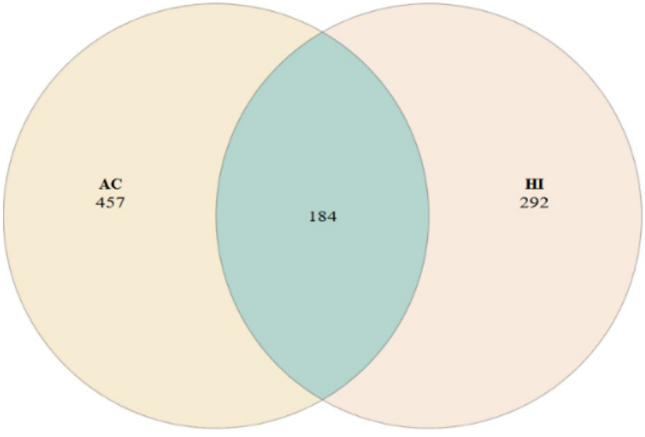

Figure 3. Venn diagram showing the number of bacterial OTUs in *A. cerana* (AC) and *H. itama* (HI) honey

Based on the abundance data of the top species of *A. cerana* and *H. itama* honey, a heatmap was made (Figure 5). The abundance of bacterial communities found in both honeys were *Acetilactobacillus jinshanensis, Acinetobacter nectaris, Aquabacterium parvum, Brachybacterium paraconglomeratum, Brevibacterium casei, B. celere, B. sanguinis, Brevibacterium, Fructilactobacillus fructivorans, Limosilactobacillus fermentum, Limosilactobacillus, Methylorubrum aminovorans, Paenibacillus glucanolyticus, P. lautus, P. uliginis. Paenibacillus, Philodulcilactobacillus myokoensis, Pseudomonas aeruginosa, Pseudomonas, Sinomonas atrocyanea*, and *Stutzerimonas stutzeri*.

Bacteria that were only found in *A. cerana* honey were *Acinetobacter oryzae, Thalassoporum komareki, Holzapfeliella floricola, Brachybacterium saurashtrense, Brevibacterium ammoniilyticum, Sinomonas echigonensis, Sinomonas flava, Methylorubrum extorquens, Acinetobacter baretiae, Idiomarina baltica, Acinetobacter johnsonii, Methylorubrum, Acinetobacter, Sinomonas mesophile, Brachybacterium conglomeratum, Brachybacterium, Micrococcus lylae*, and *Corynebacterium bouchesdurhonense*. In contrast, the bacteria detected only in *H. itama* were *Liquorilactobacillus, Nicoliella spurrieriana, Lactobacillus, Lactobacillus acidophilus, Paracoccus speluncae, Fructobacillus*, and *Paraburkholderia fungorum*.

In addition, the bacterial community in both honey samples consisted mainly of Gram-positive bacteria such as *Acetilactobacillus jinshanensis, Philodulcilactobacillus myokoensis, Limosilactobacillus, Limosilactobacillus*

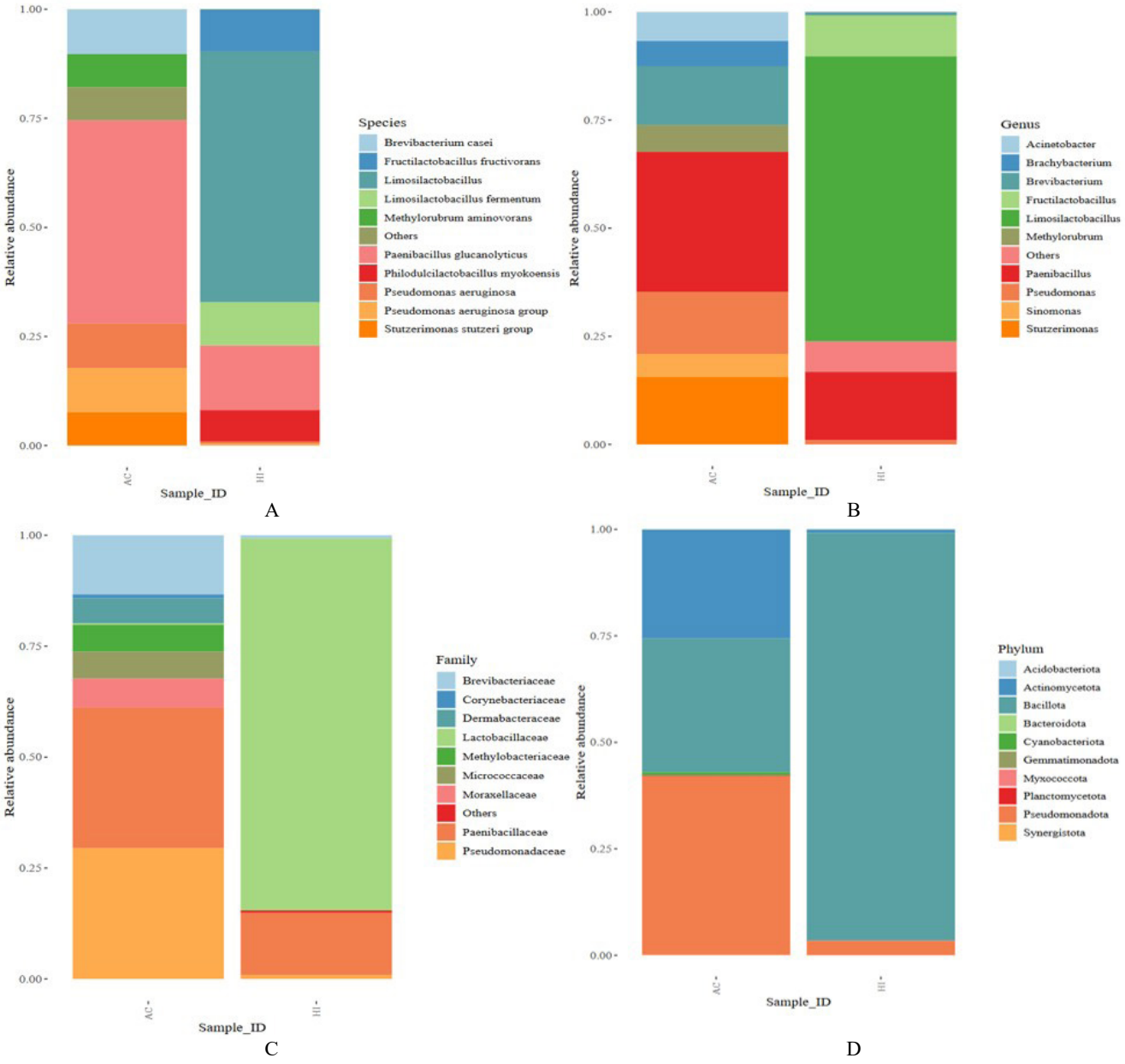

Figure 4. Top ten relative abundance from Species level (A), Genus leve (B), Family level (C), and Phyla level (D) in *A. cerana* (AC) and *H. itama* (HI) honey

fermentum, Fructilactobacillus fructivorans, Liquorilactobacillus, Lactobacillus, Lactobacillus acidophilus, Fructobacillus, Paenibacillus uliginis, Paenibacillus glucanolyticus, Paenibacillus, P. lautus, B. casei, B. sanguinis, B. celere, B. ammoniilyticum, Brevibacterium, Sinomonas atrocyanea, S. echigonensis, S. flava, S. mesophile, B. paraconglomeratum, B. saurashtrense, B. conglomeratum, Brachybacterium, Corynebacterium bouchesdurhonense, and *Micrococcus lylae.* Meanwhile Gram-negative bacteria identified included *Aquabacterium parvum, Paracoccus speluncae, Paraburkholderia fungorum, Stutzerimonas stutzeri, Pseudomonas aeruginosa, Pseudomonas, Methylorubrum aminovorans, Methylorubrum extorquens, Methylorubrum, Acinetobacter nectaris, A. oryzae, A. baretiae, A. johnsonii, Acinetobacter, Thalassoporum komareki, Idiomarina baltica,and Holzapfeliella floricola*. Furthermore, several lactic acid bacteria (LAB) were also detected, including *Acetilactobacillus jinshanensis, Limosilactobacillus, Philodulcilactobacillus myokoensis, Limosilactobacillus fermentum, Fructilactobacillus fructivorans,*

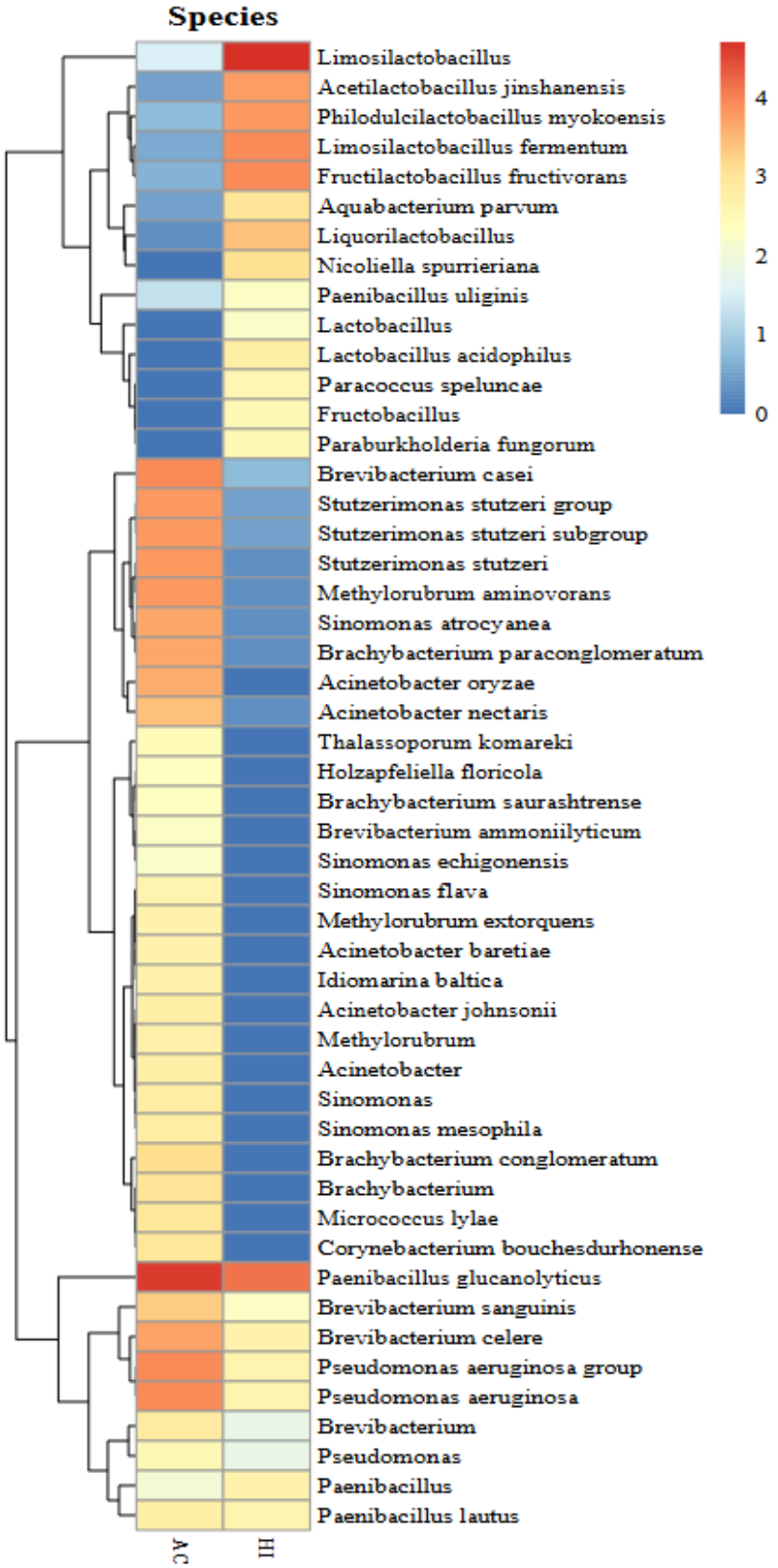

Figure 5. Heatmap displaying the differences in the relative abundance of top 50 species of *A. cerana* (AC) and *H. itama* (HI) honey. The colors indicate the relative abundance of the taxon, ranging from blue (low) to red (high)

Liquorilactobacillus, Lactobacillus, Lactobacillus acidophilus, and *Fructobacillus*.

Actinobacteria were also found in *A. cerana* and *H. itama* honey. Actinobacteria found in both species were *B. sanguinis, B. celere, B. casei, Brevibacterium* sp., *B. paraconglomeratum*, and *S. atrocyanea*. In addition, several actinobacteria were only found in *A. cerana* honey, such as *B. saurashtrense, B. ammoniilyticum, S. echigonensis, S. flava, S. mesophile, M. lylae, C. bouchesdurhonense*, and *B. conglomeratum*.

4. Discussion

Metabarcoding analysis of honey revealed differences in both the diversity and abundance of bacteria present in *A. cerana* and *H. itama* honey. The diversity of the number of bacteria was higher in *A. cerana* honey compared to *H. itama* honey (Figure 3). Meanwhile, the abundance of bacteria in *A. cerana* honey and *H. itama* honey was categorized as medium and low, respectively, based on the Shannon and Simpson indices (Figure 2). There is dominance in *A. cerana* and *H. itama* honey, namely *Paenibacillus glucanolyticus* and *Limosilactobacillus* (Figure 4A). If one or two dominate a community, the diversity is considered low, even though the total number of individuals in the ecosystem is large. It is also known that the dominance value has an inverse relationship with the level of diversity; the higher the level of dominance, the lower the level of diversity (Haq *et al.* 2021). Shannon measures the abundance and evenness among species; the higher the Shannon value, the more diverse and balanced the community (Willis 2019). The Shannon value will be low if a dominant species was found (Almeida *et al.* 2023).

A. cerana and *H. itama* honey samples were collected from the same bee farm. Still, they revealed diverse bacterial communities, and each honey sample contained unique bacteria, namely those found only in one type of honey. These bacteria are included in the dominant phylum in each honey. *A. cerana* honey has three dominant phyla with insignificant relative abundance, namely *Pseudomonadota* (41.95%), *Bacillota* (31.4%), and *Actinomycetota* (25.59%), while *H. itama* honey showed significant relative abundance, namely *Bacillota* (95.67%), *Pseudomonadota* (3.37%), and *Actinomycetota* (0.85%) (Figure 4). These results are consistent with those of a study conducted on green honey from Malaysia, which identified three dominant phyla: *Pseudomonadota* (35%), *Bacillota* (31%), and *Actinomycetota* (16%) (Ullah *et al.* 2023). Our data show different percentages for those three phyla. Furthermore, the different phyla reported for *A. mellifera* honey from Kenya were found to have Firmicutes, Proteobacteria, and Bacteroidetes as dominant phyla (Njoroge *et al.* 2024). *A. cerana* honey collected from healthy bees from Vietnam has two dominant phyla, namely Firmicutes (50%) and Proteobacteria (49%) (Doung *et al.* 2020; Lanh *et al.* 2024).

Several species of bacteria are found in *A. cerana* and *H. itama* honey, including Gram-positive, Gram-negative, Lactic acid bacteria, and actinobacteria species (Figure 5). Green honey from Malaysia was found to contain a diverse range of bacterial genera, including Gram-positive and negative bacteria, lactic acid bacteria, and actinobacteria, such as *Salmonella, Klebsiella, Lactobacillus, Paenibacillus, Micromonospora, Bifidobacterium*, and *Streptomyces* (Ullah *et al.* 2023). *Lactobacillus kunkeei, Fructobacillus, Gilliamella apicola*, and *Lactobacillus lactis* were found in *A. mellifera* honey from Kenya (Njoroge *et al.* 2024). Lactic acid bacteria were also detected in small numbers of *A. cerana* honey from Vietnam, primarily consisting of three bacterial genera: *Lactobacillus, Fructobacillus*, and *Weissella* (Doung *et al.* 2020; Lanh *et al.* 2024). Fir honey is dominated by lactic acid bacteria of the genus *Lactobacillus*, followed by members of the genus *Bradyrhizobium* (Stavropoulou *et al.* 2023). Although several distinct genera and species of bacteria were discovered, our data also revealed groups of bacteria that were the same.

Another study using 16S rRNA sequencing on *A. cerana* honey in Vietnam identified 116 bacterial species from four major phyla: Proteobacteria (70.7%), Actinobacteria (10.7%), Firmicutes (10.3%), and Bacteroidetes (8.4%) (Doung *et al.* 2020). These microbes, including lactic acid bacteria such as *Lactobacillus kunkeei, Fructobacillus fructosus*, and *Bifidobacterium asteroides*, play a crucial role in the fermentation process of nectar into honey, contributing to the quality and flavor of the honey (Zheng *et al.* 2019). The interaction between microbes in honey can influence the flavor profile. Honey from *A. cerana* and *H. itama* has a distinctive sour taste due to fermentation activities performed by microbes such as *Lactobacillus*. Additionally, microbes can produce volatile compounds that contribute to the aroma of the honey. Differences in microbial communities between

these two bee species may result in variations in the flavor and aroma of the honey produced (Ngalimat *et al.* 2019). Meanwhile, *H. itama* honey from Malaysia identified seven different plant/pollen species, reflecting the diversity of nectar sources collected by these bees. The microbial composition of this honey is also influenced by microbes from the bee's digestive tract and the surrounding environment (Huda *et al.* 2023).

Lactic acid bacteria (LAB), such as *Limosilactobacillus*, are commonly found in honey. The abundance of LAB in honey samples originates from various sources, including flower nectar, the journey from flower to hive (Saraiva *et al.* 2015), the honeycomb environment (Mattila *et al.* 2012), and the honey bee stomach (Martinson *et al.* 2012). Environmental factors, such as geographic location (Hroncova *et al.* 2015), air temperature (Russell and McFrederick 2022), and other microorganisms present in the collected nectar (Ludvigsen *et al.* 2015), influence the composition of honey. The presence of LAB in honey indicates that honey serves as a microbial habitat for bacteria with unique abilities (Fatma *et al.* 2022). In honey, such as *Lactobacillus*, LAB can survive in acidic pH conditions and adapt to honey's high sugar content (Tajabadi *et al.* 2013; Almasaudi 2021). LAB also plays a role in the initial fermentation process of honey, before the water content drops and microbial activity stops, helping to protect honey from contamination by pathogenic microbes (Tajabadi *et al.* 2013; Fatma *et al.* 2022). It can potentially serve as a natural probiotic that benefits human health (Hill *et al.* 2014; Silva *et al.* 2016), contributing to honey's unique chemical and bioactive characteristics (Anderson *et al.* 2011).

These microbes, which affect honey's flavor and aroma, have potential as natural probiotics that support human health (Olofsson and Vásquez 2008; Kwong and Moran 2016). Although both bee species produce honey with beneficial microbial content, there are significant differences in the composition and function of their microbiota. *A. cerana* honey is dominated by Firmicutes and Proteobacteria phyla, while *H. itama* honey contains LABs that can produce important vitamins, such as folic acid and riboflavin (Doung *et al.* 2020; Huda *et al.* 2023). Our findings show that *A. cerana* and *H. itama* honey have distinct bacterial dominance and diversity, despite originating from the same farm. The diversity of lactic acid bacteria, Gram-positive and negative bacteria, and actinobacteria live together in both types of honey. The important information for future exploration to obtain a more diverse bacterial culture, focusing not only on lactic acid bacteria but also on isolating other bacteria and assaying the activity of each bacterium found.

Based on this genomic data, it was found that *A. cerana* honey shows higher bacterial diversity than *H. itama*. The diversity of actinobacteria was also higher in *A. cerana* honey than in *H. itama* honey. The dominant bacteria in *A. cerana* and *H. itama* honey were *Paenibacillus* and *Limosilactobacillus*. Lactic acid bacteria were found more in *H. itama* honey than in *A. cerana*. Saveral actinobacteria were only found in *A. cerana* honey. The result is the first genomic finding of bacterial diversity found in *A. cerana* and *H. itama* honey that live sympatrically on the same farm.

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References

Almasaudi, S., 2021. The antibacterial activities of honey. *Saudi J. Biol. Sci.* 28, 2188-2196. https://doi.org/10.1016/j.sjbs.2020.10.017

Almeida, E.L., Ribiere, C., Frei, W., Kenny, D., Coffey, M.F., O'Toole, P.W., 2023. Geographical and seasonal analysis of the honeybee microbiome. *Microb. Ecol.* 85, 765-778. https://doi.org/10.1007/s00248-022-01986-x

Anderson, K.E., Sheehan, T.H., Eckholm, B.J., Mott, B.M., DeGrandi-Hoffman, G., 2011. An emerging paradigm of colony health: microbial balance of the honey bee and hive (*Apis mellifera*). *Insec. Soc.* 58, 431-444. https://doi.org/10.1007/s00040-011-0194-6

Bonilla-Rosso, G., Engel, P., 2018. Functional roles and metabolic niches in the honey bee gut microbiota. *Curr. Opin. Microbiol.* 43, 69-76. https://doi.org/10.1016/j.mib.2017.12.009

Castillo, D., Abella, E., Sinpoo, C., Phokasem, P., Chantaphanwattana, T., Yongsawas, R., Cervancia, C., Baroga-Barbecho, J., Attasopa, K., Noirungsee, N., Disayathanoowat. T., 2025. Gut microbiome diversity in European honeybees (*Apis mellifera* L.) from La Union Northern Luzon, Philippines. *Insects.* 16, 112. https://doi.org/10.3390/insects16020112

De Coster, W., D'Hert, S., Schultz, D.T., Cruts, M., Van Broeckhoven, C., 2018. NanoPack: visualizing and processing long-read sequencing data. *Bioinformatics.* 34, 2666-2669. https://doi.org/10.1093/bioinformatics/bty149

Duong, B.T.T., Lien, N.T.K., Thu, H.T., Hoa, N.T., Lanh, P.T., Yun, B.R., Yoo, M.S., Cho, Y.S., Quyen, D.V., 2020. Investigation of the gut microbiome of *Apis cerana* honeybees from Vietnam. *Biotechnol. Lett.* 42, 2309-2317. https://doi.org/10.1007/s10529-020-02948-4

Engel, P., Martinson, V.G., Moran, N.A., 2012. Functional diversity within the simple gut microbiota of the honey bee. *PNAS.* 109, 11002–11007. https://doi.org/10.1073/pnas.1202970109

Fatma, I.I., Nuraida, L., Faridah, D.N., 2022. Probiotic potential of lactic acid bacteria from honey of three different type honey bees. *JTIP.* 33, 189-199. https://doi.org/10.6066/jtip.2022.33.2.189

Forsgren, E., Olofsson, T.C., Vásquez, A., Fries, I., 2010. Novel lactic acid bacteria inhibiting *Paenibacillus larvae* in honey bee larvae. *Apidologie.* 41, 99–108. https://doi.org/10.1051/apido/2009065

Frank, J.A., Reich, C.I., Sharma, S., Weisbaum, J.S., Wilson, B.A., Olsen, G.J., 2008. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl. Environ. Microbiol.* 74, 2461–2470. https://doi.org/10.1128/AEM.02272-07

Gairola, A., Tiwari, P., Tiwari, J.K., 2013. Physico-chemical properties of *Apis cerana indica* F. Honey from Uttarkashi district of Uttarakhand India. *J. Global Biosci.* 2, 20-25. https://www.mutagens.co.in/jgb/vol.02/1/04.pdf

Hadisoesilo, S., 2001. Review: the diversity of indigenous honey bee species of Indonesia. *Biodiversitas.* 2, 123-128. https://doi.org/10.13057/biodiv/d020107

Haq, H.U., Li, Y., Jin, L., Zhang, T., Cheng, L., Li, Z., Tian, B., 2021. Effect of chicken manure-based fertiliser on bacterial communities and diversity of tomato endosphere microbiota. *Agriculture.* 67, 144-154. https://doi.org/10.2478/agri-2021-0013

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., Calder, P., Sanders, M.E., 2014. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* 11, 506-514. https://doi.org/10.1038/nrgastro.2014.66

Hroncova, Z., Havlik, J., Killer, J., Doskocil, I., Tyl, J., Kamler, M., Titera, D., Hakl, J., Mrazek, J., Bunesova, V., Rada, V., 2015. Variation in honey bee gut microbial diversity affected by ontogenetic stage, age, and geographic location. *PLoS One*. 10, e0118707. https://doi.org/10.1371/journal.pone.0118707

Huda, N., Ullah, S., Wahab, R.A., Lani, M.N., Daud, N.H.A., Shariff, A.H.M., Ismail, N.I., Hamid, A.A.A., Mohamad, M.A.N., Huyop, F., 2023 The first ITS2 sequence data set of eDNA from honey of Malaysian giant honeybees (*Apis dorsata*) and stingless bees (*Heterotrigona itama*) reveals plant species diversity. *BMC Res. Notes.* 16, 211. https://doi.org/10.1186/s13104-023-06495-9

Kahono, S., Chantawannakul, P., Engel, M.S., 2018. Social bees and the current status of beekeeping in Indonesia, in: Chantawannakul, P., Williams, G., Neumann, P. (Eds.), *Asian Beekeeping in the 21st Century.* Springer, Singapore, pp. 287–306. https://doi.org/10.1007/978-981-10-8222-1_13

Kim, D., Song, L., Breitwieser, F.P., Salzberg, S.L., 2016. Centrifuge: rapid and sensitive classification of metagenomic sequences. *Genome Res.* 26, 1721-1729. https://doi.org/10.1101/gr.210641.116

Kwong, W.K., Moran, N.A., 2016. Gut microbial communities of social bees. *Nat. Rev. Microbiol.* 14, 374–384. https://doi.org/10.1038/nrmicro.2016.43

Lanh, P.T., Duong, B.T.T., Thu, H.T., Hoa, N.T., Quyen, D.V., 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. http://doi.org/10.7717/peerj.17157

Ludvigsen, J., Rangberg, A., Avershina, E., Sekelja, M., Kreibich, C., Amdam, G., Rudi, K., 2015. Shifts in the midgut/pyloric microbiota composition within a honey bee apiary throughout a season. *Microbes Environ.* 30, 235-244. https://doi.org/10.1264/jsme2.ME15019

Manyi-Loh, C.E., Ndip, R.N., Clarke, A.M., 2011. Volatile compounds in honey: a review on their involvement in aroma botanical origin determination and potential biomedical activities. *Int. J. Mol. Sci.* 12, 9514-9532. https://doi.org/10.3390/ijms12129514

Martinson, V.G., Moy, J., Moran, N.A., 2012. Establishment of characteristic gut bacteria during development of the honeybee worker. *Appl. Enviromental Microbiol.* 78, 2830-2840. https://doi.org/10.1128/AEM.07810-11

Mattila, H.R., Rios, D., Walker-Sperling, V.E., Roeselers, G., Newton, I.L.G., 2012. Characterization of the active microbiotas associated with honey bee reveals healthier and broader communities when colonies are genetically diverse. *PLoS One.* 7, e32962. https://doi.org/10.1371/journal.pone.0032962

Melia, S., Juliyarsi, I., Kurnia, Y.F., Aritonang, S.N., Rusdimansyah, R., Sukma, A., Setiawan, R.D., Pratama, Y.E., Supandil, D., 2023. Profile of stingless bee honey and microbiota produced in West Sumatra Indonesia by several species (Apidae Meliponinae). *Vet. World.* 17, 785–795. https://doi.org/10.14202/vetworld.2024.785-795

Mohammad, S.M., Mahmud-Ab-Rashid, N.K., Zawawi, N., 2021. Stingless bee collected pollen (bee bread): chemical and microbiology properties and health benefits. *Molecules.* 26, 957. https://doi.org/10.3390/molecules26040957

Ngalimat, M.S., Rahman, R.N.Z.R.A., Yusof, M.T., Syahir, A., Sabri, S., 2019. Characterisation of bacteria isolated from the stingless bee *Heterotrigona itama* honey, bee bread and propolis. *PeerJ.* 7, e7478. https://doi.org/10.7717/peerj.7478

Njoroge, J.K., Moses, N., Maina, J., Mwirichia, R., Nyabuga, F.N., Mugweru, J., 2024. Bacterial diversity in honey bee environment: Embu County, Kenya. *Sci. Afr.* 23, e02036. https://doi.org/10.1016/j.sciaf.2023.e02036

Olofsson, T.C., Vásquez, A., 2008. Detection and identification of a novel lactic acid bacterial flora within the honey stomach of the honeybee *Apis mellifera. Curr. Microbiol.* 57, 356–363. DOI: 10.1007/s00284-008-9202-0

Pathiraja, D., Cho, J., Kim, J., Choi, I.G., 2023. Metabarcoding of eDNA for tracking the floral and geographical origins of bee honey. *Int. Food Res.* 164, 112413. https://doi.org/10.1016/j.foodres.2022.112413

Raffiudin, R., Dyahastuti, M., Nugraha, R., Sayusti, T., Djuita, N.R., Suwananda, E., Allvioningrum, V., Mardhony, R., Biagioni, S., Setyaningsih, C.A., Prasetyo, L.B., Priawandiputra, W., Atmowidi, T., Saad, A., Behling, H., 2024. The effect of land cover on the foraging behavior and pollen in the honey of the giant bee *Apis dorsata* in Sumatra. *Front. Bee Sci.* 2, 1366287. https://doi.org/10.3389/frbee.2024.1366287

Russell, K.A., McFrederick, Q.S., 2022. Elevated temperature may affect nectar microbes, nectar sugars, and bumble bee foraging preference. *Environ. Microbiol.* 84, 473-482. https://doi.org/10.1007/s00248-021-01881-x

Saraiva, M.A., Zemolin, A.P.P., Franco, J.L., Boldo, J.T., Stefenon, V.M., Triplett, E.W., Camargo, F.A.D.O., Roesch, L.F.W., 2015. Relationship between honeybee nutrition and their microbial communities. *Antonie Van Leeuwenhoek.* 107, 921-933. https://doi.org/10.1007/s10482-015-0384-8

Silva, M.S., Rabadzhiev, Y., Iliev, I., Ivanova, I., Eller, M.R., Santana, W.C., 2016. Microorganisms in honey, in: De Toledo, V.D.A.A. (Eds.), *Honey Analysis*. inTech, Croatia, pp. 233–258.

Stavropoulou, E., Remmas, N., Voidarou, C., Vrioni, G., Konstantinidis, T., Ntougias, S., Tsakris, A., 2023. Microbial community structure among honey samples of different pollen origin. *Antibiotics.* 12, 101. https://doi.org/10.3390/antibiotics12010101

Tajabadi, N., Mardan, M., Manap, M.Y.A., Mustafa, S., 2013. Molecular identification of *Lactobacillus* spp. isolated from the honey comb of the honey bee (*Apis dorsata*) by 16S rRNA gene sequencing. *J. Apicul. Res.* 52, 235-241. https://doi.org/10.3896/IBRA.1.52.5.10

Trianto, M., Arisuryanti, T., Purwanto, H., Ubaidillah, R., 2024. Taxonomic study on selected species of stingless bees (Hymenoptera: Apidae: Meliponini) in Sulawesi Island Indonesia. *Biodiversitas.* 25, 2290-2306. https://doi.org/10.13057/biodiv/d250547

Ullah, S., Wahab, R.A., Oyewusi, H.A., Hamid, A.A.A., Mohamad, M.A.N., Huyop, F., 2023. Exploring the microbial communities in green honey from Banggi Island, Sabah, Malaysia using amplicon sequencing analysis. *Access Microbiol.* 000624-v3. [Preprint] https://doi.org/10.1099/acmi.0.000624.v3

Wick, R.R., Judd, L.M., Holt, K.E., 2019. Performance of neural network basecalling tools for Oxford Nanopore sequencing. *Genome Biol.* 20, 129. https://doi.org/10.1186/s13059-019-1727-y

Willis, A.D., 2019. Rarefaction, alpha diversity and statistics. *Front. Microbiol.* 10, 2407. https://doi.org/10.3389/fmicb.2019.02407

Zheng, H., Perreau, J., Powell, J.E., Han, B., Zhang, Z., Kwong, W.K., Tringe, S.G., Moran, N.A., 2019. Division of labor in honey bee gut microbiota for plant polysaccharide digestion. *PNAS.* 116, 25909-25916. https://doi.org/10.1073/pnas.1916224116