ISTANBUL UNIVERSITY CERRAHPAŞA

Identification of Bacterial Diversity of Bee Collected Pollen and Bee Bread Microbiota by Metagenomic Analysis

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Abstract

This study investigated the bacterial diversities of bee-collected pollen and bee bread of *Apis mellifera* in Turkey. The bacterial community structure of 14 bee pollen from Bingöl, Konya, and Hakkari and 11 bee bread samples from Bingöl were studied using 16 S rRNA amplicon sequencing and metagenomic analysis. The dominant bacterial phylum in pollen and bee bread samples was Firmicutes, followed by Proteobacteria. In pollen and bee bread samples, Bacillaceae, Clostridiaceae, Enterococcaceae, and Enterobacteriaceae were identified as dominant bacterial families. At the genus level, *Bacillus*, *Clostridium sensu stricto*, and *Enterococcus* were dominant bacteria in both pollen and bee bread samples. The most abundant species was *Clostridium perfringens* in both pollen and bee bread samples. *Escherichia vulneris*, *Enterococcus faecalis*, *Bacillus cereus*, *Enterococcus casseliflavus*, and *Cronobacter malonaticus* were identified with high reads in pollen samples. In bee bread samples, *E. faecalis*, *Clostridium bifermentans*, and *Pantoea calida* were abundant bacterial species. Alpha diversity showed that pol-3 sample had the highest diversity. Beta-diversity plots separated the pollen samples into four main groups and bee bread samples into three main groups. Our results indicated that the culture-independent metagenomic analysis will be a valuable tool for determining the microbial diversity of bee products produced in Bingöl-Turkey one of the important centers of apiculture.

Keywords: Bee bread, next-generation sequencing, metagenomics, pollen

Introduction

Next-generation omics technologies have been applied to the comprehensive assessment of microbiome characterization for agricultural sustainability (Yang et al., 2021). Specifically, next-generation sequencing (NGS) was applied to bee products including honey, propolis, royal jelly, bee collected pollen, and bee bread to explore the microbial diversity in different environments (Kafantaris et al., 2021; Kwong et al., 2017). Metagenomic research for bee products is an emerging field that has primarily focused on honey bee gut microbiota as a model system (Romero et al., 2019; Zheng et al., 2018; Zitvogel et al., 2015).

The microbiota is a group of microorganisms or microbial communities that live together in great diversity (Gagliardi et al., 2018). Honey bee microbiota plays a critical role in metabolic functions that contribute to numerous biochemical and physiological processes (Nowak et al., 2021). The microbial community of the honey bee gut and beehive products has previously been explored. In particular, the microbiota of honey bees (Engel et al., 2012; Zheng et al., 2019) and the beehive-associated products, including royal jelly (Asama et al., 2015), pollen (Moreno Andrade et al., 2018), and bee bread (Didaras et al., 2020; Disayathanoowat et al., 2020) has been investigated in the previous studies from different parts of the World.

The economically important honey bees *Apis mellifera, Apis cerana*, *Apis dorsata*, *Apis florea,* and *Apis andreniformis* have been studied for the gut microbial community diversity (Kwong et al., 2017), specifically, *A. mellifera* L. is a critical species that contribute to the food production (Engel et al., 2012) and the bee products including honey, bee pollen, bee bread, and royal jelly (Asama et al., 2015).

Research interest in bee collected pollen and bee bread has been increased due to their nutritional and health properties. Honey bee-collected pollen and bee bread are called "a new health-oriented product" due to their nutritional value (Kieliszek et al., 2018). Bee

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pollen is a product collected from honey bees from a wide variety of flower sources (Alippi et al., 2022), and also pollen and nectar are used as the food sources from bee colonies (Saraiva et al., 2015). As a healthy bee product, pollen is the source of proteins, carbohydrates, lipids, vitamins, minerals, crude fibers, and phenolic compounds (Kieliszek et al., 2018; Mărgăoan et al., 2010). Bee bread, "a fermented food for the worker bees and larvae" is the product of metabolic transformations linked with the honey bee gut microbial community and plant-derived sources, including pollen and nectar and also secretions of bees' salivary glands (Lee et al., 2015; Mărgăoan et al., 2019; Vásquez & Olofsson, 2009). Bee bread is the leading supplier of carbohydrates, essential amino acids, fatty acids, free sugars, organic acids, vitamins, and minerals (Bakour et al., 2019; Čeksteryte et al., 2016; Dranca et al., 2020). Moreover, the chemical composition, total phenolic content, phenolic composition, fatty acids, and microbial metabolites of the microbial community of bee collected pollen and bee bread contribute to the bioactive properties (Di Cagno et al., 2019; Didaras et al., 2020; Disayathanoowat et al., 2020).

Bee bread contains phenolic compounds including gallic acid, protocatechuic acid, ρ-hydroxybenzoic acid, vanillic acid, caffeic acid, chlorogenic acid, ρ-coumaric acid, rosmarinic acid, myricetin, quercetin, and kaempferol (Dranca et al., 2020; Isidorov et al., 2009; Urcan et al., 2018). The bioactive compounds of bee bread contribute to the bioactive properties including antimicrobial, antioxidant, anti-carcinogenic, and anti-inflammatory activities (Bakour et al., 2019; Mărgăoan et al., 2019). Bee pollen also exhibits antimicrobial (Fatrcová-Šramková et al., 2013), antioxidant (Kostić et al., 2019; Leja et al., 2007), and anti-carcinogenic (Wu & Lou, 2007) activities. The biological activity depends on various constituents such as phenolic acids, including hydroxybenzoic acids, hydroxycinnamic acids, and flavonoids including flavones, flavonols, flavanones, and isoflavones (Rzepecka-Stojko et al., 2015).

The chemical composition of the bee bread is not the same as the bee pollen; it is more acidic than the pollen and it contains antimicrobial metabolites which preserve the comb from disease (Lee et al., 2015; Saraiva et al., 2015). Pollen and nectar have different chemical compositions depending on the plant species, and this may affect the growth of lactic acid bacteria (LAB), which corresponds to the fermentation process in the bee bread and also the quality of the product (Vásquez & Olofsson, 2009). Bee bread is a product of the fermentation process and metabolic transformation. Since the exact mechanism of fermentation has not been fully identified, the generally accepted concept was the metabolic transformation of bee bread was related to microbial communities of bees, including bacteria, yeast, or both of them. They play an important role in the process specifically in anaerobic microorganisms and LAB (Lee et al., 2015; Vásquez & Olofsson, 2009).

The aim of this study was to determine the bacterial community structure and dominant bacterial populations present in the microbiota of bee pollen and bee bread samples collected from Turkey by NGS (a culture-independent DNA-based method) and metagenomic analysis.

Methods

Materials

Pollen ($n=14$) and bee bread ($n=11$) samples of *A. mellifera* were examined in this study. Bee collected pollen samples were collected using pollen traps that were placed in front of the hives. Bee bread samples were obtained from fresh honeycombs using a steel needle. *A. mellifera* colonies come from *A. mellifera* anatoliaca and *A. mellifera* caucasica. The samples were collected from Bingöl in two seasons (November and April) from November 2019 to November 2020. In addition, five pollen samples were obtained from the local markets in Konya and Hakkari (Table 1). All samples were stored at +4°C until DNA extraction. The examples of the samples are shown in Figure 1 and the sampling places are described in Table 1.

DNA Extraction

Total DNA extraction was carried out directly from homogenized and pre-enriched pollen and bee bread samples. First, pollen and bee bread samples (5 g) were homogenized in 45 mL buffered peptone water (Oxoid, UK) using a stomacher (Interscience) for 5 minutes at room temperature. Direct DNA extraction was applied by DNeasy® PowerFood® Microbial kit (Qiagen, Germany) using 1.8 mL homogenized pollen and bee bread samples. Second, for the pre-enrichment procedure, 1 mL homogenate of each pollen and bee bread samples was added into 9 mL Brain Heart Infusion broth (Oxoid) and incubated at 35°C for 24 h by shaking at 200 rpm. After that, 1 mL

Table 1.
Pollen and Bee Bread Sample Codes and Collection Places

Pollen Samples	Collection Place	Coordinates*
pol-1	Adaklı-Bingöl	39.19563 N, 40.46313 E
pol-2	Adaklı-Bingöl	39.22884 N, 40.48325 E
pol-3	Adaklı-Bingöl	39.27543 N, 40.57696 E
pol-4	Yayladere-Bingöl	39.21314 N, 40.07813 E
pol-5	Bingöl City Centre	38.88569 N, 40.49608 E
pol-6	Genç-Bingöl	38.74850 N, 40.53755 E
pol-7	Genç-Bingöl	38.74994 N, 40.55627 E
pol-8	Karlıova-Bingöl	39.29788 N, 41.01421 E
pol-9	Kığı-Bingöl	39.31001 N, 40.34909 E
pol-10	Hakkari City Centre	37.60631 N, 43.73525 E
pol-11	Konya City Centre	37.86556 N, 32.48734 E
pol-12	Konya City Centre	37.88150 N, 32.48704 E
pol-13	Konya City Centre	37.87012 N, 32.50089 E
pol-14	Konya City Centre	37.87630 N, 32.48457 E
Bee Bread Samples	**Collection Place**	**Coordinates***
AE-1	Bingöl City Centre	38.88569 N, 40.49608 E
AE-2	Yedisu-Bingöl	39.43372 N, 40.54642 E
AE-3	Bingöl City Centre	38.88569 N, 40.49608 E
AE-4	Karlıova-Bingöl	39.29788 N, 41.01421 E
AE-5	Karlıova-Bingöl	39.29788 N, 41.01421 E
AE-6	Karlıova-Bingöl	39.29788 N, 41.01421 E
AE-7	Bingöl City Centre	38.88569 N, 40.49608 E
AE-8	Sancak-Bingöl	39.09511 N, 40.40147 E
AE-9	Sancak-Bingöl	39.09511 N, 40.40147 E
AE-10	Sancak-Bingöl	39.09511 N, 40.40147 E
AE-11	Sancak-Bingöl	39.09511 N, 40.40147 E

*Sampling coordinates were given based on Google Maps locations.

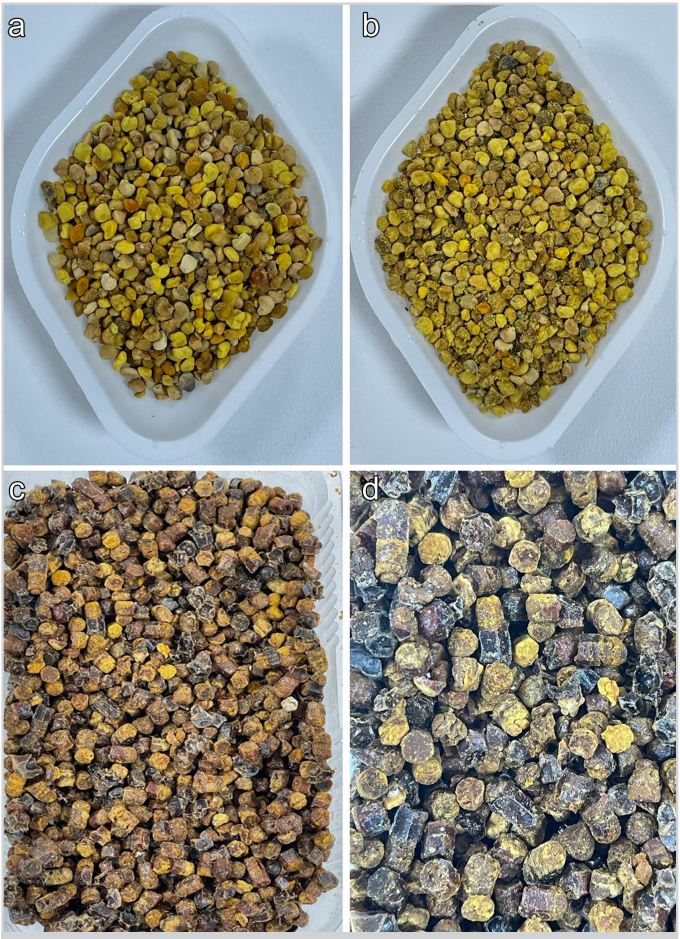

Figure 1.
The Samples Used in this Study. (a and b) Bee Collected Pollen Samples. (c and d) Bee Bread Samples Collected from Bingöl-Turkey.

of the pre-enriched pollen and bee bread cultures were centrifuged (10 000 rpm, 5 minutes), and the pellet was resuspended in 0.5 mL 1×TE (10 mM Tris-HCl, 1 mM EDTA) containing 4 mg/mL lysozyme (Applichem, Germany). According to Liu et al. (2004), total DNA extraction from the grown culture was carried out using the phenol/chloroform/isoamyl alcohol method. The DNA extracts from directly studied samples by the commercially available kit and pre-enriched samples by the manual method were quantitated using Take3 plate of the microplate reader (Epoch2, BioTek, USA) at 260/280 nm.

Next-Generation Sequencing

16S amplicon sequencing and DNA library preparation were carried out according to the 16S metagenomic sequencing library preparation guide (Illumina, Inc., San Diego, California, USA). The primers; F-primer: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′ and R-primer: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′ were used. 16S rRNA V3-V4 regions were amplified using KAPA HiFi HS Mix (Roche, Germany). Polymerase chain reaction (PCR) products from each sample were indexed with dual indexes using the Nextera® XT Index Kit v2 Set-A (Illumina). All samples were cleaned by AMPure XP beads (Beckman Coulter, Inc. Brea, California, USA). The prepared equimolar proportions of samples were pooled, and they were diluted to 10 mM and finally to a 35 pM DNA library. The prepared library (20 µL) containing 5% (v/v) PhiX control DNA (Illumina) was loaded into an iSeq100 v1 cartridge (Illumina). The sequencing was carried out using the iSeq100 system (Illumina) by the pair-end read type and the two reads of 151 bp read length.

Metagenomic Analysis

The sequencing data were analyzed using 16S Metagenomics, Version: 1.1.0 in BaseSpace Sequence Hub (Illumina), and an operational taxonomic unit (OTU) approach was used to identify bacteria from the kingdom to the species level. Then, the Shannon species diversity index, the number of identified species, evenness and Principal coordinate analysis (PCoA) of pollen and bee bread samples were determined by 16S Metagenomics software Version 1.1.0 (Illumina) using RefSeq RDP 16S v3 May 2018 DADA2 32 bp taxonomical interference and the Ribosomal Database Project Classifier (Wang et al., 2007).

Results

In the present study, a total of 14 pollen and 11 bee bread samples of *A. mellifera* were used in the culture-independent NGS

Table 2.
Shannon Species Diversity, the Number of Reads and the Identified Species, and Evenness Values of Pollen (pol) and Bee Bread (AE) Samples Obtained by NGS and Metagenomic Analysis

Sample ID	Number of Reads	Shannon Species Diversity	Number of Identified Species	Evenness
pol-1	27741	1.096	179	0.211
pol-2	9305	0.872	111	0.185
pol-3	11397	0.422	140	0.085
pol-4	8638	1.285	138	0.261
pol-5	19043	1.115	154	0.221
pol-6	20245	1.327	163	0.261
pol-7	32933	0.535	216	0.100
pol-8	18455	0.440	160	0.087
pol-9	21281	0.949	110	0.202
pol-10	12515	0.603	109	0.129
pol-11	14018	0.586	85	0.132
pol-12	20348	1.422	164	0.279
pol-13	19906	1.104	145	0.222
pol-14	9625	1.013	105	0.218
AE-1	13609	0.891	103	0.192
AE-4	27099	1.302	191	0.248
AE-5	13649	0.810	161	0.159
AE-6	10448	0.897	104	0.193
AE-7	19529	1.058	163	0.208
AE-8	38280	1.067	147	0.214
AE-9	22266	0.988	101	0.214
AE-10	22891	1.105	130	0.227
AE-11	20072	0.977	121	0.204

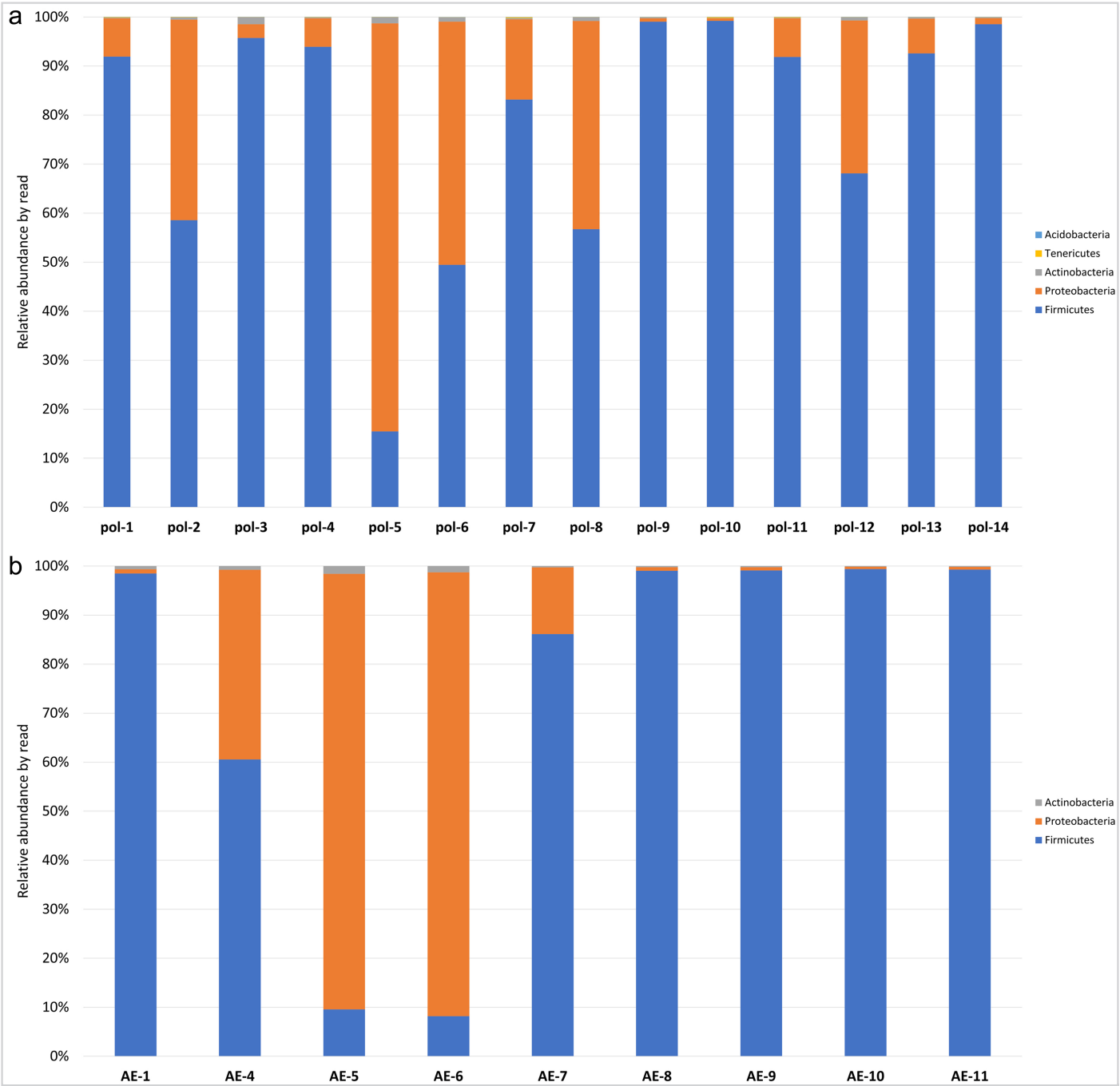

Figure 2.
Bacterial Microbiota Composition of the Pre-Enriched Bee Collected Pollen (a) and Bee Bread Samples (b) at the Phylum Level.

method and metagenomic analysis. In the directly studied pollen and bee bread samples, as well as the pre-enriched bee bread samples AE-2 and AE-3, a 16S rRNA amplicon could not be obtained in PCR experiments. For this reason, only pre-enriched and 16S rRNA gene amplified 14 pollen and 9 bee bread samples included in this study. Next-generation sequencing from pre-enriched samples resulted in a total of 245,450 and 187,843 high-quality sequencing reads for pollen and bee bread samples, respectively (Table 2). The relative abundances of bacterial OTUs belonging to the phylum, the family, the genus, and the species levels of bee collected pollen and bee bread samples were shown in Figures 2–5, respectively. The main bacterial phylum identified in the pollen and bee bread samples was Firmicutes. Second, Proteobacteria was detected with high read numbers, especially in pol-5, AE-5, and AE-6 samples. Moreover, Actinobacteria, Tenericutes, and Acidobacteria phyla were also identified with low read numbers in the pollen samples (Figure 2). The percentages of bacterial OTUs belonging to the family level are given in

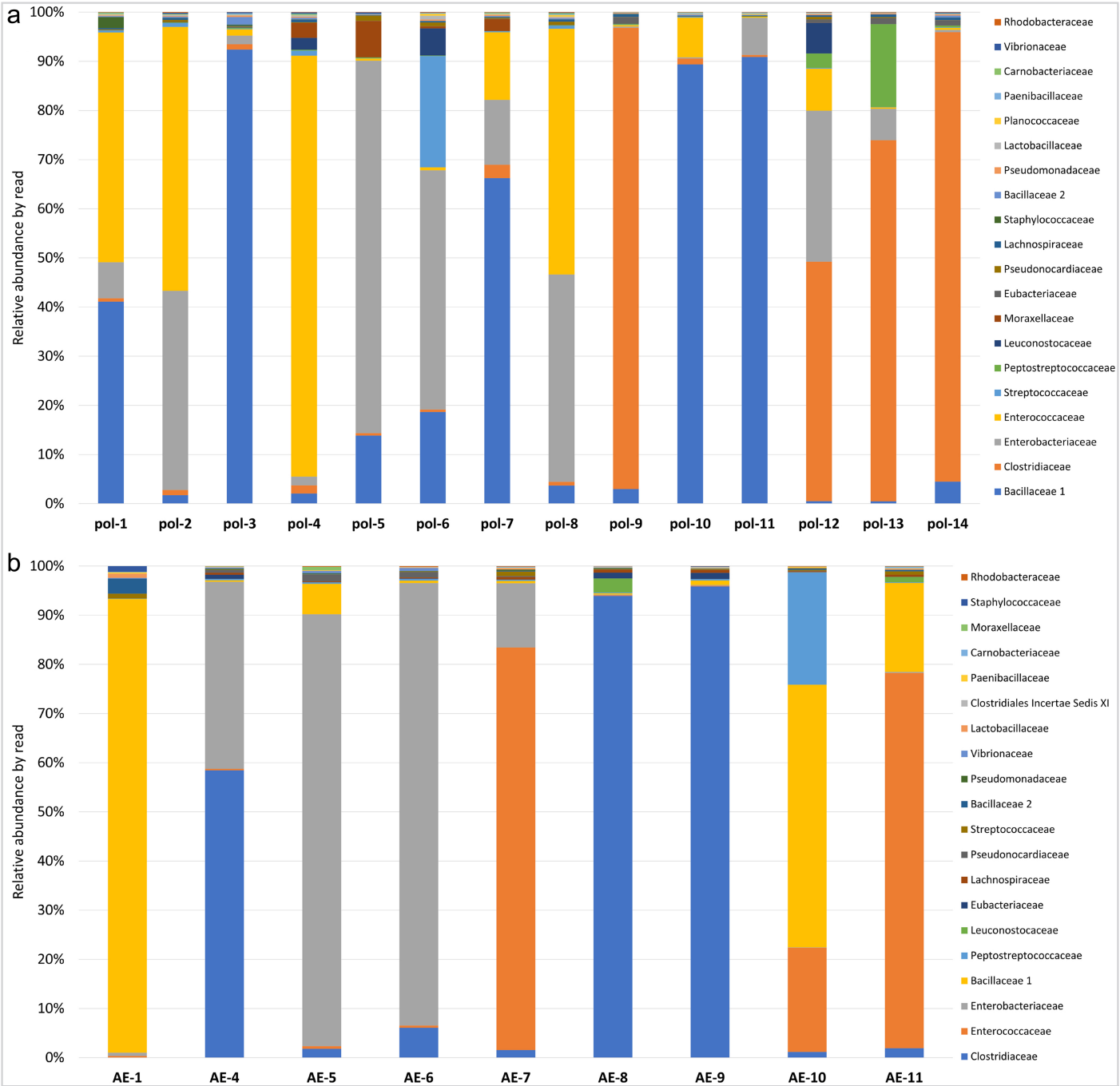

Figure 3.
The Family-Level Taxonomic Distribution of Bacterial Diversity Present in the Pre-Enriched Bee Collected Pollen (a) and Bee Bread (b) Microbiota.

Figure 3. The most abundant bacterial families identified were Clostridiaceae and Bacillaceae in both pollen and bee bread samples. Enterobacteriaceae, Enterococcaceae, Streptococcaceae, Peptostreptococcaceae, and Leuconostocaceae were also present in the pollen and bee bread microbiota (Figure 3). At the genus level taxonomic analysis of the samples showed that in pol-9, pol-12, pol-13, pol-14, AE-4, AE-8, and AE-9 samples *Clostridium sensu stricto* and in pol-1, pol-3, pol-7, pol-10, pol-11, AE-1, and AE-10 samples, *Bacillus* were dominant (Figure 4). Furthermore, *Enterococcus* were detected in both pollen (pol-1, pol-2, pol-4, and pol-8) and bee bread (AE-7, AE-10, and AE-11) samples with high sequencing read. Interestingly, in AE-5 and AE-6 bee bread samples, *Pantoea* was dominant at the genus level (Figure 4). The dominant bacterial species was *Clostridium perfringens* in four pollen and three bee bread samples (Figure 5). *E. faecalis* was abundant in pol-1 and pol-4 pollen samples and in AE-7, AE-10, and AE-11 bee bread samples. Different bacterial species were identified at the species-level taxonomic analysis (Figure 5).

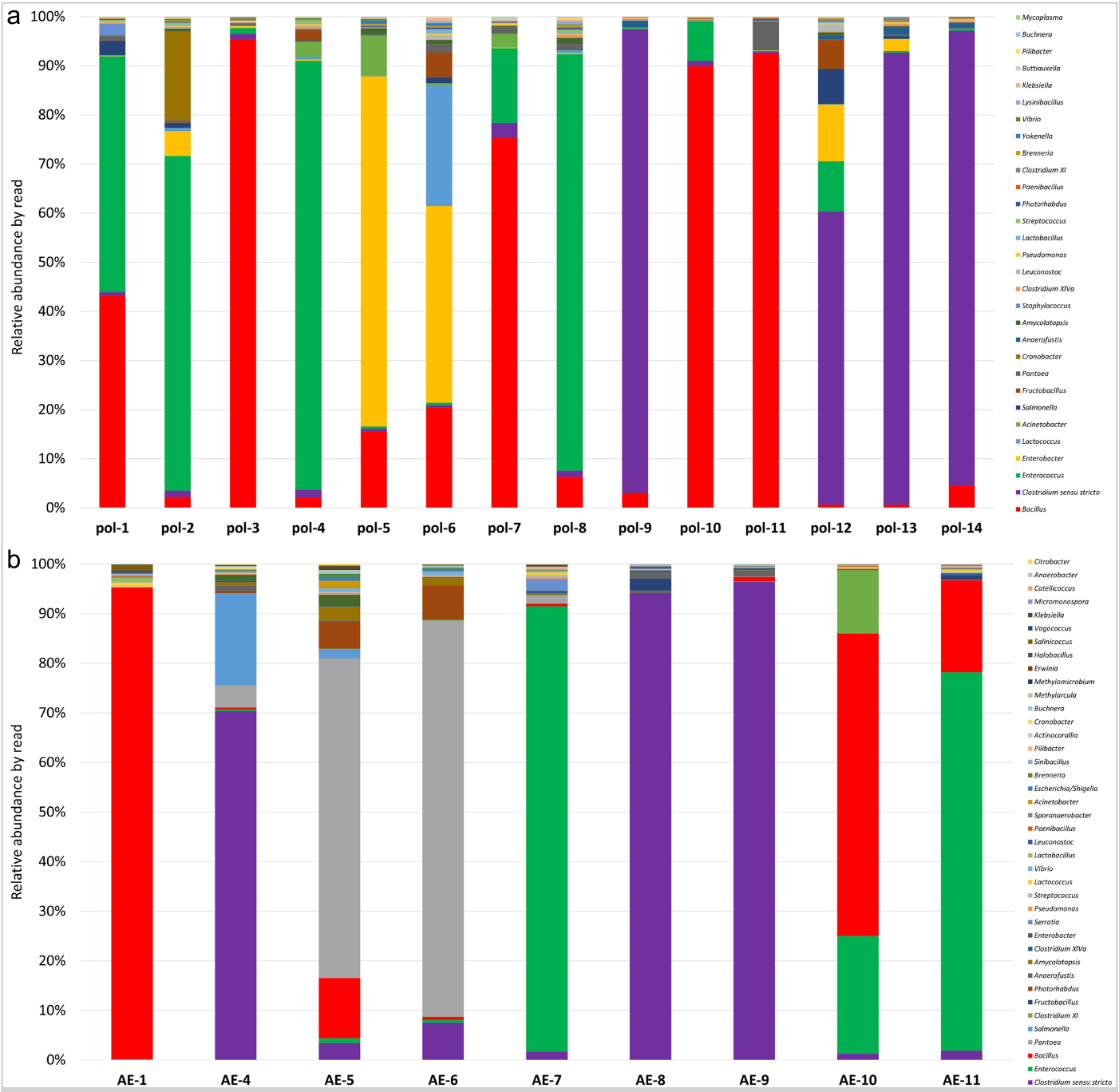

Figure 4.
Bacterial Microbiota of the Pre-Enriched Bee Collected Pollen (a) and Bee Bread (b) Samples at the Genus Level.

Alfa diversity (Shannon species diversity) and evenness results demonstrated that the most diverse pollen sample was pol-3 collected from Adaklı-Bingöl with an evenness value of 0.085. The lowest diversity was found in the pol-12 sample from Konya City Centre with an evenness value of 0.279 (Table 2). The bee bread sample collected from Karlıova-Bingöl AE-5 was the most diverse and AE-4 was the lowest diversity present among bee bread samples with the evenness values of 0.159 and 0.248, respectively (Table 2). In the case of the number of identified species, the highest number of species (216) was detected in pol-7 and the lowest (85) was found in pol-11 pollen samples. AE-4 sample contained 191 species (the highest among bee bread samples) and AE-9 was found to contain the lowest number of species with 101 (Table 2). PCoA plots demonstrated beta diversity results of pollen and bee bread samples. At the genus level, beta diversity results showed that pol-1 was separated from the other three main groups. The pollen samples (pol-3, pol-5, pol-6, pol-7, pol-10, and pol-11) had a close association (Figure 6A). In the case of bee bread samples, three main groups were determined

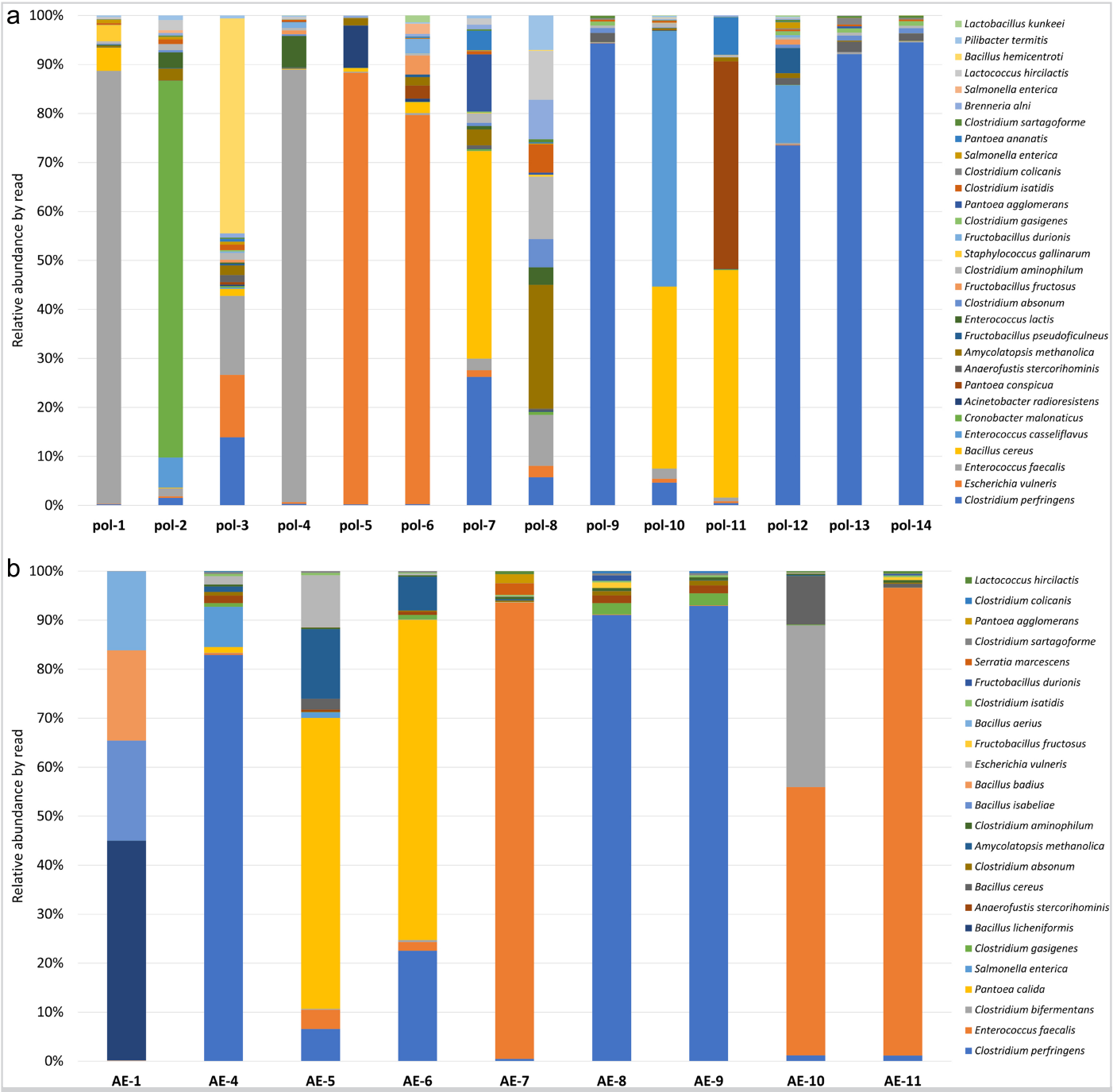

Figure 5.
The Species-Level Taxonomic Distribution of Bacterial Diversity Present in the Pre-Enriched Bee Collected Pollen (a) and Bee Bread (b) Microbiota.

in beta diversity at the genus level. AE-5 and AE-6 samples were distinguished from the other samples. However, AE-4, AE-8, and AE-9 samples were closely related (Figure 6B).

Discussion

In terms of bacterial community structure, several factors can affect the microbial composition of bee collected pollen, and bee bread including honey bee type (Disayathanoowat et al., 2020), land-use changes (De Palma et al., 2016), floral nectar, stored food, alimentary tract (Anderson et al., 2013), and seasonal variations (Anderson et al., 2014; Danner et al., 2017). Honey bees have long been studied not only for economic value for bee products and agricultural crop pollinators but also for microbiota research due to their similarities to mammals. Previously, the significant similarities and differences between the gut microbiota of honey bees and the gut microbiota of humans have been reviewed (Zheng et al., 2019). *A. mellifera* was used as a model organism because its microbial community

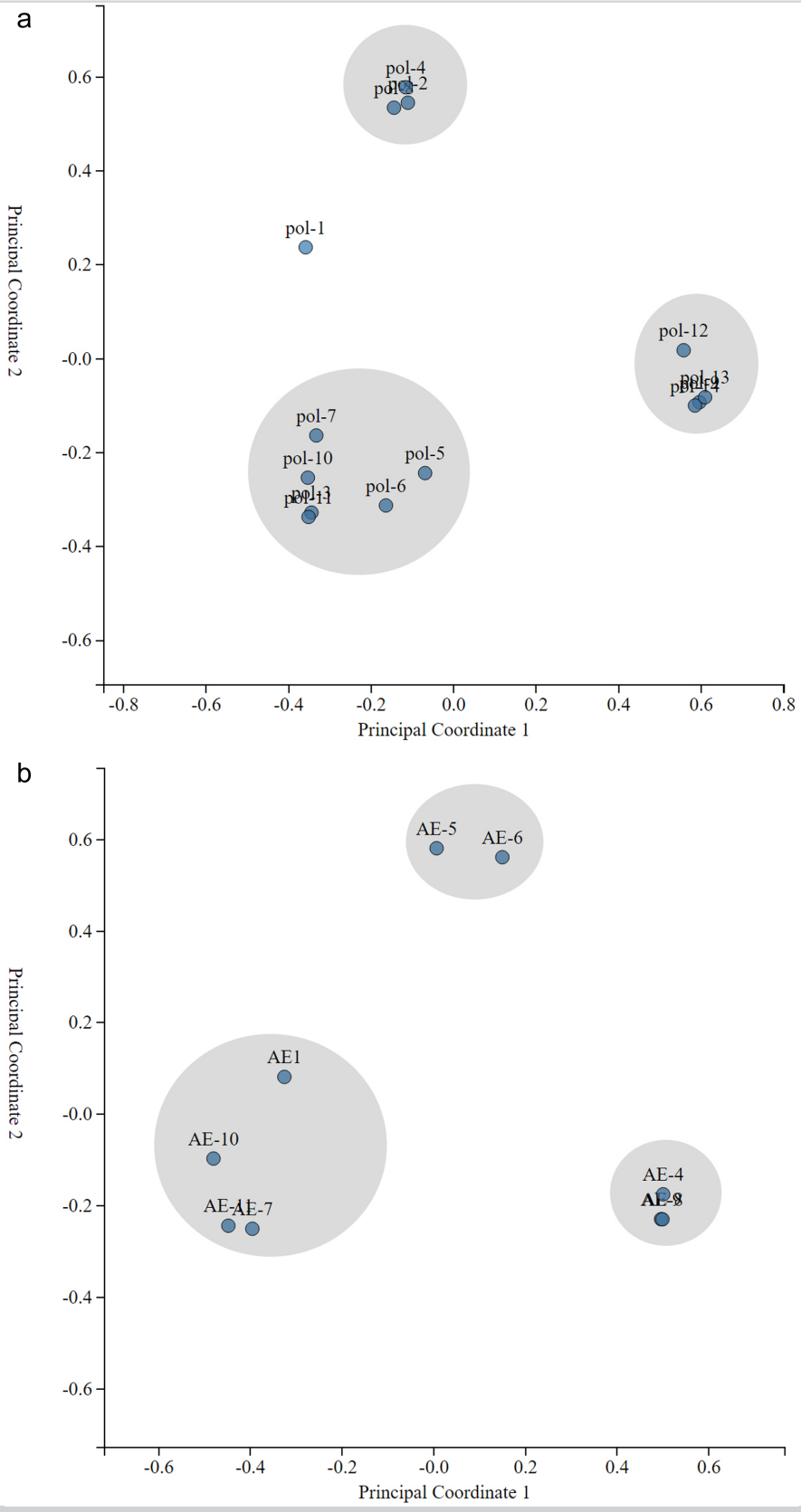

Figure 6.
The Scatterplots of PCoA Show Beta Diversity of Pollen (a) and Bee Bread Samples (b) at the Genus Taxonomic Level.

display high adaptation to various environments (Engel et al., 2012; Nowak et al., 2021). Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes are dominant bacterial phyla found in honey bee guts and their hive environments (Engel et al., 2012; Zheng et al., 2019). The results of the study by Engel et al. (2012) support that the bacteria in honey bees' gut microbiota take part in the nutrition, pathogen defense mechanism, and colony health.

Microbial genera of bee products can be related to the microbial communities of the food source of *Apis* bees. A few studies regarding the microbial diversity in bee bread and bee-collected pollen samples have been reported in the specific areas (Anderson et al., 2013; Anderson et al., 2014; Asama et al., 2015; De-Melo et al., 2015; Disayathanoowat et al., 2020; Saraiva et al., 2015). Disayathanoowat et al. (2020) investigated the bacterial genera of corbicular pollen and hive-stored bee bread collected from commercial honey bees in China, including *A. mellifera* and *A. cerana*. The core gut bacteria were reported in both corbicular pollen and bee bread, whereas the population in bee bread from commercial *A. mellifera* was higher than *A. cerana*. Moreover, for the bee bread samples, the bacterial genera population for *Rosenbergiella, Pantoea, Paracoccus,* and *Escherichia/ Shigella* were found in the two different bee species. The proportion of microbial diversity of both corbicular pollen and bee bread was significantly different. The major genera of bacterial populations that have been identified are *Acinetobacter* followed by *Buttiauxella* and *Pantoea* (Disayathanoowat et al. 2020). In the case of our study, *Clostridium sensu stricto*, *Bacillus,* and *Enterococcus* were the top three dominant bacterial genera. *Pantoea* was detected in the samples (pol-1, pol-6, pol-7, pol-8, pol-11, AE-4, AE-5, AE-6, and AE-7). *Acinetobacter* was found in the samples (pol-5, pol-7, and AE-5) and *Buttiauxella* was detected only the in pol-7 sample (Figure 4). Most probably the reasons for these differences in dominant bacteria were the environmental conditions, bacterial diversities of flowering plants, handling, and storage conditions of bee products.

Bacterial populations in honey, stomach, whole gut, and honey bee products, including bee pollen, bee bread, and royal jelly were identified by 16S rRNA gene sequencing (Asama et al., 2015). The sequence abundance of bee bread at the phylum level were Firmicutes and Proteobacteria, and bee pollen was Firmicutes, Proteobacteria, and Actinobacteria. Furthermore, 18 *Lactobacillus* species were found in honey, bee pollen, bee bread, and royal jelly. *Lactobacillus kunkeei* was the dominant species in bee bread, bee pollen, royal jelly, honey, whole gut, and honey stomach, 99.5, 98.6, 99.7, 98.9, 0.6, and 57.1%, respectively. Moreover, the study reported that *Lb. kunkeei* YB38, isolated from honey bee products, may improve IgA production in humans. In our study, *Lb. kunkeei* was detected only in two pollen samples (pol-6 and pol-12) and it was not abundant.

Saraiva et al. (2015) assessed the microbial community of Africanized honey bee gut and bee bread using the 16S rRNA sequencing. In bee bread samples, a total of 10 bacterial phyla were identified. Similar to our study, the dominant phyla in bee bread samples were Firmicutes and Proteobacteria. Moreover, Acidobateria, Actinobacteria, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Proteobacteria, Verrucomicrobia, and Tenericutes phyla were also present. The identified main families were Neisseriaceae, Acetobacteraceae, Lactobacillaceae, and Flavobacteriaceae. In another study, Anderson et al. (2013) reported the bacterial families present in the bee bread samples using 16S rRNA sequencing from the United States. The bacterial families in the bee bread samples were similar to our examined bee bread samples, including Streptomycetaceace, Pseudonocardiaceace, Corynebacteriaceace, Staphylococcaceae, Bacillaceace, Leuconostocaceace, Lachnospiraceace, Enterobacteriaceae, Cornabacteriaceace, Enterococcaceae, and Lactobacillaceace. This similarity was most probably due to the wild flora of plants and crops. Indeed, honey bees are natural pollinators of crops and the microbiota of plants, therefore, shaping the microbiota of bee products.

Microbial populations differed greatly depending on the supplier sources. Bee pollen, due to its nature, is affected by various environmental factors, and its nutritional compositions provide a favorable microhabitat for bacterial and yeast communities (Anderson et al., 2013; De Palma et al., 2016; Disayathanoowat et al., 2020; Saraiva et al., 2015). The study of Dharampal et al. (2020) evaluated the bacterial diversity of pollen from two different commercial bumblebee hives from the United States and Canada. Four phyla, including Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria identified in the pollen samples. Firmicutes community was higher in the USA-collected samples whereas the samples collected from Canada had a higher abundance of Proteobacteria. Similar to our study, the most abundant phyla Proteobacteria and Firmicutes were found in the pollen samples collected from Mexico, Europe, and Chile (Moreno Andrade et al., 2018). Bacillacae, Planococcaceae, Thermoactinomycetaceae, and Paenibacillaceae were also identified at the family level. In the case of our taxonomic analysis results at the family level, Bacillacae was the most abundant bacteria.

Lactobacillaceae were taught to contribute to the fermentation process and release the secondary metabolites. Additionally, the study Disayathanoowat et al. (2020) detected the presence of core gut bacteria in corbicular pollen and bee bread from *A. mellifera*. Antimicrobial metabolites produced by bifidobacteria and LAB include acetic acid, lactic acid, formic acid, bacteriocins, hydrogen peroxide, diacetyl, and benzoate (Vásquez & Olofsson, 2009). The study performed by Lee et al. (2015) reported the γ-Proteobacteria, Bacilli, and Actinobacter as dominant bacteria in the bee gut microbiome that produce glycosidases and peptidases. These bacterial classes are predicted to be involved in the fermentation process for the breakdown of the polysaccharides and polypeptides, resulting in fermentation products and biosynthesis of secondary metabolites such as organic acid, fatty acids, and alcohols (Saraiva et al., 2015). Zheng et al. (2019) identified *Bifidobacterium* and *Gilliamella* bacterial species that are able to digest polysaccharides, including hemicellulose and pectin. Moreover, Gilliam et al. (1989) reported the specific mold flora that produces enzymes from bee bread and bee pollen for metabolic activities. Saraiva et al. (2015) also predicted the microbial genes associated with the pollen breakdown process in the bee bread. Recently, Zhang et al. (2022) reported the effect of the fermentation process on the content of fermented pollen. The fermentation process is able to break the pollen wall and enhances the release of active ingredients. The content of primary metabolites, including amino acids and their derivatives, organic acids, polyunsaturated fatty acids, and secondary metabolites such as phenolic acids increased in fermented pollen.

Conclusion and Recommendations

The results of this study contribute to the international knowledge of the diversity of bacteria associated with honey bee-collected pollen and bee bread obtained mainly from Bingöl, the main apiculture

center in Turkey. Microbiota of these products plays a crucial role in nutrition supply and maintains the defense system in the hive to avoid the spread of pathogens among honey bees. The communities of bee-collected pollen and bee bread-associated bacteria identified in this study were similar to the previous reports. In fact, bee type, environmental factors, climate, geographic locations, crop, and plant/flower microbiota influence and shape bacterial populations in bee products. Future studies and investigations related to honey products such as propolis, bee bread, pollen, and royal jelly collected from different geographic locations elucidate the effects of bacterial populations on bee health and the quality of bee products.

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