Caste-specific gut symbionts contribute to the different adult longevity in the honeybee

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Abstract

Background

Honeybees are important pollinators, and their health is important to agricultural production and ecosystem. Queen-bees contain same genome as worker-bees, but live longer and healthier than worker-bees; thus, queen and worker pairs are natural biological models for studying longevity. Concerns are increasing regarding the relationship between gut microbes and honeybee health. We compared the hindgut microflora of queen and worker (Apis mellifera carpatica) by sequencing the bacterial 16S DNA, then salvaging the caste-specific microbes using LEfSe analysis and predicting the microbial functions using Tax4Fun, hoping to find potential gut symbionts associated with longevity.

Results

The hindgut microflora of queens differed from those of worker. Queens had higher abundances of Commensalibacter, Lactobacillus and Bifidobacterium than workers. The dominant microflora in the worker hindguts were Gilliamella (29.37%), Lactobacillus (15.28%), Commensalibacter (13.65%), Snodgrassella (11.56%), Bifidobacterium (6.07%) and Frischella (3.51%). The dominant microflora in the queen hindguts were Commensalibacter (44.89%), Lactobacillus (38.42%), Bifidobacterium (6.74%), Gilliamella (2.44%) and Bombella (2.41%). Queen-specific microbe was Bombella genus, and worker-specific microbe included Snodgrassella alvi, Frischella perrara and Gilliamella apicola. Queen and worker hindgut microbes exhibited diverse functions in lipid metabolism, neurodegenerative diseases, endocrine system, nervous system and immune system; those functions were linked to honeybee fitness and longevity. The queen-specific symbiont, Bombella, was predicted to be involved in host endocrine and immune regulation, which may contribute to queens living longer and healthier than worker bees.

Conclusion

The supernal Acetobacteraceae (especially Commensalibacter and Bombella), Lactobacillus and Bifidobacterium in the queen-hindgut help the queen bees live longer and healthier than the worker bees. This study may help determine the mechanisms of queen longevity and enable further understanding the positive roles of gut symbionts in honeybee fitness.

Introduction

Honeybees are highly eusocial insects, and their colonies consist of the queen, workers and drones. Queen and worker bees are both female and have the same genome but differ in reproduction, immunity and other physiological functions [1–3]. Interestingly, queen and worker bee lifespans vary considerably, with queens living up to 10–20 times longer than workers,[4, 5] suggesting that queen bees are healthier than worker bees. Thus, honeybees have long been studied as models of aging [6]. The variations in their lifespans are mostly attributed to different diets. Throughout their lifetime, the queens primarily eat royal jelly, which is functionally analogous to mammalian breast milk and supplies their complete nutrition, including antioxidant, antimicrobial, and immunoregulatory properties. Conversely, worker bees eat bee bread, the fermentation product of honey and pollen, for most of their short lives, except during the larval stage. Feeding royal jelly to worker bees enables them to live longer [7]. The diet regulates bodily health via many mechanisms. However, we believe that food first affects the gut microbes because the digestive tract is where food initially enters the bees’ bodies. Food provides nutrition for the bees as well as a substrate for intestinal bacterial survival. Therefore, honeybees are a natural biological model for studying the relationship between gut bacteria and host health and longevity.

Intestinal bacteria are important to host health. The gut microflora community structure and characteristics of honeybees have been fully and systematically analyzed over the last 15 years. Nine distinctive bacterial phylotypes dominate the guts of honeybee workers: Gilliamella apicola (Gamma-1), Frischella perrara (Gamma-2), Snodgrassella alvi (Beta), Bartonella apis (Alpha-1), Commensalibacter (Alpha 2.1), Parasaccharibacter and Bombella (Alpha 2.2), Bifidobacterium and Lactobacillus (Firm-4, Firm-5)[8–11]. The dominant species and abundances vary among various niches of the digestive tract. Few bacterial phylotypes with low abundances exist in the foregut, but rich phylotypes with high abundances exist in the hindgut, accounting for over 99% of the microbes in the entire gut. However, most of these findings were determined from studies on worker bees. Few studies have examined the composition of the queen bee gut microflora. Queen bee core bacteria include Acetobacteraceae (Alpha 2.1 and Alpha 2.2) and Firm-4 and Firm-5 Lactobacillus; whereas worker guts comprise mostly Proteobacteria (Gamma-1, Gamma-2 and Beta) [12–14]. Our latest study found that sucrose as overwintering feed increased the Acetobacteraceae abundance in worker guts compared with that of honey and fructose syrup, and honeybee colonies with higher Acetobacteraceae abundances exhibited less overwintering loss. These results suggest that increasing the Acetobacteraceae abundance in honeybee guts maybe prolong the life of the bees[15]. In summary, associations between microbial community compositions and longevity remain uncertain.

Determining the caste-specific gut microflora, especially members of Acetobacteraceae, is important. Queen-specific bacteria may be closely related to honeybee health and longevity. To determine the specific bacteria associated with honeybee health and longevity, we systematically compared the hindgut microflora of the mature queen and worker bees (Apis mellifera carpatica, Kaqian black ring strain) and predicted the functions of the different taxa. The Kaqian black ring strain, which exhibits stronger adaptability, less food consumption, higher honey production and better overwintering ability than does Apis mellifera L., was bred from Apis mellifera carpatica by pure breed selection under the ecological conditions of the Changbai Mountain area in China and was thus used in this study.

Materials And Methods
Animals and sampling

Three sister queen colonies (Apis mellifera carpathica, Kazjan black ring strain) were used in our experiments. The queens and worker bees were reared as per the methods of Wang and Begna [16, 17]. Briefly, the spawning queen and an empty wax comb frame were confined to a cage for 12 hours, through which the workers could pass freely, but the queen could not. After the queen laid enough eggs, the frame was transferred to the upper successor box of the colony to incubate for 3 days. Some of the first-instar larvae were transferred from the worker cells to queen cell cups in a queen-rearing frame, then put into a queenless colony for further development. Seven days after emerging from the cells, five queen bees per colony, which had not finished mating but were sexually mature with an established gut microflora community, were collected into 2-mL microcentrifuge tubes. The remaining larvae remained in worker cells, allowing them to develop into worker bees. Worker bees were marked with color after emerging from the cells. The colored 7-day-old worker bees (n = 10/colony), whose gut microflora community was stably established, were collected into 2-mL microcentrifuge tubes. Under aseptic conditions, the hindguts of the 7-day-old virgin queens and 7-day-old worker bees were sampled to extract the gut microbial DNA [15].

Gut microflora genomic DNA extraction and sequencing

Microbial DNA was extracted using HiPure Stool DNA Kits (Magen, Guangzhou, China) per the manufacturer’s protocols. The DNA concentration, purity and integrity were verified using a NanoDrop (Thermo, USA) (1.8 < A260/A280 < 2.0) and 1% agarose gels. The 16S rDNA V3–V4 regions of the ribosomal RNA genes were amplified via PCR using primers 341F: CTCACGGGNGGCWGCAG and 806R: GGACTACHVGGGTATCTAAT. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) per the manufacturer’s instructions and quantified using the ABI StepOnePlus Real-Time PCR System (Life Technologies, Foster City, CA, USA). Purified amplicons were pooled in equimolar quantities, then were paired-end sequenced using an Illumina platform to generate 250-bp paired-end reads (PE250).

Bioinformatics analysis of 16S rDNA sequencing data

The raw reads were further filtered to remove reads containing more than 10% unknown nucleotides and reads in which < 50% of the bases had a quality (Q-value) > 20 according to the following rules using FASTP (version 0.18.0)[18]. Paired-end clean reads were merged as raw tags using FLASH (version 1.2.11) [19] with a minimum 10-bp overlap and 2% mismatch error rate. To obtain high-quality clean tags, noisy sequences of raw tags were filtered via the QIIME pipeline (version 1.9.1) [20]. Clean tags were searched against the reference database (version r20110519, http://drive5.com/uchime/uchime_download.html) to perform reference-based chimera checking using the UCHIME algorithm [21]. All chimeric tags were removed, then the resulting effective tags were further analyzed. The effective tags were clustered and classified into operational taxonomic units (OTUs) at an identity threshold of 97% similarity using UPARSE software (version 9.2.64)[22]. The representative OTU sequences were classified into taxonomic categories as per the SILVA database (version 132) [23].

Statistical analysis and software

Statistical comparisons between two measurements were analyzed using unpaired two-tailed Student’s t-tests in SAS 9.1. The stacked bar plot of the community composition was visualized using the R project ggplot2 package (version 2.2.1). A heatmap of the species abundance was plotted using the R project ggplot2 package (version 2.2.1). Multivariate statistical techniques, including principal coordinates analysis (PCoA) of the Bray-Curtis distances and pairwise analysis of similarities (ANOSIM) tests were performed using the R project Vegan package (version 2.5.3) to determine significant differences between the worker and queen gut microbial communities. R values in ANOSIM were used to detect the community overlap [24] (R > 0.75: well-separated; 0.50 < R ≤ 0.75: separated but overlapped; 0.25 < R ≤ 0.50: separated but strongly overlapped; and R ≤ 0.25, barely separated). P-values indicated significant differences between the groups (**P < 0.01, ***P < 0.001). The Kyoto Encyclopedia of Genes and Genomes pathway analysis of the OTUs was inferred using Tax4Fun (version 1.0). Functional differences between groups were calculated via Welch’s t-test in the R project Vegan package (version 2.5.3). Pearson correlation analysis between taxa and functions was calculated in the R project psych package (version 1.8.4). P-values were obtained via Fisher’s Z transformation.

Results

Bacterial sequences and classification

We obtained 641,352 16S rRNA V3-V4 region raw paired-end (PE) reads from the 6 samples (3 queen bee and 3 worker bee samples). After filtering, we retained 626,318 clean PE reads (Table S1). Tag assembly and quality control yielded an average of 85445 effective tags per sample. Of those, an average of 20529 tags (24%) were singletons, and 46993 tags (55%) were unique. The taxon tags averaged 64916 (76%) and were clustered into an average of 669 OTUs per sample (Table S2). Those OTUs were mainly classified into three phyla (Proteobacteria, Firmicutes and Actinobacteria), which accounted for more than 99% of all the gut bacteria (Fig S1A).

Queen and worker bees had different gut microbial profiles

To compare the microflora communities of the workers and queens, the diversity and distances of the samples were analyzed. PCoA based on Bray-Curtis showed differences in gut bacterial compositions between the worker and queen bees with a profound difference along the PC01 axis (reaching 59.47% of overall variation, P = 0.003684). The aggregation of samples within groups and dispersion of samples between groups indicated higher similarity within groups with a clear difference between groups (Fig. 1A). ANOSIM results (Fig. 1B) showed that the intergroup distance between the worker and queen bees was greater than the intragroup distance between the worker and queen bees (R = 0.926, P = 0.028). The distance index between the worker and queen samples was larger than that among samples in either the worker or queen bees (Fig. 1C). Larger distance indexes indicated greater distances between samples. These results confirmed that the worker and queen gut microflora communities differed.
Gut microbial caste-specific patterns

The worker and queen gut microfloras exhibited both differences and commonalities. Venn diagrams were constructed to analyze the common and unique OTUs between worker and queen bees. The worker and queen hindguts contained 339 common OTUs (50.67%); the worker hindguts contained 266 unique OTUs (43%), and the queen hindguts contained 346 unique OTUs (50.51%). The common OTUs were mostly annotated to dominant bacterial taxa in both the worker and queen guts (Table S3). Fewer unique OTUs were found (< 40 for workers, < 20 for queens; Tables S4 and S5).

Queen and worker bees have similar taxonomic types of dominant gut microflora at the phylum level, including Proteobacteria (78.34% for workers, 53.92% for queens), Firmicutes (15.33% for workers, 38.73% for queens) and Actinobacteria (6.08% for workers, 6.81% for queens) (Fig. S1A and Fig. 3A). However, the dominant genera in the worker and queen bee guts differed. The dominant microflora in the worker guts were Gilliamella (29.37%), Lactobacillus (15.28%), Commensalibacter (13.65%), Snodgrassella (11.56%), Bifidobacterium (6.07%) and Frischella (3.51%; Figs S1C and 3B). The dominant microflora in the queen guts were Commensalibacter (44.89%), Lactobacillus (38.42%), Bifidobacterium (6.74%), Gilliamella (2.44%) and Bombella (2.41%; Figs S1D and 3B).

Compared with the worker bees, queens had higher abundances of Alphaproteobacteria Acetobacteriales Actinobacteriaceae (Commensalibacter and Bombella), Firmicutes Bacillales Lactobacillaceae (Lactobacillus) and Actinobacteria Bifidobacteriales Bifidobacteriaceae (Bifidobacterium). However, the abundances of Proteobacteria, Gammaproteobacteria, Orbales, Orbaceae (Gilliamella and Frischella), Betaproteobacteria, Neisseriaceae and Snodgrassella were much richer in the worker guts (Figs S2 and 3AB).

LEFSe analysis (Linear Discriminant Analysis, LDA > 4) was performed to determine the caste-specific biomarker taxa. Queen-specific biomarker taxa included Bombella intestini (Fig. 3C), and the worker-specific biomarker taxa were S. alvi, F. perrara and G. apicola (Figs. 4 and 3C).

Landscape and inter-caste differences in intestinal microbiota functions

The worker hindgut and queen hindgut microfloras differed. To understand the functional changes in these differences in microbial construction, we predicted the functional profiles of the gut microbiome based on 16S RNA sequencing data using Tax4Fun (v1.0). The honeybee gut microbiomes were enriched in six functions at level 1: metabolism, environmental information processing, genetic information processing, cellular processes, human diseases and organismal systems (Fig. 5A). The significant changes (P < 0.05) in hindgut microbiota gene functions at level 2 between workers and queens focused on lipid metabolism, neurodegenerative diseases, the endocrine system, nervous system and immune system. We further compared the more detailed gut microbiota functional differences at level 3 between the worker and queen bees (Fig. 5B). Twenty-seven functions significantly differed at level 3 (P < 0.05), of which, only five functional abundances were higher in queens than in workers: fructose and mannose metabolism, taurine and hypotaurine metabolism, bacterial toxins, alpha-linolenic acid metabolism and the insulin signaling pathway. Seven caste-specific species (Lactobacillus sp SF6D, Lactobacillus kuehnei, Lactobacillus apis, Lactobacillus sp Adhmt19, B. intestini, F. perrara, and S. alvi; Figs. 3C and 4) were targeted to predict their associated functions at level 2 (Fig. 6). Five functions were significantly associated with S. alvi (r > 0.8 and P < 0.05): folding, sorting and degradation; glycan biosynthesis and metabolism; signaling molecules and interaction; endocrine system; and excretory system, of which, folding, sorting and degradation; glycan biosynthesis and metabolism; and endocrine system were positively correlated, while the others were negatively correlated. The endocrine system and excretory system functions were significantly associated with B. intestini (r > 0.8 and P < 0.05). The r between immune system and B. intestini was 0.8015, but P was > 0.05. The most relevant function for L. sp. Adhmt19 was immune system (r = 0.9196 and P = 0.009432). The functional profiles of L. sp. SF6D, L. kuehnei and L. apis were similar, and their more relevant functions (0.5 < r < 0.8) included amino acid metabolism, metabolism of cofactors and vitamins, environmental adaptation, circulatory system and immune system diseases.

To further study the potential functions of the caste-specific taxa, correlations between the caste-specific taxa and the level 3 functions from the significantly related functions at level 2 were analyzed using Pearson correlation analysis. Figure 6B shows the level 3 functions with r > 0.5. Eight tertiary functions were significantly related to B. intestini, 9 were significantly related to L. sp. Adhmt19, 6 were significantly related to F. perrara, and 11 were significantly related to S. alvi (r > 0.8; P < 0.05). The tertiary functions closely related to B. intestini and L. sp. Adhmt19 primarily comprised antigen recognition and innate immunity (RIG-I-like receptor signaling pathway, cell antigens, antigen processing and presentation, and NOD-like receptor signaling pathway), biosynthesis and degradation of polysaccharides (N-glycan biosynthesis, glycosaminoglycan degradation and glycosphingolipid biosynthesis) and endocrine signaling pathways (PPAR signaling and adipocytokine signaling pathways). The functions closely related to S. alvi included ubiquinone and other terpenoid-quione biosynthesis, proximal tubule bicarbonate reclamation, bacterial toxins, alpha-linolenic acid metabolism and the insulin signaling pathway. F. perrara functions mainly included ubiquinone and other terpenoid-quione biosynthesis, alpha-linolenic acid metabolism, insulin signaling pathway, sulfur relay system, ubiquitin system and chaperones, and folding catalysts. These predictive functions of the caste-specific taxa indirectly indicated that the host bees (workers and queens) may differ in physiological functions, substrate metabolism, and immune recognition and helped clarify the differences between workers and queens.

Discussion
Honeybee gut microbiotas are distinct from those of solitary bee species, whose guts mainly include *Burkholderia*, and the pervasive insect associate *Wolbachia* [25, 26]. However, previous studies found that bacteria in honeybee guts are highly conserved despite subspecies differences among hosts [9, 25–28]. Previously characterized results on *Apis mellifera ligustica* showed that the main core phyotypes in worker guts included Gammaproteobacteria (*G. apicola*), Betaproteobacteria (*S. alvi*), Firmicutes (*Lactobacillus Firm-4* and *Lactobacillus Firm-5*) and *Bifidobacterium* spp. (listed in order of highest to lowest abundance). Other studies analyzed the gut microbiomes of other species and *Apis* subspecies, including *A. dorsata*, *A. andreniformis*, *A. mellifera carnica*, *A. mellifera capensis*, and *A. mellifera scutellata* [9, 25, 27]. The results showed that the gut microbes differed only slightly among the various honeybee species and subspecies. Most core bacterial taxa in the *Apis* guts included the nine dominant phyotypes mentioned above; however, some subtle differences were noted: Alpha-2.1, Firm-4 and Firm-5, which were common in *Apis mellifera ligustica* guts, were undetected in *A. dorsata*, while *A. andreniformis* was universally colonized with high abundances of Alpha-1, Beta, Firm-4, and Gamma-1 [25]. *Simonsiella* was detected in *A. mellifera scutellata*, and *Gluconacetobacter*, *Serratia*, and *Simonsiella* were detected in *A. mellifera capensis* [9]. *Gluconacetobacter*, *Curvibacter*, *Comamonas*, *Ralstonia*, *Simonsiella*, *Salmonella* and *Serratia* occurred sporadically in the *A. mellifera carnica* guts [28], but these bacteria were rare in the *A. mellifera ligustica* guts. In the present study, the dominant microflora in the worker guts (*A. mellifera carpathica*, *Kaqian black ring strain*) were *Gilliamella* (Gamma-1; 29.37%), *Lactobacillus* (Firm-5; 15.28%), *Commensalibacter* (Alpha-2.1; 13.65%), *Snodgrassella* (Beta; 11.56%), *Bifidobacterium* (6.07%) and *Frischella* (Gamma-2; 3.51%); no *Lactobacillus Firm-4* was identified. *Apis* species or subspecies taxa, in addition to methodological and environmental factors, may have contributed to these subtle differences.

**Queen bee guts contained more Acetobacteraceae, Lactobacillus and Bifidobacterium, differing from the worker bee guts**

In eusocial insects, there were many different phenotypes between queen and worker, including morphology, genitalia and longevity. Studies of the differences in longevity between castes are valuable because individual longevity is often closely associated with physical fitness. Recent studies regarding how microbial communities in bee guts are involved in pathogen protection and nutrition metabolism have drawn attention to the impact of the microbiota on bee fitness. Thus, we examined which differences in the gut microbial communities of the queen and worker bees would contribute to differences in their lifespans. Previous studies of the gut microbial communities of queen and worker bees showed that queens lack the stable core microbiotas that are associated with the workers, although these studies used queens of different ages and reproductive stages (i.e., 4–6-month-old, 16–18-month-old, 4-day-old virgin, 14-day-old spawning and 7-day-old virgin queens) (Kapheim et al. 2015; Anderson et al. 2018; Powell et al. 2018). Our study of caste-specific microbiotas in *A. mellifera carpathica* (*Kaqian black ring strain*) reconfirms the clear distinctions between castes. The same phenomenon was found in termites, which are also social insects (Poulsen et al. 2014; Otani et al. 2019). Diverse gut microbial communities between infertile and royal termite castes were thought to be associated with caste-specific diets and lifestyles (Otani et al. 2019). The same also applies to honeybees. Compared with the worker gut microbial data reported by Kwong & Moran (2016), Anderson et al. (2018) found that the queen-specific microflora of *A. mellifera ligustica* included *Parasaccharibacter apium* (Alpha-2.2) and *Lactobacillus kunkeei* (Firm-5); worker-specific microflora included *Bartonella apis*, *F. perrara*, *S. alvi* and *G. apicola*, and shared core microflora were *Lactobacillus* Firm-4, *Lactobacillus* Firm-5, *Bifidobacterium* asteroids and *Acetobacteraceae* Alpha 2.1. Our results showed that the queen-specific taxon was *B. intestini* (Alpha 2.2; Fig. 3C), the worker-specific taxa were *S. alvi*, *F. perrara* and *G. apicola* (Figs. 4 and 3C), and queens had higher abundances of *Commensalibacter*, *Lactobacillus* and *Bifidobacterium* than worker bees (Fig. 3B). However, *B. intestini* was not the most abundant bacterium in the queens, but the fifth most abundant, with *Commensalibacter* (44.89%; Alpha 2.1), *Lactobacillus* (38.42%), *Bifidobacterium* (5.44%), and *Gilliamella* (2.44%) ranking before it (2.41%). Powell et al. (2018) showed that *Acetobacteraceae* (Alpha-2.1 and Alpha-2.2) and *Lactobacillus* Firm-5 dominated queen *Apis mellifera* guts. Kapheim (2015) reported similar results in that the top four most abundant bacteria were *P. apium*, Alpha-2.1, *Lactobacillus* Firm-4 and *Lactobacillus* Firm-5. These studies collectively suggest that queen guts harbor more abundant *Acetobacteraceae*, especially Alpha-2.2, than do worker guts, likely related to the honeybee diets. Previous research concluded that *Acetobacteraceae* Alpha 2.2 were prolific in the crops, hypopharyngeal glands of nurse bee, royal jelly and larva fed on royal jelly, but were negligible in the nurse and forager midguts and hindguts (Anderson et al. 2013; Vojvodic, Rehan & Anderson 2013; Corby-Harris et al. 2014). Some niches were characterized by the availability of royal jelly, which is the main food of queen bees and differs from the bee bread and honey eaten by the workers. Thus, queen bee guts are a niche accessible to royal jelly, suggesting that royal jelly may promote *Acetobacteraceae* Alpha 2.2 proliferation. The widespread distribution of Alpha 2.2 in many niches of honeybee indicates some specialized biological functions in the hosts. Our latest study found that overwintering honeybee colonies with higher abundances of *Acetobacteraceae* exhibited a lower rate of overwintering loss (Wang et al. 2020a), suggesting that *Acetobacteraceae* plays a positive role in honeybee fitness. One Alpha 2.2 isolate was shown to increase honeybee larval survival in vitro (Corby-Harris et al. 2014). *Acetobacteraceae* are symbionts of various insects. Studies on *Anopheles* and *Drosophila* indicated that *Acetobacteraceae* provided nutrition to the host (Crotti et al. 2010), benefited host growth and development [29–31], and modulated host immunity (Ryu et al. 2008). So it was inferred that the abundant *Acetobacteraceae*, including *Commensalibacter* and *Bombella*, in queen bee guts might be another important beneficial bacteria enabling queen bees to live healthier and longer lives than worker bees, except for the well-known beneficial symbiotic bacteria *Lactobacillus* and *Bifidobacterium*.

**Gut microbial functions in queen bees differed from those of worker bees**

Regarding the integral functions of gut microbes, the microbiota gene functions that differed between workers and queens at level 2 included lipid metabolism, neurodegenerative diseases, and the endocrine, nervous and immune systems (Fig. 5). Of these, lipid metabolism and the endocrine and immune systems are correlated with honeybee longevity. Regarding lipid metabolism, the fatty acid composition of the phospholipid membrane affects bee longevity [32]. The membrane phospholipids of adult worker bees include richer polyunsaturated fatty acids (PUFAs) and increase with age. However, the membrane phospholipids in adult queens remain highly monounsatuated throughout the bees adult life [32, 33]. PUFAs are 1,000 times more likely to oxidize than are monounsaturated fatty acids [34]. Accumulation of lipid oxidative damage over a lifetime is one main cause of aging [35, 36]. Studies in mice have shown that gut bacteria can alter the saturation and length of host fatty acids [37]. Therefore, gut microbes likely influence the lifespans of queens and workers by regulating lipid metabolism.
Regarding the endocrine system, the insulin pathway was shown to be associated with caste differentiation in female honeybees [38]. In the present study, the honeybee gut microbes were predicted to be functionally associated with host insulin signaling, consistent with previous studies [31, 39]. Reducing insulin/insulin-like growth factor signaling (IIS) activity inhibits juvenile hormone secretion, thereby increasing vitellogenin expression [40, 41]. Upregulating vitellogenin expression can prolong bee longevity [42]. Studies have shown that older queen bees have lower IIS activity than do older worker bees [40], and the insulin signaling abundance associated with gut microbes was predicted to be lower in queens than in worker bees. Thus, queen bees live longer than do worker bees. Studies of model organisms have shown that reducing IIS activity increases longevity [43–45]. Thus, bee gut microbes likely affect host longevity via endocrine pathways, particularly insulin signaling.

Regarding the immune system, a common characteristic among aging animals is reduced immunity [46], which also occurs in aging bees [3, 47, 48]. That is, factors that affect bee immune performance tend to affect their longevity. Studies on insects have shown that gut microbes play key roles in establishing and regulating the host immune system [49–52], suggesting that gut microbes may affect host longevity via immunoregulation. Differences in lipid metabolism and endocrine and immune function abundance in gut microbes between queen and worker bees indicate that gut microbes contribute to the differences in longevity between queen and worker bees.

Another gut microbial function predicted to differ between female castes is folate biosynthesis. Folic acid functions as a one-carbon unit carrier involved in substance metabolism and synthesis. The nitrogen 5-trimethyl-tetrahydrofolic acid (N5-CH3-FH4) of the one-carbon units provides methyl for homocysteine to produce methionine. Methionine is activated into S-adenosylmethionine, which is the methyl donor in DNA methylation [53, 54]. DNA methylation is one of the main molecular mechanisms for female bee castes differentiation [55, 56]. Methionine as a methyl donor also plays a regulatory role in differentiating female honeybee castes [57]. Thus, gut microbes may be involved in regulating the ontogenetic trajectory of female bees.

**Bombella positively affected honeybee fitness**

The functions of the caste-specific taxa, *L. sp. SF60, L. kullabergensis, L. apis, L. sp. Adhmto19, B. intestini, F. perrara, and S. alvi*, were predicted using Tax4Fun. As worker bee-specific taxa, the functions of *F. perrara* and *S. alvi* were previously investigated [58, 59]; these microbes are involved in immunity [60], defense [61] and maintaining the anaerobic fermentation environment [39]. However, less is known about the microbial functions associated with queen bees. In this study, five queen-specific taxa were found, including four species of *Lactobacillus* and *B. intestini*. The benefits of *Lactobacillus* on animal fitness have been well documented. However, little is known about *B. intestini* originating from honeybees. In the present study, *B. intestini* appeared as a queen-specific gut microbe; thus, it received our attention. *B. intestini*, which was first isolated from bumblebee crops [62], is part of a clade of acetic acid bacteria (a group within the family Acetobacteraceae). To date, *B. intestini* from honeybees is unreported. However, *Bombella apis*, another member of the genus *Bombella* with 98% sequence similarity to *B. intestini*, was detected from honeybee midguts [63]. However, little is known about the role of *genus Bombella* in honeybee fitness. Whole-genome sequencing of *B. intestini* and *B. apis* disclosed the general genomic features and functional annotations of the coding gene [64, 65]. Fusaric acid resistance (FUSC) genes were found in the *B. apis* genome. Several fungal species produce fusaric acid [66]. FUSC genes in the *B. apis* genome indicate that *B. apis* in honeybees may protect honeybees from fungal infection [67]. In this study, *B. intestini* was predicted to be associated with immune and endocrine functions, including the RIG-I-like receptor signaling pathway, cell antigens, antigen processing and presentation, the NOD-like receptor signaling pathway, N-glycan biosynthesis, glycosaminoglycan degradation and glycosphingolipid biosynthesis, the PPAR signaling pathway and the adipocytokine signaling pathway. Of these, RIG-I-like and NOD-like receptors are members of pattern-recognition receptor families that sense nucleic acids derived from viruses and trigger antiviral innate immune responses [68, 69]. Therefore, honeybee symbiotic bacteria belonging to *Bombella* may function by resisting pathogen infection and play important roles in maintaining honeybee health. Further studies and more direct evidence are required.

**Declarations**

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**Author contributions**

Hongfang Wang and Baohua Xu participated in the conception and design of the study. Hongfang Wang drafted the manuscript and performed the experiments. Hongfang Wang, Zhenguo Liu, Xuepeng Chi and Kai Han collecting the samples. Ying Wang, Lanting Ma and Baohua Xu revised the paper. All authors read and approved the final manuscript.

**Declaration of conflict of interests**

All the authors declare no conflict of interests.

**Availability of data and materials**

The dataset supporting the conclusions of this article is available in the NCBI Sequence Read Archive database (Accession Number: SUB8289109).

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### Tables

#### Table S1
Data preprocessing statistics and quality control

| Sample ID | Raw PE | Clean PE | Raw Tags | Clean Tags | Effective Tags | Effective Ratio (%) |
|-----------|--------|----------|----------|------------|----------------|---------------------|
| W1        | 103476 | 101069   | 91686    | 90820      | 84863          | 82.01               |
| W2        | 107307 | 104395   | 93183    | 91580      | 81020          | 75.5               |
| W3        | 112197 | 109653   | 99427    | 97833      | 81654          | 72.78              |
| Q1        | 101310 | 98798    | 89335    | 87254      | 84261          | 83.17              |
| Q2        | 110332 | 108004   | 98695    | 97394      | 92397          | 83.74              |
| Q3        | 106730 | 104399   | 95001    | 93649      | 88479          | 82.9               |

Note: PE, paired-end; W, worker; Q, queen

#### Table S2
Numbers of tags and OTUs

| Sample ID | Total Tags | Unique Tags | Taxon Tags | Unclassified Tags | Singleton Tags | OTUs |
|-----------|------------|-------------|------------|-------------------|----------------|------|
| W1        | 84863      | 52552       | 61387      | 0                 | 23476          | 667  |
| W2        | 81020      | 50489       | 59019      | 0                 | 22001          | 694  |
| W3        | 81654      | 47897       | 63612      | 0                 | 18042          | 632  |
| Q1        | 84261      | 37079       | 71120      | 0                 | 13141          | 615  |
| Q2        | 92397      | 47327       | 70363      | 0                 | 22034          | 732  |
| Q3        | 88479      | 46614       | 63998      | 0                 | 24481          | 679  |
| Avg       | 85445      | 46993       | 64916      | 0                 | 20529          | 669  |

Note: W, worker; Q, queen

Table S3 The common OTUs in queen bees and worker bees hindguts
| Otu | Worker | Queen | taxonomy |
|-----|--------|-------|----------|
| Otu00001 | 1947 | 9371 | Rootk_Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacteriales;f__Acetobacteraceae;g__Commensalibacter |
| Otu00002 | 2745 | 8555 | Rootk_Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacteriales;f__Acetobacteraceae;g__Commensalibacter |
| Otu00003 | 10326 | 931 | Rootk_Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Orbales;f__Orbaceae;g__Gilliamella |
| Otu00004 | 4398 | 616 | Rootk_Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae |
| Otu00005 | 843 | 4103 | Rootk_Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacteriales;f__Acetobacteraceae;g__Commensalibacter |
| Otu00006 | 2501 | 2421 | Rootk_Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus |
| Otu00007 | 1896 | 2178 | Rootk_Bacteria;p__Proteobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium |
| Otu00008 | 3672 | 327 | Rootk_Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Beta proteobacteriales;f__Neisseriaceae;g__Snodgrassella |
| Otu00009 | 3268 | 377 | Rootk_Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae |
| Otu00010 | 306 | 3053 | Rootk_Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus |
| Otu00011 | 1170 | 1897 | Rootk_Bacteria;p__Proteobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium |
| Otu00012 | 646 | 2418 | Rootk_Bacteria;p__Proteobacteria;c__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus |
| Otu00013 | 1040 | 1266 | Rootk_Bacteria;p__Proteobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium |
| Otu00014 | 416 | 1859 | Rootk_Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Orbales;f__Orbaceae;g__Gilliamella |
| Otu00015 | 1985 | 192 | Rootk_Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Orbales;f__Orbaceae;g__Gilliamella |
| Otu00016 | 1833 | 303 | Rootk_Bacteria;p__Proteobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium |
| Otu00017 | 593 | 1311 | Rootk_Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Orbales;f__Orbaceae;g__Frischella;h__Frischella_perrara |
| Otu00018 | 662 | 923 | Rootk_Bacteria;p__Proteobacteria;c__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus |
| Otu00019 | 1245 | 282 | Rootk_Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Acetobacteriales;f__Acetobacteraceae;g__Commensalibacter |
| Otu00020 | 89 | 1437 | Rootk_Bacteria;p__Proteobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium |
| Otu00021 | 61 | 1382 | Rootk_Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacteriales;f__Acetobacteraceae;g__Bombella |
| Otu00022 | 1252 | 132 | Rootk_Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Beta proteobacteriales;f__Neisseriaceae;g__Snodgrassella |
| Otu00023 | 139 | 1178 | Rootk_Bacteria;p__Proteobacteria;c__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus |
| Otu00024 | 136 | 1179 | Rootk_Bacteria;p__Proteobacteria;c__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus |
| Otu00025 | 224 | 1050 | Rootk_Bacteria;p__Proteobacteria;c__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus |
| Otu00026 | 245 | 989 | Rootk_Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacteriales;f__Acetobacteraceae;g__Commensalibacter |
| Otu00027 | 93 | 1128 | Rootk_Bacteria;p__Proteobacteria;c__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus |
| Otu00028 | 269 | 924 | Rootk_Bacteria;p__Proteobacteria;c__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus |
| Otu00029 | 480 | 684 | Rootk_Bacteria;p__Proteobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium |
| Otu00030 | 1005 | 101 | Rootk_Bacteria;p__Proteobacteria;c__Gamma proteobacteria;o__Orbales;f__Orbaceae;g__Gilliamella |
| Otu00031 | 971 | 90 | Rootk_Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Orbales;f__Orbaceae;g__Gilliamella |
| Otu00032 | 966 | 77 | Rootk_Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Orbales;f__Orbaceae;g__Gilliamella |
| Otu00033 | 156 | 852 | Rootk_Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacteriales;f__Acetobacteraceae;g__Commensalibacter |
| Otu00034 | 616 | 378 | Rootk_Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacteriales;f__Acetobacteraceae;g__Commensalibacter |
| Otu00035 | 900 | 61 | Rootk_Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Orbales;f__Orbaceae;g__Gilliamella |
| Otu00036 | 817 | 85 | Rootk_Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Orbales;f__Orbaceae;g__Gilliamella |
| Otu00037 | 408 | 485 | Rootk_Bacteria;p__Proteobacteria;c__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus |
| Otu00038 | 92 | 742 | Rootk_Bacteria;p__Proteobacteria;c__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus |
| Otu00039 | 649 | 167 | Rootk_Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Orbales;f__Orbaceae;g__Gilliamella |
| Otu00040 | 121 | 689 | Rootk_Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacteriales;f__Acetobacteraceae;g__Commensalibacter |
| Otu00041 | 121 | 647 | Rootk_Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacteriales;f__Acetobacteraceae;g__Commensalibacter |
| Otu00042 | 134 | 611 | Rootk_Bacteria;p__Proteobacteria;c__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus |
| Otu00043 | 686 | 52 | Rootk_Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Beta proteobacteriales;f__Neisseriaceae;g__Snodgrassella |
| OTU     | Rank         | Taxonomy                                                                 |
|---------|--------------|---------------------------------------------------------------------------|
| Otu000088 | 6            | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000089 | 5            | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000090 | 91           | Root_k_Bacteria;p_Proteobacteria;c_Gamma proteobacteria                    |
| Otu000091 | 2            | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000092 | 13           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000093 | 86           | Root_k_Bacteria;p_Proteobacteria;c_Gamma proteobacteria                    |
| Otu000094 | 2            | Root_k_Bacteria;p_Firmicutes;c_Alphaproteobacteria;o_Acetobacteralesf_Acetobacteraceae_g_Bombella |
| Otu000095 | 4            | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000096 | 60           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000097 | 3            | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000098 | 68           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000099 | 12           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000100 | 2            | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000101 | 15           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000102 | 65           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000103 | 29           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000104 | 15           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000105 | 28           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000106 | 12           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000107 | 10           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000108 | 3            | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000109 | 61           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000110 | 58           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000111 | 55           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000112 | 11           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000113 | 7            | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000114 | 38           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000115 | 8            | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000116 | 48           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000117 | 46           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000118 | 44           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000119 | 46           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000120 | 39           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000121 | 42           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000122 | 44           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000123 | 5            | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000124 | 39           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000125 | 10           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000126 | 10           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000127 | 5            | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000128 | 29           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000130 | 37           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000131 | 35           | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| Otu000132 | 5            | Root_k_Bacteria;p_Firmicutes;c_Bacillio_Lactobacillalesf_Lactobacillaceae_g_Lactobacillus |
| OTU | Percentage | Taxonomy |
|-----|------------|----------|
| Otu000133 | 18 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacterales, Enterobacteriaceae, Escherichia-Shigella |
| Otu000134 | 26 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Bacteroidales, Bacteroidaceae, Parabacteroides |
| Otu000135 | 33 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Betaproteobacteria, Neisseriaceae, Neisseria |
| Otu000136 | 4 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Acidobacteriales, Acidobacteriaceae, Acidobacterium |
| Otu000137 | 20 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Actinobacteria, Actinobacteriales, Actinobacteriaceae, Actinomyces |
| Otu000138 | 3 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000139 | 14 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacterales, Enterobacteriaceae, Escherichia-Shigella |
| Otu000140 | 15 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Entero bacteriales, Entero bacteriaceae, Escherichia-Shigella |
| Otu000141 | 25 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000142 | 4 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacterales, Enterobacteriaceae, Escherichia-Shigella |
| Otu000143 | 29 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacterales, Enterobacteriaceae, Escherichia-Shigella |
| Otu000144 | 29 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacterales, Enterobacteriaceae, Escherichia-Shigella |
| Otu000145 | 28 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000146 | 1 | 29 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacterales, Enterobacteriaceae, Escherichia-Shigella |
| Otu000147 | 1 | 29 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacterales, Enterobacteriaceae, Escherichia-Shigella |
| Otu000148 | 24 | 6 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000149 | 27 | 2 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacterales, Enterobacteriaceae, Escherichia-Shigella |
| Otu000150 | 5 | 24 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000151 | 24 | 5 | Root, Bacteria, Proteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000152 | 21 | 7 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000153 | 17 | 11 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000154 | 19 | 9 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000155 | 27 | 1 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000156 | 2 | 25 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000157 | 5 | 22 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000158 | 10 | 17 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000159 | 23 | 3 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000160 | 18 | 8 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000161 | 6 | 19 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000162 | 23 | 1 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000163 | 18 | 5 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000164 | 18 | 5 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000165 | 22 | 1 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000166 | 13 | 3 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000167 | 22 | 1 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000168 | 19 | 4 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000169 | 8 | 13 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000170 | 20 | 1 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000171 | 19 | 2 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000172 | 6 | 15 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000173 | 4 | 16 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000174 | 3 | 17 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000175 | 8 | 11 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000176 | 5 | 15 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000177 | 8 | 11 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000178 | 5 | 15 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000179 | 8 | 11 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu000180 | 2 | 25 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Rhizobium |
| Otu     | Rank    | Description                                                                 |
|---------|---------|-----------------------------------------------------------------------------|
| Otu000184 | 10 9 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacteriaceae, Lactobacillus, Lactobacillus_sp_SFT |
| Otu000185 | 18 1 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacteriaceae, Gilliamella |
| Otu000186 | 14 4 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacteriaceae, Pantoehaeae |
| Otu000187 | 7 11 | Root, Bacteria, Actinobacteria, Bifidobacteriaceae, Bifidobacterium |
| Otu000188 | 16 2 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacteriaceae, Gilliamella |
| Otu000189 | 2 16 | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus |
| Otu000190 | 2 15 | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus |
| Otu000192 | 14 3 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiales |
| Otu000193 | 7 10 | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus |
| Otu000195 | 7 9  | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus |
| Otu000196 | 1 15 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Acetobacteraceae, Commensalibacte |
| Otu000197 | 3 13 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Acetobacteraceae, Commensalibacte |
| Otu000199 | 6 10 | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus |
| Otu000200 | 1 15 | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus |
| Otu000201 | 15 1 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacteriaceae |
| Otu000205 | 3 12 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Acetobacteraceae, Commensalibacte |
| Otu000206 | 5 10 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Acetobacteraceae, Commensalibacte |
| Otu000211 | 1 13 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Acetobacteraceae, Commensalibacte |
| Otu000212 | 1 13 | Root, Bacteria, Firmicutes, Clostridia, Lachnospiraceae, Blautia, Lachnospiraceae, Blautia |
| Otu000213 | 1 13 | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus, Lactobacillus_kullab |
| Otu000214 | 2 12 | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus |
| Otu000215 | 13 1 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Orbaeae, Frischella, Frischella_perrara |
| Otu000216 | 6 8  | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus |
| Otu000217 | 13 1 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Orbaeae, Gilliamella |
| Otu000218 | 1 13 | Root, Bacteria, Bacteroidetes, Bacteroidia, Prevotellaceae, Prevotella_9s |
| Otu000219 | 6 8  | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus |
| Otu000220 | 9 4  | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus |
| Otu000221 | 1 12 | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus |
| Otu000222 | 6 7  | Root, Bacteria, Actinobacteria, Actinobacteria, Bifidobacteriaceae, Bifidobacterium |
| Otu000223 | 2 11 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Acetobacteraceae, Commensalibacte |
| Otu000224 | 10 3 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Orbaeae, Frischella, Frischella_perrara |
| Otu000228 | 12 1 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Orbaeae, Gilliamella |
| Otu000229 | 12 1 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Orbaeae, Gilliamella |
| Otu000230 | 3 9  | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Acetobacteraceae, Commensalibacte |
| Otu000231 | 6 6  | Root, Bacteria, Actinobacteria, Bifidobacteriaceae, Bifidobacterium |
| Otu000233 | 11 1 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Betaproteobacteria, Neisseriaceae, Snodgrassella |
| Otu000234 | 4 8  | Root, Bacteria |
| Otu000235 | 10 2 | Root, Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiales |
| Otu000236 | 10 2 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Orbaeae, Gilliamella |
| Otu000237 | 3 9  | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus |
| Otu000239 | 10 1 | Root, Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacteriaceae |
| Otu000240 | 1 11 | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus, Lactobacillus_sp_Aatl |
| Otu000241 | 3 9  | Root, Bacteria, Actinobacteria, Bifidobacteriaceae, Bifidobacteriaceae, Bifidobacterium |
| Otu000242 | 8 3  | Root, Bacteria, Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus |
| OTU ID | Rank     | Category                        | Description                                          |
|--------|----------|---------------------------------|------------------------------------------------------|
| Otu000244 | 3 | 9 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales|f_Acetobacteraceae|g_Caenobacterales|s_Lactobacillus |
| Otu000245 | 1 | 10 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales|f_Acetobacteraceae|g_Caenobacterales|s_Lactobacillus |
| Otu000246 | 2 | 9 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales|f_Acetobacteraceae|g_Caenobacterales|s_Lactobacillus |
| Otu000247 | 1 | 10 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales|f_Acetobacteraceae|g_Caenobacterales|s_Lactobacillus |
| Otu000248 | 1 | 10 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000250 | 3 | 7 | Root_k_Bacteria;p_Actinobacteria;c_Acinetobacteriales|f_Bifidobacteriaceae|g_Bifidobacterium |
| Otu000251 | 3 | 8 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000252 | 3 | 8 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales|f_Acetobacteraceae|g_Caenobacterales|s_Lactobacillus |
| Otu000253 | 2 | 8 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales|f_Acetobacteraceae|g_Caenobacterales|s_Lactobacillus |
| Otu000255 | 10 | 1 | Root_k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Betaproteobacteriales|f_Neisseriaaceae|g_Snodgrassella |
| Otu000256 | 3 | 8 | Root_k_Bacteria;p_Firmicutes;c_Negativicutes|o_Selenomonadales|f_Vellionellaceae|g_Megaphera |
| Otu000257 | 4 | 6 | Root_k_Bacteria;p_Actinobacteria;c_Acinetobacteriales|f_Bifidobacteriaceae|g_Bifidobacterium |
| Otu000259 | 6 | 4 | Root_k_Bacteria |
| Otu000260 | 2 | 8 | Root_k_Bacteria;p_Actinobacteria;c_Acinetobacteriales|f_Bifidobacteriaceae|g_Bifidobacterium |
| Otu000262 | 3 | 6 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000268 | 4 | 6 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000269 | 4 | 5 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000271 | 1 | 8 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000278 | 1 | 8 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales|f_Acetobacteraceae|g_Caenobacterales |
| Otu000279 | 8 | 1 | Root_k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Orbales|f_Orbaceae|g_Gilliamella |
| Otu000280 | 7 | 2 | Root_k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Orbales|f_Orbaceae|g_Frischella |
| Otu000282 | 3 | 5 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000287 | 3 | 5 | Root_k_Bacteria;p_Actinobacteria;c_Acinetobacteriales|f_Bifidobacteriaceae|g_Bifidobacterium |
| Otu000289 | 7 | 1 | Root_k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Betaproteobacteriales|f_Neisseriaaceae|g_Snodgrassella |
| Otu000292 | 7 | 1 | Root_k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Orbales|f_Orbaceae|g_Gilliamella |
| Otu000293 | 1 | 7 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000294 | 7 | 1 | Root_k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Orbales|f_Orbaceae|g_Gilliamella |
| Otu000297 | 1 | 7 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000298 | 2 | 6 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000302 | 7 | 1 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Streptococcaecae|g_Streptococcus|s_Gallis |
| Otu000303 | 6 | 2 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000304 | 2 | 5 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000308 | 4 | 3 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales|f_Acetobacteraceae|g_Caenobacterales |
| Otu000309 | 2 | 6 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000311 | 5 | 2 | Root_k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Orbales|f_Orbaceae|g_Frischella |
| Otu000313 | 2 | 6 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales|f_Acetobacteraceae|g_Caenobacterales |
| Otu000318 | 1 | 6 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000320 | 1 | 6 | Root_k_Bacteria;p_Epsilonbacteriota;c_Campylobacteriota;c_Campylobacteriota|f_Campylobacteraceae|g_Campylobacteriota |
| Otu000322 | 2 | 5 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000324 | 3 | 4 | Root_k_Bacteria;p_Firmicutes;c_Bacillio_lactobacillales|f_Lactobacillaceae|g_Lactobacillus |
| Otu000326 | 2 | 5 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales|f_Acetobacteraceae|g_Caenobacterales |
| Otu000327 | 1 | 5 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales|f_Acetobacteraceae|g_Caenobacterales |
| Otu000328 | 4 | 2 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales|f_Acetobacteraceae|g_Caenobacterales |
| Otu000329 | 4 | 2 | Root_k_Bacteria |
| Otu000330 | 1  | 5 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus_s_Lactobacillus_sp_SF6 |
| Otu000331 | 1  | 5 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales:f_Acetobacteraceae_g_Consensalbacte |
| Otu000337 | 1  | 5 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus_s_Lactobacillus_apis |
| Otu000338 | 1  | 5 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000342 | 5  | 1 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales:f_Acetobacteraceae_g_Consensalbacte |
| Otu000344 | 1  | 4 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales:f_Acetobacteraceae_g_Consensalbacte |
| Otu000346 | 3  | 3 | Root_k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales:f_Bifidobacteriaceae_g_Bifidobacterium |
| Otu000347 | 3  | 3 | Root_k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales:f_Bifidobacteriaceae_g_Bifidobacterium |
| Otu000348 | 3  | 3 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000351 | 1  | 4 | Root_k_Bacteria;p_Proteobacteria;o_Acetobacterales:f_Acetobacteraceae_g_Consensalbacte |
| Otu000354 | 4  | 1 | Root_k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Orbales:f_Orbaceae_g_Gilliamella_s_Gilliamella_apicola |
| Otu000356 | 4  | 1 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000357 | 2  | 3 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales:f_Acetobacteraceae_g_Consensalbacte |
| Otu000363 | 1  | 4 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000365 | 1  | 4 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales:f_Acetobacteraceae_g_Consensalbacte |
| Otu000366 | 2  | 3 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000367 | 4  | 1 | Root_k_Bacteria |
| Otu000375 | 1  | 4 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus_s_Lactobacillus_sp_SF6 |
| Otu000376 | 1  | 4 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000382 | 1  | 3 | Root_k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Micrococcales:f_Intrasporangiaceae |
| Otu000383 | 1  | 3 | Root_k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Orbales:f_Orbaceae |
| Otu000385 | 1  | 3 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus_s_Lactobacillus_sp_SF6 |
| Otu000389 | 1  | 3 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000391 | 1  | 3 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000395 | 1  | 3 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales:f_Acetobacteraceae_g_Consensalbacte |
| Otu000398 | 1  | 3 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000400 | 3  | 1 | Root_k_Bacteria |
| Otu000401 | 3  | 1 | Root_k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Orbales:f_Orbaceae_g_Gilliamella |
| Otu000402 | 3  | 1 | Root_k_Bacteria;p_Proteobacteria |
| Otu000403 | 3  | 1 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales:f_Rhizobiacae |
| Otu000405 | 2  | 2 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000407 | 1  | 2 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000408 | 1  | 3 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales:f_Acetobacteraceae_g_Consensalbacte |
| Otu000411 | 1  | 3 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales:f_Acetobacteraceae_g_Consensalbacte |
| Otu000415 | 1  | 3 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000426 | 3  | 1 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000428 | 1  | 3 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus_s_Lactobacillus_sp_SF6 |
| Otu000433 | 3  | 1 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus |
| Otu000434 | 1  | 2 | Root_k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales:f_Lactobacillaceae_g_Lactobacillus_s_Lactobacillus_sp_SF6 |
| Otu000435 | 1  | 3 | Root_k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales:f_Lachnospiraceae |
| Otu000436 | 1  | 3 | Root_k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales:f_Acetobacteraceae_g_Consensalbacte |
| Otu000437 | 2  | 1 | Root_k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales:f_Bifidobacteriaceae_g_Bifidobacterium_s_Bifi |
| Otu000438 | 1  | 3 | Root_k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales:f_Bifidobacteriaceae_g_Bifidobacterium |
| Otu000439 | 1  | 2 | Root_k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales:f_Bifidobacteriaceae_g_Bifidobacterium |
| OTU          | Value | Taxonomy                                                                 |
|-------------|-------|--------------------------------------------------------------------------|
| OTu000444   | 1     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000445   | 1     | Rootk_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales;f_Acetobacteraceae;g_Clostridium |  
| OTu000447   | 2     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000450   | 1     | Rootk_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales;f_Acetobacteraceae;g_Clostridium |  
| OTu000451   | 2     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000452   | 1     | Rootk_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Betaproteobacteria;f_Bifidobacteriales;g_Bifidobacterium |  
| OTu000456   | 2     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000462   | 2     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000464   | 1     | Rootk_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales;f_Acetobacteraceae;g_Clostridium |  
| OTu000465   | 2     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000472   | 1     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000478   | 2     | Rootk_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales;f_Acetobacteraceae;g_Clostridium |  
| OTu000480   | 1     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000481   | 2     | Rootk_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales;f_Acetobacteraceae;g_Clostridium |  
| OTu000490   | 2     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000491   | 2     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000496   | 2     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000504   | 1     | Rootk_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales;f_Acetobacteraceae;g_Clostridium |  
| OTu000509   | 2     | Rootk_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales;f_Acetobacteraceae;g_Clostridium |  
| OTu000514   | 1     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000521   | 1     | Rootk_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales;f_Acetobacteraceae;g_Clostridium |  
| OTu000525   | 1     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000527   | 1     | Rootk_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales;f_Acetobacteraceae;g_Clostridium |  
| OTu000538   | 1     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000542   | 1     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000543   | 1     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000545   | 1     | Rootk_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales;f_Acetobacteraceae;g_Clostridium |  
| OTu000557   | 1     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000568   | 1     | Rootk_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales;f_Acetobacteraceae;g_Clostridium |  
| OTu000570   | 1     | Rootk_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales;f_Bifidobacteriaceae;g_Bifidobacterium |  
| OTu000588   | 1     | Rootk_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Acetobacterales;f_Acetobacteraceae;g_Clostridium |  
| OTu000617   | 1     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  
| OTu000618   | 1     | Rootk_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;sp_SF6 |  

Table S4 The worker bee-specific OTUs
| Otu       | Worker | taxonomy                                                                 |
|----------|--------|--------------------------------------------------------------------------|
| Otu000128| 38     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; |  f\_Orbaceae |
| Otu000161| 25     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; |  f\_Orbaceae |
| Otu000164| 23     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Betaproteobacteriales; f\_Neisseriaceae |
| Otu000170| 22     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria |
| Otu000171| 21     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria |
| Otu000173| 21     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Betaproteobacteriales |
| Otu000180| 20     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Betaproteobacteriales; f\_Neisseriaceae |
| Otu000183| 19     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria |
| Otu000194| 17     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Betaproteobacteriales; f\_Neisseriaceae; g\_Snodgrassella |
| Otu000198| 16     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Alphaproteobacteria; o\_Rhizobiales; f\_Rhizobiaceae |
| Otu000203| 15     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae |
| Otu000204| 15     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae |
| Otu000208| 14     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae; g\_Frischella; s\_Frischella_perrara |
| Otu000210| 15     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria |
| Otu000225| 13     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Betaproteobacteriales; f\_Neisseriaceae; g\_Snodgrassella; s\_Snodg |
| Otu000226| 13     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria |
| Otu000238| 12     | Root\_K\_Bacteria |
| Otu000243| 11     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae; g\_Gilliamella; s\_ |
| Otu000249| 10     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae |
| Otu000258| 10     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria |
| Otu000265| 9      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae; g\_Gilliamella; s\_ |
| Otu000266| 9      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Betaproteobacteriales; f\_Neisseriaceae; g\_Snodgrassella; s\_Snodg |
| Otu000267| 10     | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Betaproteobacteriales; f\_Neisseriaceae; g\_Snodgrassella; s\_Snodg |
| Otu000270| 9      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae; g\_Gilliamella; s\_ |
| Otu000271| 9      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae; g\_Frischella; s\_Frischella_perrara |
| Otu000272| 9      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae; g\_Gilliamella |
| Otu000274| 9      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae; g\_Gilliamella |
| Otu000276| 8      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Alphaproteobacteria; o\_Rhizobiales; f\_Rhizobiaceae |
| Otu000283| 8      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae; g\_Gilliamella |
| Otu000286| 8      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Betaproteobacteriales; f\_Neisseriaceae; g\_Snodgrassella; s\_Snodg |
| Otu000291| 8      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria |
| Otu000296| 8      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Betaproteobacteriales; f\_Neisseriaceae; g\_Snodgrassella; s\_Snodg |
| Otu000301| 8      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae; g\_Gilliamella |
| Otu000305| 7      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae; g\_Frischella; s\_Frischella_perrara |
| Otu000307| 7      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Betaproteobacteriales; f\_Neisseriaceae; g\_Snodgrassella; s\_Snodg |
| Otu000310| 7      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae; g\_Gilliamella |
| Otu000312| 7      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae |
| Otu000314| 7      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Betaproteobacteriales; f\_Neisseriaceae |
| Otu000315| 7      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria |
| Otu000316| 7      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae; g\_Gilliamella |
| Otu000319| 7      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria; o\_Orbales; f\_Orbaceae |
| Otu000321| 7      | Root\_K\_Bacteria; p\_Proteobacteria; c\_Gammaproteobacteria |
| Otu000323| 7      | Root\_K\_Bacteria |
| OTU            | Identified Taxonomy                                                                 |
|---------------|-----------------------------------------------------------------------------------|
| Otu000992     | Root; k__Bacteria                                                                  |
| Otu001000     | Root; k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Orbales; f__Orbaceae; g__Frischella; s__Frischella_perrara |
| Otu001001     | Root; k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Orbales; f__Orbaceae |

Table S5 The queen bee-specific OTUs
| Otu   | Queen taxonomy                                                                 |
|-------|-------------------------------------------------------------------------------|
| Otu000191 | 17 Rootk_Bacteria,p_Bacteroidetes,c_Bacteroidia,o_Bacteroidiales,f_Bacteroidaceae,g_Bacteroides |
| Otu000202 | 15 Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacterales,f_Acetobacteraceae |
| Otu000207 | 14 Rootk_Bacteria,p_Proteobacteria,c_Gammaproteobacteria,o_Betaproteobacterales,f_Burkholderiaceae,g_Curvibacter |
| Otu000209 | 15 Rootk_Bacteria,p_Cyanobacteria,c_Oxyphotobacteria,o_Chloroplast,f_Selaginella_moellendorfii,g_s_ |
| Otu000227 | 13 Rootk_Bacteria,p_Firmicutes,c_Cacillio_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus,s_Lactobacillus_kunkeei |
| Otu000232 | 12 Rootk_Bacteria,p_Cyanobacteria,c_Oxyphotobacteria,o_Chloroplast |
| Otu000254 | 11 Rootk_Bacteria,p_Bacteroidetes,c_Bacteroidia,o_Bacteroidiales,f_Bacteroidaceae,g_Bacteroides,s_ |
| Otu000261 | 10 Rootk_Bacteria,p_Firmicutes,c_Bacillio_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus |
| Otu000263 | 10 Rootk_Bacteria,p_Bacteroidetes,c_Bacteroidia,o_Bacteroidiales,f_Bacteroidaceae,g_Bacteroides,s_Bacteroides_stercoris_ATCC, |
| Otu000264 | 9 Rootk_Bacteria,p_Proteobacteria,c_Gammaproteobacteria,o_Pseudomonadales,f_Moraxellaceae,g_Acinetobacter,s_ |
| Otu000273 | 9 Rootk_Bacteria,p_Proteobacteria,c_Gammaproteobacteria,o_Pseudomonadales,f_Pseudomonadaceae,g_Pseudomonas |
| Otu000276 | 9 Rootk_Bacteria,p_Firmicutes,c_Bacillio_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus |
| Otu000277 | 9 Rootk_Bacteria,p_Bacteroidetes,c_Bacteroidia,o_Bacteroidiales,f_Bacteroidaceae,g_Bacteroides,s_Bacteroides_massiliensis |
| Otu000284 | 9 Rootk_Bacteria,p_Gemmationadetes,c_Gemmationadetes,c_Gemmationadates,o_Gemmationadales,f_Gemmationadaceae,g_s_ |
| Otu000285 | 8 Rootk_Bacteria,p_Firmicutes,c_Negativicutes,o_Selenomondales,f_Acidaminococcaceae,g_Phascolarctobacterium |
| Otu000288 | 8 Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacterales,f_Acetobacteraceae,g_Combinsalibacter,s_ |
| Otu000290 | 8 Rootk_Bacteria,p_Planctomycetes,c_Planctomycetacia,o_Gemmatales,f_Gemmataceae,g_s_ |
| Otu000295 | 8 Rootk_Bacteria,p_Acidobacteria,c_Subgroup_60,f_g_s_ |
| Otu000299 | 8 Rootk_Bacteria,p_Proteobacteria,c_Gammaproteobacteria,o_Pseudomonadales,f_Moraxellaceae,g_Enhydrobacter,s_ |
| Otu000300 | 8 Rootk_Bacteria,p_Bacteroidetes,c_Bacteroidia,o_Bacteroidiales,f_Bacteroidaceae,g_Bacteroides |
| Otu000306 | 8 Rootk_Bacteria,p_Firmicutes,c_Clostridio_o_Clostridiales,f_Ruminococcaceae,g_Subdoligranulum,s_ |
| Otu000317 | 7 Rootk_Bacteria,p_Bacteroidetes,c_Bacteroidia,o_Bacteroidiales,f_Prevotellaceae_g_Prevotella_9a_ |
| Otu000332 | 6 Rootk_Bacteria,p_Proteobacteria,c_Gammaproteobacteria,o_Pseudomonadales,f_Pseudomonadaceae |
| Otu000333 | 6 Rootk_Bacteria,p_Actinobacteria,c_Actinobacteria,o_Micrococcales,f_Micrococcaceae |
| Otu000335 | 6 Rootk_Bacteria,p_Chloroflexi,c_Anaerolineae_g_Anaerolineales,f_Anaerolineaceae,g_s_ |
| Otu000343 | 5 Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacterales,f_Acetobacteraceae,g_Combinsalibacter,s_ |
| Otu000352 | 5 Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacterales,f_Acetobacteraceae,g_Combinsalibacter,s_ |
| Otu000358 | 5 Rootk_Bacteria,p_Planctomycetes,c_Physicisthea,o_Physicistheales,f_Physicistheaeae,g_s_ |
| Otu000360 | 5 Rootk_Bacteria,p_Bacteroidetes,c_Bacteroidia,o_Bacteroidiales,f_Bacteroidaceae,g_Bacteroides |
| Otu000361 | 5 Rootk_Bacteria,p_Firmicutes,c_Bacillio_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus |
| Otu000364 | 5 Rootk_Bacteria,p_Firmicutes,c_Bacillio_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus |
| Otu000377 | 4 Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacterales,f_Acetobacteraceae,g_Combinsalibacter,s_ |
| Otu000378 | 5 Rootk_Bacteria,p_Proteobacteria,c_Deltaproteobacteria,o_Desulfuromondales,f_Geobacterales,g_Geobacter |
| Otu000379 | 5 Rootk_Bacteria,p_Actinobacteria,c_Coriobacteria,o_Coriobacteriaeae,g_Collinsella,s_ |
| Otu000380 | 5 Rootk_Bacteria,p_Firmicutes,c_Clostridio_o_Clostridiales,f_Lachnospiraceae,g_Eubacterium_hallii,g_s_ |
| Otu000381 | 5 Rootk_Bacteria,p_Firmicutes,c_Clostridio_o_Clostridiales,f_Lachnospiraceae,g_Blautia |
| Otu000386 | 4 Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Caulobacterales,f_Caulobacteraceae,g_Brevundimonas |
| Otu000387 | 5 Rootk_Bacteria,p_Actinobacteria,c_Actinobacteria,o_Proponibacterales,f_Nocardiaoidaceae,g_Nocardioides |
| Otu000390 | 4 Rootk_Bacteria,p_Firmicutes,c_Bacillio_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus |
| Otu000392 | 4 Rootk_Bacteria,p_Firmicutes,c_Bacillio_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus,s_Lactobacillus_sp_SF6D |
| Otu000396 | 4 Rootk_Bacteria,p_Firmicutes,c_Clostridio_o_Clostridiales,f_Lachnospiraceae,g_Ruminococcus_torques_group |
| Otu000399 | 4 Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacterales,f_Acetobacteraceae,g_Combinsalibacter,s_ |
| Otu000409 | 3 Rootk_Bacteria,p_Firmicutes,c_Bacillio_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus |
Root\_Bacteria;p\_Bacteroidetes;c\_Bacteroidia;o\_Bacteroidales;f\_Bacteroidaceae;g\_Bacteroides;s\_Bacteroides_caccae

Root\_Bacteria;p\_Proteobacteria;c\_Gammaproteobacteria;o\_Betaproteobacteriales;f\_Burkholderiaceae;g\_Parasutterella;s__

Root\_Bacteria;p\_Firmicutes;c\_Clostridia;o\_Clostridiales;f\_Ruminococcaceae;g\_Ruminococcaceae_UCG-005;s__

Root\_Bacteria;p\_Proteobacteria;c\_Alphaproteobacteria;o\_Acetobacterales;f\_Acetobacteraceae;g\_Commensalibacter;s__

Root\_Bacteria;p\_Bacteroidetes;c\_Bacteroidia;o\_Bacteroidales;f\_Bacteroidaceae;g\_Bacteroides;s\_Bacteroides_fragilis

Root\_Bacteria;p\_Firmicutes;c\_Bacilli;o\_Lactobacillales;f\_Lactobacillaceae;g\_Lactobacillus

Root\_Bacteria;p\_Proteobacteria;c\_Alphaproteobacteria;o\_Acetobacterales;f\_Acetobacteraceae;g\_Commensalibacter;s__

Root\_Bacteria;p\_Proteobacteria;c\_Alphaproteobacteria;o\_Acetobacterales;f\_Acetobacteraceae;g\_Commensalibacter;s__

Root\_Bacteria;p\_Proteobacteria;c\_Alphaproteobacteria;o\_Acetobacterales;f\_Acetobacteraceae;g\_Commensalibacter;s__

Root\_Bacteria;p\_Proteobacteria;c\_Alphaproteobacteria;o\_Acetobacterales;f\_Acetobacteraceae;g\_Commensalibacter;s__
Otu000602 2  Rootk_Bacteria,p_Firmicutes,c_Clostridia,o_Clostridiales,f_Lachnospiraceae

Otu000603 1  Rootk_Bacteria,p_Actinobacteria,c_Actinobacteria,o_Bifidobacteriales,f_Bifidobacteriaceae,g_Bifidobacterium

Otu000606 1  Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacteriales,f_Acetobacteriaceae,g_Commensalibacter,s_

Otu000607 2  Rootk_Bacteria,p_Firmicutes,c_Bacilli,o_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus

Otu000608 2  Rootk_Bacteria,p_Bacteroidetes,c_Bacteroidia,o_Bacteroidales,f_Prevotellaceae

Otu000610 2  Rootk_Bacteria,p_Proteobacteria,c_Gamma proteobacteria,o_Pseudomonadales,f_Moraxellaceae,g_Acinetobacter,s_Acinetobacter

Otu000611 2  Rootk_Bacteria,p_Bacteroidetes,c_Bacteroidia,o_Flavobacteriales,f_Flavobacteriaceae,g_Flavobacterium

Otu000612 2  Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacteriales,f_Acetobacteriaceae,g_Commensalibacter,s_

Otu000613 2  Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacteriales,f_Acetobacteriaceae,g_Commensalibacter,s_

Otu000615 1  Rootk_Bacteria,p_Firmicutes,c_Clostridia,o_Clostridiales,f_Lachnospiraceae

Otu000616 1  Rootk_Bacteria,p_Firmicutes,c_Bacilli,o_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus apis

Otu000622 2  Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacteriales,f_Acetobacteriaceae,g_Commensalibacter,s_

Otu000623 1  Rootk_Bacteria,p_Bacteroidetes,c_Bacteroidia,o_Bacteroidales,f_Bacteroidaceae,g_Bacteroides,s_Bacteroides coprophilus_DS

Otu000624 2  Rootk_Bacteria,p_Firmicutes,c_Bacilli,o_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus

Otu000627 2  Rootk_Bacteria,p_Firmicutes,c_Clostridia,o_Clostridiales,f_Lachnospiraceae,g_Lachnospiraceae_NK4A136_group,s_

Otu000628 2  Rootk_Bacteria,p_Actinobacteria,c_Actinobacteria,o_Micrococcales,f_Intrasporangiae

Otu000629 2  Rootk_Bacteria,p_Clostridia,o_Clostridiales,f_Christensenellaceae,g_Christensenellaceae_R-7_group,s_

Otu000630 2  Rootk_Bacteria,p_Planctomycetes,c_Planctomycetacia,o_Gemmatales,f_Gemmataceae,g_Gemmata,s_

Otu000631 1  Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacteriaceae,f_Acetobacteraceae,g_Commensalibacter,s_

Otu000632 2  Rootk_Bacteria,p_Proteobacteria,c_Deltaproteobacteria,o_Myxococcales,f_Blii41,g_s_

Otu000633 2  Rootk_Bacteria,p_Firmicutes,c_Bacilli,o_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus apis

Otu000634 1  Rootk_Bacteria,p_Firmicutes,c_Bacilli,o_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus

Otu000635 1  Rootk_Bacteria,p_Firmicutes,c_Bacilli,o_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus apis

Otu000636 1  Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacteriales,f_Acetobacteraceae,g_Commensalibacter

Otu000638 1  Rootk_Bacteria,p_Bacteroidetes,c_Bacteroidia,o_Bacteroidales,f_Rikenellaceae,g_Alistipes

Otu000639 1  Rootk_Bacteria,p_Firmicutes,c_Bacilli,o_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus

Otu000640 1  Rootk_Bacteria,p_Firmicutes,c_Bacilli,o_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus

Otu000643 1  Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacteriaceae,f_Acetobacteraceae,g_Commensalibacter,s_

Otu000644 1  Rootk_Bacteria,p_Actinobacteria,c_Actinobacteria,o_Bifidobacteriales,f_Bifidobacteriaceae,g_Bifidobacterium

Otu000645 1  Rootk_Bacteria,p_Firmicutes,c_Bacilli,o_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus

Otu000646 1  Rootk_Bacteria,p_Actinobacteria,c_MB-A2-108,o_f,g_s_

Otu000648 1  Rootk_Bacteria,p_Proteobacteria,c_Gamma proteobacteria,o_CCD24,f_g_s_

Otu000649 1  Rootk_Bacteria,p_Firmicutes,c_Clostridia,o_Clostridiales,f_Ruminococcaceae,g_Ruminococcaceae_UCG-002,s_

Otu000650 1  Rootk_Bacteria,p_Actinobacteria,c_Actinobacteria,o_Pseudonocardiaceae,f_Pseudonocardiaceae,g_Pseudonocardia

Otu000653 1  Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Orbales,f_Orbaceae,g_Gilliamellales,s_Gilliamella apicola

Otu000658 1  Rootk_Bacteria,p_Verrucomicrobia,c_Verrucomicrobiaceae,g_Verrucomicrobiaceae,g_Akkermansiacaeae,g_Akkermansia,s_

Otu000659 1  Rootk_Bacteria,p_Actinobacteria,c_Bifidobacteriaceae,f_Bifidobacteriaceae,g_Bifidobacterium

Otu000660 1  Rootk_Bacteria,p_Actinobacteria,c_Coriobacteria,o_Coriobacteriales,f_Eggerthellaceae,g_Eggerthella,s_

Otu000662 1  Rootk_Bacteria,p_Firmicutes,c_Bacilli,o_Lactobacillales,f_Lactobacillaceae,g_Lactobacillus_sp_Adhirnto19

Otu000664 1  Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacteriales,f_Acetobacteriaceae,g_Commensalibacter,s_

Otu000665 1  Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacteriales,f_Acetobacteriaceae,g_Commensalibacter,s_

Otu000667 1  Rootk_Bacteria,p_Proteobacteria,c_Alphaproteobacteria,o_Acetobacteriales,f_Acetobacteriaceae,g_Commensalibacter,s_

Otu000668 1  Rootk_Bacteria,p_Actinobacteria,c_Actinobacteria,o_Micrococcales,f_Micrococcales,g_Kocuria,s_Kocuria_palustris

Otu000669 1  Rootk_Bacteria,p_Proteobacteria
| OTU       | Relative Abundance | Taxonomy                                                                 |
|-----------|--------------------|--------------------------------------------------------------------------|
| Otu000926 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Alphaproteobacteria,o__Acetobacterales,f__Acetobacteraceae,g__Commensalibacter |
| Otu000932 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Rhodobacterales,f__Rhodobacteraceae,g__HIMB11,s__ |
| Otu000933 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Betaproteobacteria,o__Bacteroidiales,f__Burkholderiaceae,g__Pelomonas,s__ |
| Otu000936 | 1                  | Rootk_Bacteria,p__Halanaerobacterales,o__Halanaerobiales,f__Halanaerobeae,g__Halanaerobium,s__Halanaerobi |
| Otu000940 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Alphaproteobacteria,o__Acetobacterales,f__Acetobacteraceae,g__Commensalibacter |
| Otu000941 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Clostridia,o__Clostridiales,f__Lachnospiraceae,g__Coprococcus_1,s__ |
| Otu000942 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Bacillio_Lactobacillales,f__Lactobacillaceae,g__Lactobacillus |
| Otu000943 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Acetobacterales,f__Acetobacteraceae,g__Commensalibacter,s__ |
| Otu000944 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Bacillio_Lactobacillales,f__Lactobacillaceae,g__Lactobacillus |
| Otu000945 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Bacillio_Lactobacillales,f__Lactobacillaceae,g__Lactobacillus |
| Otu000947 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Alphaproteobacteria,o__Acetobacterales,f__Acetobacteraceae,g__Bombella,s__Bombella_intes |
| Otu000950 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Bacillio_Lactobacillales,f__Lactobacillaceae,g__Lactobacillus_sp_SF6D |
| Otu000951 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Alphaproteobacteria,o__Acetobacterales,f__Acetobacteraceae,g__Commensalibacter,s__ |
| Otu000954 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Bacillio_Lactobacillales,f__Lactobacillaceae,g__Lactobacillus |
| Otu000957 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Clostridia,o__Clostridiales,f__Lachnospiraceae,g__Anaerostipes |
| Otu000960 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Gammaproteobacteria,o__Pseudomonadales,f__Moraxellaceae,g__Acinetobacter,s__Acinetoba |
| Otu000967 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Clostridia,o__Clostridiales,f__Lachnospiraceae,g__Coprococcus_3,s__ |
| Otu000973 | 1                  | Rootk_Bacteria,p__Bacteroidetes,c__Bacteroidia,o__Bacteroidales,f__Bacteroidiaceae,g__Bacteroides |
| Otu000974 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Clostridia,o__Clostridiales,f__Lachnospiraceae,g__Eisenbergiella,s__ |
| Otu000976 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Alphaproteobacteria,o__Acetobacteraceae,f__Acetobacteraceae |
| Otu000982 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Clostridia,o__Clostridiales,f__Ruminococcaceae,g__Ruminococcus_2 |
| Otu000985 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Alphaproteobacteria,o__Acetobacteraceae,f__Acetobacteraceae,g__Commensalibacter,s__ |
| Otu000986 | 1                  | Rootk_Bacteria,p__Bacteroidetes,c__Bacteroidia,o__Bacteroidales,f__Prevotellaceae,g__Prevotella_1,s__ |
| Otu000987 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Bacillio_Lactobacillales,f__Lactobacillaceae,g__Lactobacillus |
| Otu000988 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Alphaproteobacteria,o__Rhizobiales,f__Rhizobiales,g__Ochrobactrum |
| Otu000989 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Bacillio_Lactobacillales,f__Lactobacillaceae,g__Lactobacillus |
| Otu000990 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Alphaproteobacteria,o__Bifidobacteriales,f__Bifidobacteriaceae,g__Bifidobacterium |
| Otu000991 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Gammaproteobacteria,o__Bacteroidiales,f__Burkholderiaceae,g__Massilia,s__ |
| Otu000993 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Bacillio_Lactobacillales,f__Enterococcaceae,g__Enterococcus |
| Otu000996 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Bacillio_Lactobacillales,f__Lactobacillaceae,g__Lactobacillus |
| Otu000997 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Bacillio_Bacillales,f__Paenibacillaceae,g__Brevibacillus |
| Otu000998 | 1                  | Rootk_Bacteria,p__Bacteroidetes,c__Bacteroidia,o__Bacteroidales,f__Prevotellaceae,g__Paraprevotella,s__ |
| Otu000999 | 1                  | Rootk_Bacteria,p__Firmicutes,c__Bacillio_Lactobacillales,f__Lactobacillaceae,g__Lactobacillus |
| Otu001003 | 1                  | Rootk_Bacteria,p__Bacteroidetes,c__Bacteroidia,o__Bacteroidales,f__Verrucomicrobiae,g__Verrucomicrobiales,f__Akermansia,g__Akermansia,s__ |
| Otu001006 | 1                  | Rootk_Bacteria,p__Bacteroidetes,c__Bacteroidia,o__Bacteroidales,f__Rikenellaceae,g__Rikenellaceae_RC9_gut_group,s__ |
| Otu001007 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Alphaproteobacteria,o__Elsterales,f__g__s__ |
| Otu001008 | 1                  | Rootk_Bacteria,p__Proteobacteria,c__Alphaproteobacteria,o__Acetobacterales,f__Acetobacteraceae,g__Commensalibacter,s__ |

**Figures**
Figure 1

Diversity differences in the gut microflora between workers and queens. A. Principal coordinate analysis (PCoA) plot based on Bray-Curtis similarity for the worker and queen samples. B. Analysis of similarities (ANOSIM) testing based on unweighted UniFrac was used to evaluate the intergroup (workers and queens) and intragroup (workers or queens) distances. $R$, interpretation degree of the different groups in the sample differences. $R=(\text{the between-group variance})/(\text{total variance})$. C. Sample distance analysis based on the Bray-Curtis distance index. Larger indices indicate greater distances between samples.
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Figure 2
Numbers of OTUs in the worker and queen guts.
Figure 3

Gut microbial abundance and taxonomic distribution of the workers and queens. A. phylum level; B. genus level; C. species level. Column diagram, relative abundances of microflora. Heatmap, relative abundances normalized by z-score.
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Figure 4

Main biomarker taxa of the microbiotas in the worker and queen guts. A. LEfSe analysis (taxa with LDA scores >4). B. Cladogram of the biomarker main taxa of the microbiotas based on LEfSe analysis. Yellow (B) represents no significant difference in taxa; green (A and B) represents significantly different taxa with the highest relative abundances in worker guts; red (A and B) represents significantly different taxa, with their highest relative abundances in the queen gut.
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Figure 5

Functional differences between worker and queen intestinal microbial communities predicted using Tax4Fun. A. Levels 1 and 2. * represents significant differences (P<0.05); B. Level 3 (P<0.05).
Figure 5

Functional differences between worker and queen intestinal microbial communities predicted using Tax4Fun. A. Levels 1 and 2. * represents significant differences (P<0.05); B. Level 3 (P<0.05).
Figure 6

Correlation between caste-specific taxa and functions using Pearson correlation analysis. A. Correlation heatmap of caste-specific taxa and functions at level 2. B. Correlation network of caste-specific taxa and functions at level 3 from the functions at level 2, which are significantly correlated with the caste-specific taxa (A). Violet node size indicates the average relative abundances of the microorganisms. Blue node size indicates the average relative abundances of the functions. Lines linked to nodes indicate significant correlations between the nodes (r>0.5), with red dotted and black solid lines showing negative and positive correlations, respectively. * represents a significant correlation (P<0.05), ** represents a significant correlation (P<0.01), *** represents a significant correlation (P<0.001).

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