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Study of *Periplaneta Americana* Microbial Community Structure and Diversity by *16S rRNA* High-Throughput Sequencing

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Abstract

Objective: *The present study probes into the microbial community structure in Periplaneta americana under different breeding conditions, using 16S rRNA high-throughput sequencing technique, in the hope of finding the microbial community structure in Periplaneta americana and their diversity under different breeding conditions.*

Methods: *In this study, we extract the microbial metagenomic DNA of 5 groups of Periplaneta americana which under different breeding conditions. Using lllumina Miseq sequencing platform, two-terminal sequencing of V3-V4 regions of 16S rRNA were sequenced; diversity of community structure was analyzed using the softwares such as fastqc, QIIME, PyNAST, fasttree and R language.*

Results: *Shannon index of samples in SG group was lower than that of the other four groups, significantly lower than that of DB group ($P<0.05$), but not significantly different from other groups. This suggested that the intake of a mixed fodder with high sugar, high fat and high protein by Periplaneta americana can reduce the diversity of microbial communities. Our findings showed that breeding intervention with different fodders may cause differences in the contents of Bacteroidetes, Proteobacteria and Firmicutes in Periplaneta americana. Results showed that long-term intake of lots of sucrose and fat may increase the proportion of Bacteroidetes in Periplaneta americana; and long-term intake of lots of sucrose may reduce the proportion of Proteobacteria in Periplaneta americana; and long-term intake of lots of fat may reduce the proportion of Firmicutes in Periplaneta*

americana. Two major dominant bacterial genera in all samples were Blattabacterium and Rickettsiella. But different feeding interventions can change the proportions of Blattabacterium and Rickettsiella.

Conclusion: *Periplaneta americana has a complex microbial community structure. Different feeding conditions may change the microbial community structure of Periplaneta americana. An important bacterial genus in Periplaneta americana, Blattabacterium is positively correlated with the intake of sucrose- and fat-rich fodder. In the breeding process of Periplaneta americana, adding sucrose and fat to fodder may increase the content and proportion of Blattabacterium in microbial communities.*

Keywords

Periplaneta americana, enteron microbial, 16S rRNA genes, next-generation sequencing

1. Introduction

Insects are an animal group in terrestrial ecosystems that has the most abundant biomasses, species and ecological habits, and also one of the groups with the highest biodiversity **(**Erwin et al., 1982; Chapman et al., 2013**)**. Due to diverse feeding characteristics and behaviors, almost all food resources on land (all substances that can be eaten by humans and provide nutrients and heat) can be consumed by insects **(**Shi et al., 2010**)**. The success of insect diversity and evolution, to a certain extent, rests upon the contributions of various beneficial microbes **(**Engel et al., 2013**)**. Symbiotic bacteria in insects have an important effect on the vital activities of insects **(**Babendreier et al., 2007; Mattila et al., 2012; Nikoh et al., 2011; Eichler et al., 2002; Philipp et al., 2012; Douglas, 1993; Engel et al., 2013; Hongoh et al., 2008**)**. For example, the physiology and evolution of insects not only improves of hosts' nutrition and helps digest food ingredients, but also prevents invasion from predators, parasites and pathogens and helps interspecific and intraspecific communications, etc. **(**Engel et al., 2013; Favia et al., 2012; Kaltenpoth et al, 2014**)**. Insect microbes are highly dependent on each other, symbiotic with hosts and involved in regulating various vital activities of hosts, thereby affecting the health of insects indirectly **(**Shi et al., 2010**)**.

Symbiotic bacteria in insects have an important association with many fields and subjects, such as agriculture, ecology and medicine **(**Engel et al., 2013**)**. Insects can be a good experimental model to study human diseases by setting up a bacterial infection model of insects **(**Ishii et al., 2014**)**. Also, we can use an insect model to study how symbiotic microbes affect the transmission efficiency and occurrence law of diseases **(**Ricci et al., 2012; Chouaia et al., 2012**)**. Meanwhile, using an insect model, we can carry out a correlational study on the interaction between intestinal symbiotic bacteria and hosts, and further promote human understanding of the symbiotic relationship between microbes and hosts **(**Lemaitre et al., 2007**)**.

To analyze *16S rRNA* in intestinal microbes, using high-throughput sequencing technique, is a common analysis method based on microbial community structure. *16S rRNA* high-throughput sequencing technique is based on high-throughput feature of next-generation sequencing technique. *16S rRNA* gene

sequences can be used to classify, identify and quantify mixed microbes in complex samples. At present, it has been widely applied to the study of microbial community structure **(**Sogin et al., 2006; Dominguez-Bello et al., 2010; Turnbaugh et al., 2009; Larsen et al., 2010; Karlsson et al., 2012**)**. However, few studies on microbial communities in Periplaneta americana, a raw material for many TCM (Traditional Chinese Medicine) varieties **(**Luo et al., 2012**)**, using 16S rRNA high-throughput sequencing technique have been reported.

The present study probes into the microbial community structure in *Periplaneta americana* under different breeding conditions, based on Illumina high-throughput sequencing platform, using *16S rRNA* high-throughput sequencing technique, in the hope of finding the microbial community structure in *Periplaneta americana* and their diversity under different breeding conditions and laying a good foundation for revealing the relationship between the microbial community structure and growth of *Periplaneta americana*.

2. Materials and Methodology

2.1 Breeding Method

Periplaneta americana used in the breeding experiment were derived from Zuoyi Insect Breeder Center of Yunnan Provincial Key Laboratory of Entomological Biopharmaceutical R&D and bred into adults with ordinary fodder in the breeding base. They had stable eating habits.

The *Periplaneta americana* adults were divided into 7 groups randomly, about 100 in each group. In a dense feeding cage, PT group mainly fed on ordinary fodder made of pumpkin, bran, corn flour and water. T group fed on pure sucrose (including 99.9% carbohydrates; Kunming Damaidi food Co., Ltd, China). ZF group fed on cooking oil (100% fat, including 16% saturated fatty acid, 25% monounsaturated fatty acid and 59% polyunsaturated fatty acid; Kerry Grain and Oil Corporation, China). DB group fed on pure pea powder (including 20.3% protein, 1.1% fat and 55.4% carbohydrate; Dali Yincang Food Co., Ltd, China). SG group fed on a three-high fodder made of pure sucrose, cooking oil and pure pea powder by a ratio of 1:1:1. 5 groups of *Periplaneta americana* above were fed at 8:00 each day for 45 days. After feeding, each group of *Periplaneta americana* must be fasting for 24 hours.

After fasting, bellies of *Periplaneta americana* were cut off using high-temperature sterile surgical scissors. Every 3 bellies of *Periplaneta americana* were combined into 1 sample. A total of 5 breeding groups, 15 samples were obtained. All samples were preserved in DNA tissue sample room-temperature preserving fluid (Sangon Biotech (Shanghai) Co., Ltd) and sent to Beijing Microread Genetics Co.,Ltd for DNA extraction, next-generation sequencing and biological information analysis.

2.2 DNA Extraction, 16S rRNA Gene Amplification and High-Throughput Sequencing

In this study, we used a kind of DNA extraction kit-QIAamp@Fast DNA Stool (QIAGEN, USA) to extract the microbial metagenomic DNA of *Periplaneta americana*. Using Illumina Hiseq2500 Sequencing System, V3-V4 regions of *16S rRNA*, a metagenomic DNA in the digestive tract of

Periplaneta americana were sequenced. The sequencing was directed. The target length of sequencing was about 460bp.

2.3 Data Analysis

After V3-V4 regions of *16S rRNA* in 15 samples from the digestive tract of *Periplaneta americana* were sequenced, the quality of raw data were first tested with Fastqc software. Adaptors and low-quality base sequences were removed. The numbers of reads and data of raw and quality-controlled samples were counted. After that, double-end reads were spliced into effective sequences, according to one-end overlapping base.

Using QIIME (Version 1.50), high-quality sequences were clustered into operational taxonomic units (OTUs). OUT clustering were performed on high-quality sequences of all samples as per a similarity of 0.97. The longest sequence in each cluster was selected as a representative sequence of OTU.

1) In the study, we adopted QIIME (Version 1.50) software to conduct a correlation analysis of Alpha diversity of samples from the digestive tract of *Periplaneta americana*. According to values of derived Alpha diversity indices, i.e., Chao1, Shannon, PD whole tree and Simpson, a corresponding rarefaction curve was generated.

2) Flag sequences were selected from each OUT. Using Uclust double sequence alignment algorithm in PyNAST software **(**Caporaso et al., 2010**)**, flag sequences were compared with a reference database (16S rRNA gene sequence). The positions of gaps in sequences were filtered. Then using Fasttree **(**Price et al., 2010**)** software, a phylogenetic tree was built, with OUT flag sequences as nodes. Then using UniFrac **(**Lozupone et al., 2012**)** algorithm, the distance matrix and PCoA of samples were calculated. According to the distance matrix of samples, a heatmap image was made using heatmap program in R language. The similarity among all samples was accounted for.

3) After the abundance of OUT in each species was calculated using QIIME software, the abundance matrix of OUT in each species was obtained. According to the derived data, generic-level taxonomic information was clustered as per sample and class and a heatmap image was generated, using heatmap program in R language. Among them, change in color represented two-dimensional matrix information. Shade of color represented the value of data. Species of high and low abundances were clustered by blocks. The similarities and differences in generic-level community composition between multiple samples were reflected by color gradient and degree of similarity.

4) Principal Pomponent Analysis (PCoA) was carried out using QIIME. One point stood for a sample. Points of the same color belonged to the same group. The closer two points were, the smaller difference between two samples in microbial communities.

5) Using UniFrac distance, a phylogenetic analysis including abundance was conducted. The abundance of bacterial genera, whose mean value was Top 100 among 5 groups of samples were selected, to make a heatmap and conduct a clustering analysis of 15 samples, so as to reflect intragroup and intergroup differences between samples.

2.4 Statistical Analysis

All experimental data involved in the present study were measured data. All average data were signified with Mean ± SD. As each breeding group had 3 sample data, Kruskal-Wallis H, a non-parametric test method with multiple independent sample rank conversion was chosen. A significance analysis was conducted of OUT number and diversity index of different breeding groups, as well as relative abundance of microbial communities in the digestive tract. After tested by Kruskal-Wallis H, the above data of each experimental group were different as whole. So a one-way ANOVA was adopted to make a pairwise comparison between all experimental groups. The statistical analysis in the present study should be implemented with R language statistical software. $P<0.05$ was regarded as statistically significant.

3. Results

3.1 Feeding Results

Finish breeding, 40 *Periplaneta americana* were randomly selected from each group, to measure their length and weight. Results showed that the average weight, length and weight-length ratio (W/L) of periplaneta americana in PT group were 1.1999±0.2070 g, 3.29±0.16 cm and 0.36±0.05 g/cm respectively. The average weight, length and weight-length ratio of periplaneta americana in T group were 1.2126±0.2442 g, 3.32±0.18 cm and 0.37±0.06 g/cm respectively. The average weight, length and weight-length ratio of periplaneta americana in ZF group were 1.0407±0.1843 g, 3.21±0.16 cm and 0.32±0.05 g/cm respectively. The average weight, length and weight-length ratio of periplaneta americana in DB group were 1.0767±0.1963 g, 3.17±0.20 cm and 0.34±0.06 g/cm respectively. The average weight, length and weight-length ratio of periplaneta americana in SG group were 1.1690±0.2765 g, 3.24±0.21 cm and 0.36±0.09 g/cm respectively. Among them, the weight-length ratio in ZF group was significantly lower than that of PT group, T group and SG group ($P<0.05$). A correspondence analysis of the weight-length ratio of periplaneta americana between five groups showed that the weight-length ratio tended to be ≥0.4 in SG group, ≥0.35 and <0.4 in PT group, as shown in Table 1 and Figure 1.

Table 1. The Samples Investigated in the Study and Information for *Periplaneta Americana*

Groups	W/L<0.3	0.3≤W/L<0.35	0.35≤W/L<0.4	W/L≥0.4	W/L (Mean±SD,g/cm)
PT	5	7	19	9	0.36±0.05
T	7	6	15	12	0.37±0.06
ZF	14	11	10	5	0.32±0.05
DB	10	9	13	8	0.34±0.06
SG	8	4	13	14	0.36±0.09

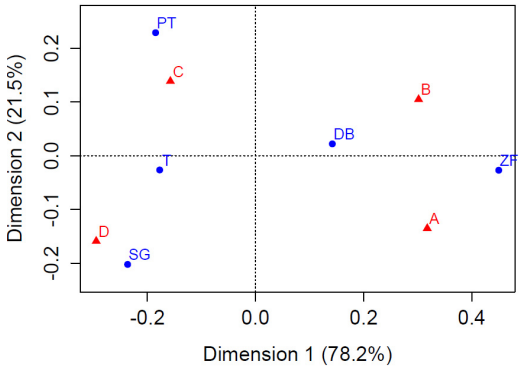

Figure 1. Correspondence Analysis for the Feeding Effect of *Periplaneta Americana*

A: W/L<0.3, B: 0.3≤W/L<0.35, C: 0.35≤W/L<0.4, D: W/L≥0.4.

3.2 Alpha Diversity Analysis

Using QIIME software, based on OTUs clustered as per a similarity of 97%, Alpha diversity value of samples was calculated. 4 diversity rarefaction curves, i.e., sparse index curves of PD whole tree rarefaction, Simpson, Chao1 and Shannon using R language software are shown in Figure 2. As shown in Tables 2 and 3, Shannon diversity indices of PT group, T group, ZF group, DB group and SG group were 4.73±0.71, 5.58±0.47, 5.01±0.50, 6.25±1.09 and 3.83±1.22 respectively. The diversity index of SG group was significantly lower than that of DB group (P<0.05), but not significantly different from other groups. And the coverages of all samples were greater than 95%.

Table 2. Alpha Diversity Analysis of the *Periplaneta Americana* Samples

Samples	Alpha diversity analysis index			
	shannon	PD whole tree	chao1	simpson
PT1	5.500	133.862	3672.942	0.827
PT2	4.628	100.253	2830.438	0.795
PT3	4.077	78.080	2031.688	0.733
T1	5.042	114.010	3661.252	0.753
T2	5.898	140.243	4729.447	0.832
T3	5.795	108.726	3747.253	0.889
ZF1	5.401	92.235	2650.316	0.896
ZF2	4.446	95.019	3098.785	0.707
ZF3	5.184	134.652	4163.358	0.764
DB1	5.636	137.807	5138.171	0.874
DB2	5.621	151.127	5554.841	0.796
DB3	7.508	160.484	5653.656	0.977
SG1	2.687	78.767	2025.597	0.534

SG2	3.694	83.191	2215.544	0.750
SG3	5.112	127.976	3851.946	0.830

Table 3. Analysis of Alpha Diversity Analysis of the *Periplaneta Americana* Samples

Group	Shannon (Mean±SD)	PD whole tree (Mean±SD)	chao1 (Mean±SD)	simpson (Mean±SD)
PT	4.73±0.71	104.07±28.09	2845.02±820.72	0.79±0.05
T	5.58±0.47	120.99±16.88	4045.98±593.46	0.82±0.07
ZF	5.01±0.50	107.30±23.73	3304.15±777.15	0.79±0.10
DB	6.25±1.09	149.81±11.40	5448.89±273.59	0.88±0.09
SG	3.83±1.22	96.64±27.22	2697.70±1004.11	0.70±0.15

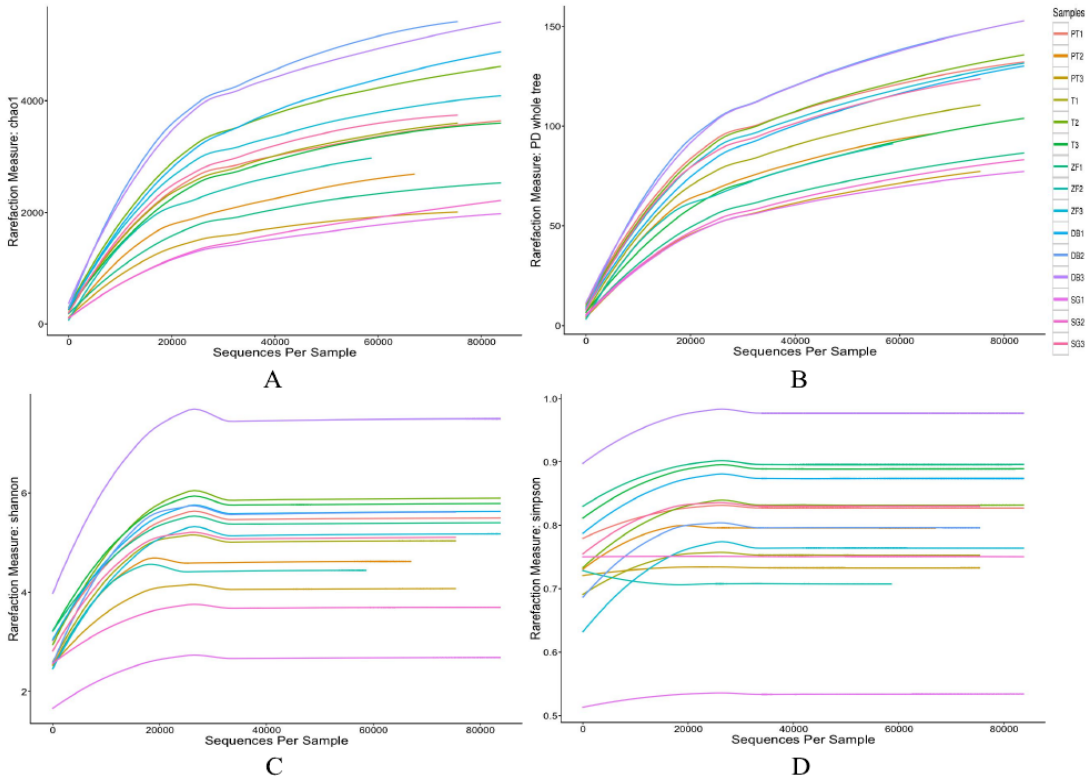

Figure 2. Rarefaction Analysis of the Different Samples

A: chao1; B: PD whole tree; C: shannon; D: simpson.

3.3 Species Distribution

In this study, a total of 1,487,612 high-quality sequences were obtained from 5 groups of *Periplaneta americana* (99,174 sequences in each sample). And 10,527 OTUs were obtained (PT group: 2777.00±374.31, T group: 2779.67±419.16, ZF group: 2213.00±596.85, DB group: 3594.33±354.41

and SG group: 1706.33±772.53). The Venn diagram of OTUs in each group of samples was shown in Figure 3. Unique-read sequences clustered as OTUs were compared with GreenGenes database. Species were annotated. A total of 397 genera of species were found (PT group: 248, T group: 245, ZF group: 245, DB group: 357 and SG group: 242). They belonged to 24 phyla, 57 classes, 104 orders and 200 families, respectively.

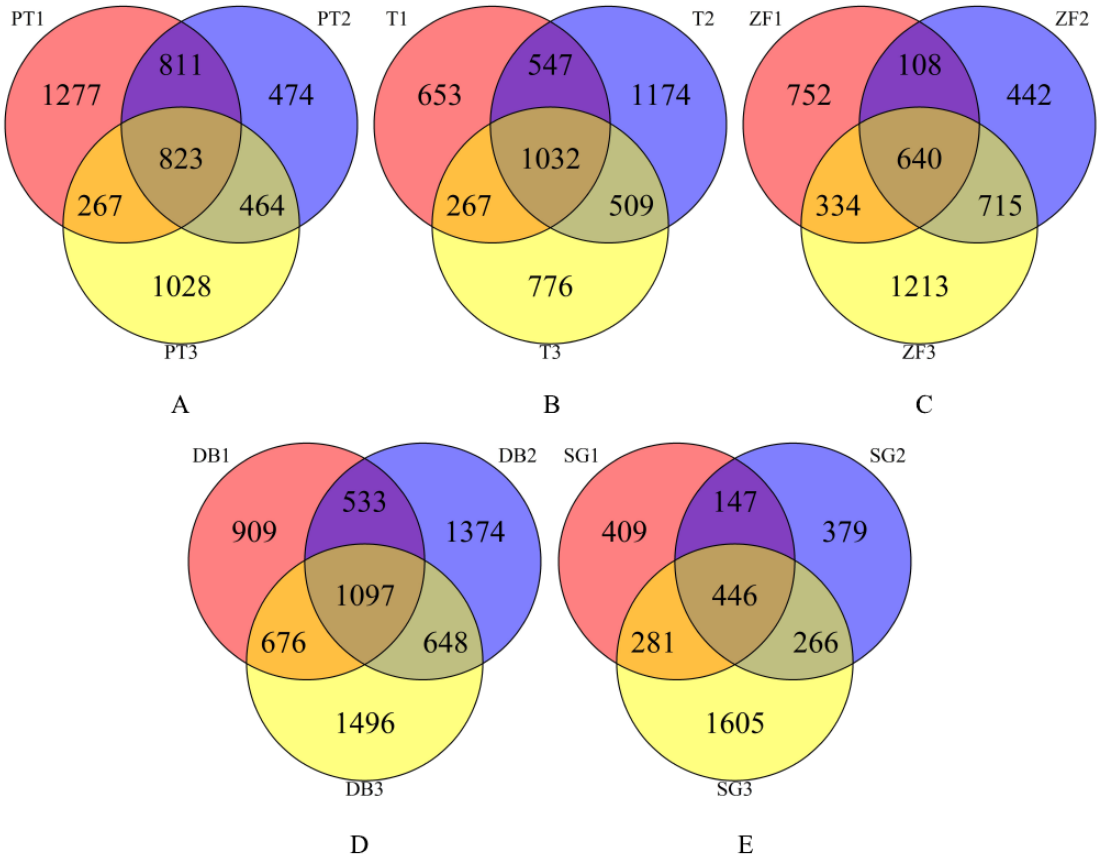

Figure 3. Venn Diagram for Shared OTU Analysis of the Different Libraries

A: PT group; B: T group; C: ZF group; D: DB group; E: SG group.

3.4 Analysis Based on the Taxonomic Category of Phylum

PT group had 22 phyla, T group had 22 phyla, ZF group had 20 phyla, DB group had 24 phyla and SG group had 22 phyla. From Table 4 and Figure 4, common dominant bacterial phyla in five groups of samples were Bacteroidetes, Proteobacteria, Firmicutes, Spirochaetes and Tenericutes. Statistical analysis showed that the proportion of the first dominant bacterial phylum—Bacteroidetes in the total flora of SG group was the highest (82.51%), followed by T group (78.88%), ZF group (65.01%), PT group (55.73%) and DB group (42.47%±38.46%), while changes in the proportion of Bacteroidetes in 5 groups of samples were not significant (P>0.05); proportion of the second dominant bacterial phylum—Proteobacteria in the total flora of PT group was the highest (39.09%, mean), followed by

DB group (31.70%, mean), ZF group (31.65%, mean), T group (12.15%, mean) and SG group (11.90%, mean), while changes in the proportion of Proteobacteria in 5 groups of samples were not significant (P>0.05); proportion of the third dominant bacterial phylum—Firmicutes in the total flora of DB group was the highest (23.44%, mean), followed by SG group (4.84%, mean), T group (4.59%, mean), PT group (4.29%, mean) and ZF group (2.44%, mean), while changes in the proportion of Firmicutes in 5 groups of samples were not significant (P>0.05).

Table 4. Analysis of Bacterial Distribution Evaluated at the Phylum Taxonomical Level

phylum	PT (Mean±SD, %)	T (Mean±SD, %)	ZF (Mean±SD, %)	DB (Mean±SD, %)	SG (Mean±SD, %)
Bacteroidetes	55.73±39.32	78.88±19.69	65.01±47.86	42.47±38.46	82.51±13.23
Firmicutes	4.29±3.18	4.59±4.71	2.44±1.02	23.44±23.21	4.84±5.99
Proteobacteria	39.09±41.30	12.15±15.39	31.65±48.42	31.70±30.59	11.90±13.25
Spirochaetes	0.23±0.33	0.08±0.06	0.20±0.26	1.54±1.23	0.30±0.24
Tenericutes	0.37±0.36	3.89±6.54	0.48±0.74	0.12±0.02	0.22±0.29

3.5 Analysis Based on the Taxonomic Category of Genus

PT group had 250 genus, T group had 247 genus, ZF group had 247 genus, DB group had 359 genus and SG group had 244 genus. From Table 5 and Figure 5, common dominant bacterial genus in five groups of samples were *Blattabacterium*, *Rickettsiella*, *Bacteroides*, *Clostridium* and *Parabacteroides*. Statistical analysis showed that the proportion of the first dominant bacterial genus—*Blattabacterium* in the total flora of SG group was the highest (79.49%, mean), followed by T group (76.54%, mean), ZF group (63.27%, mean), PT group (52.77%, mean) and DB group (37.48%, mean), while changes in the proportion of *Blattabacterium* in 5 groups of samples were not significant (P>0.05); the proportion of the second dominant bacterial genus—*Rickettsiella* in the total flora of PT group was the highest (37.65%, mean), followed by ZF group (21.23%, mean), T group (10.00%, mean), SG group (10.91%, mean) and DB group (1.12%, mean), while changes in the proportion of *Blattabacterium* in 5 groups of samples were not significant (P>0.05).

Table 5. Analysis of Bacterial Distribution Evaluated at the Genus Taxonomical Level

genus	PT (Mean±SD, %)	T (Mean±SD, %)	ZF (Mean±SD, %)	DB (Mean±SD, %)	SG (Mean±SD, %)
Bacteroides	0.78±0.71	0.54±0.13	0.42±0.21	1.44±1.20	0.85±0.48
Parabacteroides	0.38±0.51	0.23±0.26	0.16±0.16	0.69±0.62	0.16±0.14
Blattabacterium	52.77±37.19	76.54±18.79	63.27±46.85	37.48±39.77	79.49±13.96

Clostridium	0.40±0.22	0.27±0.05	0.35±0.27	15.41±25.52	0.20±0.04
Proteus	0.10±0.06	0.07±0.01	0.11±0.07	6.34±9.78	0.08±0.03
Klebsiella	0.06±0.04	0.03±0.01	0.06±0.07	3.02±5.11	0.03±0.01
Salmonella	0.00±0.01	0.02±0.02	0.01±0.01	0.00±0.00	0.01±0.02
Escherichia	0.00±0.01	0.00±0.01	0.00±0.00	0.10±0.13	0.04±0.06
Serratia	0.33±0.25	0.25±0.07	9.40±15.60	13.58±11.99	0.33±0.13
Rickettsiella	37.65±41.30	10.00±15.53	21.23±32.45	1.12±0.03	10.91±13.14

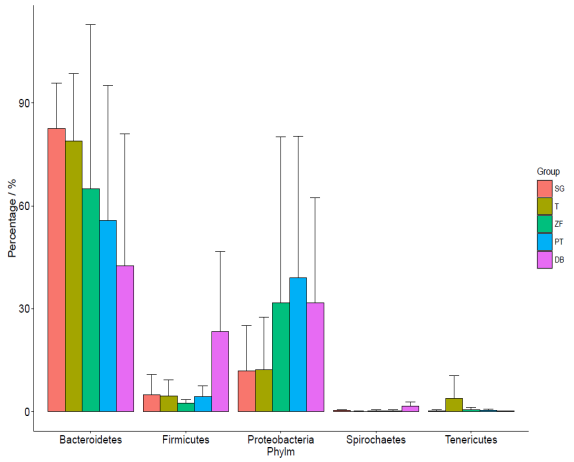

Figure 4. The Predominant Phylm of the Sequences in 5 Groups of *Periplaneta Americana*

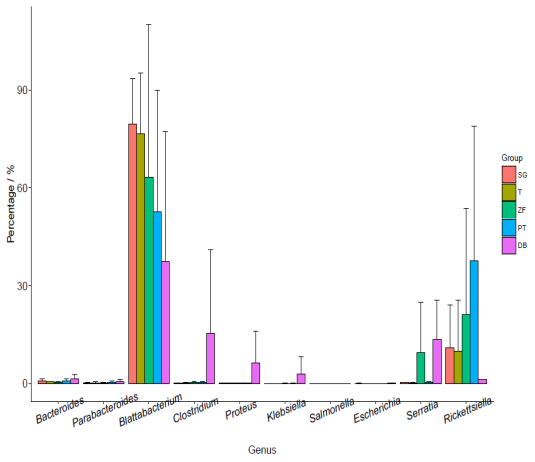

Figure 5. The Predominant Genus of the Sequences in 5 Groups of *Periplaneta Americana*

3.6 A Difference Analysis between Microbial Community Structures in the Digestive Tract of Periplaneta Americana

Through the above analysis of taxonomic levels of all floral, it was preliminarily judged that different *Periplaneta americana* samples had different microbial community structures. In order to further

analyze differences between floras in samples in terms of community complexity, using UniFrac software under QIIME platform, differences between samples were analyzed. PCoA and heatmap analyses were performed respectively.

As shown in Figure 6, from UniFrac PCoA analysis diagram under weighted UniFrac algorithm, the data of all samples in T group and SG group, as well as two samples in PT group and ZF group respectively are closely clustered, suggesting that intestinal floras between ten samples of four groups were similar and their flora structure were different from other samples. The data of DB group were dispersed, suggesting that individual differences in this group were large.

As shown in Figure 7, the clustering tree using weighted (OTU abundances considered) calculation method had two branches. Differences between 10 samples in T group, SG group, PT group and ZF group were small, but they were quite different from other samples. The results of clustering tree verified PCoA analysis.

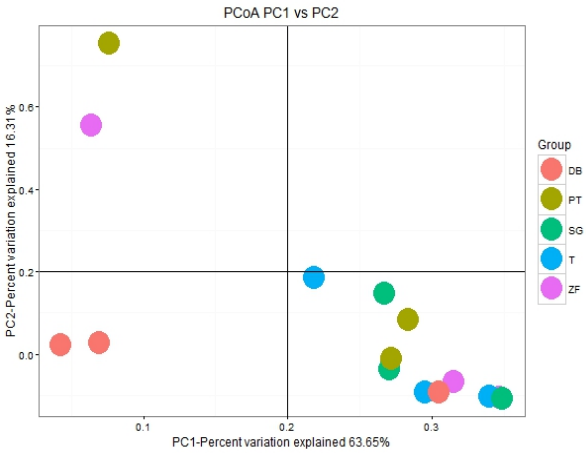

Figure 6. Weighed and Normal Principal Component Analysis (PCoA) of 5 Groups of *Periplaneta Americana*

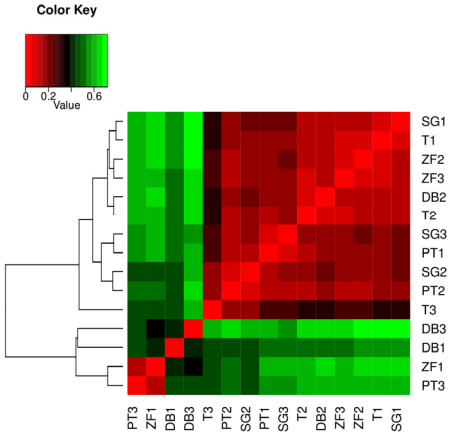

Figure 7. Heatmap: Weighted Unifrac Analysis

3.7 Clustering Analysis

From the heatmap image in Figure 8, all floras of all samples in five groups of *Periplaneta americana* were different. For example, the contents of *Rickettsiella*, *Butyricicoccus*, *Coprococcus* and *Dorea*, etc., were higher in PT3 and ZF1 samples than those of other samples, while the contents of *Blattabacterium* were higher in T1, SG1, ZF1 and ZF2 samples.

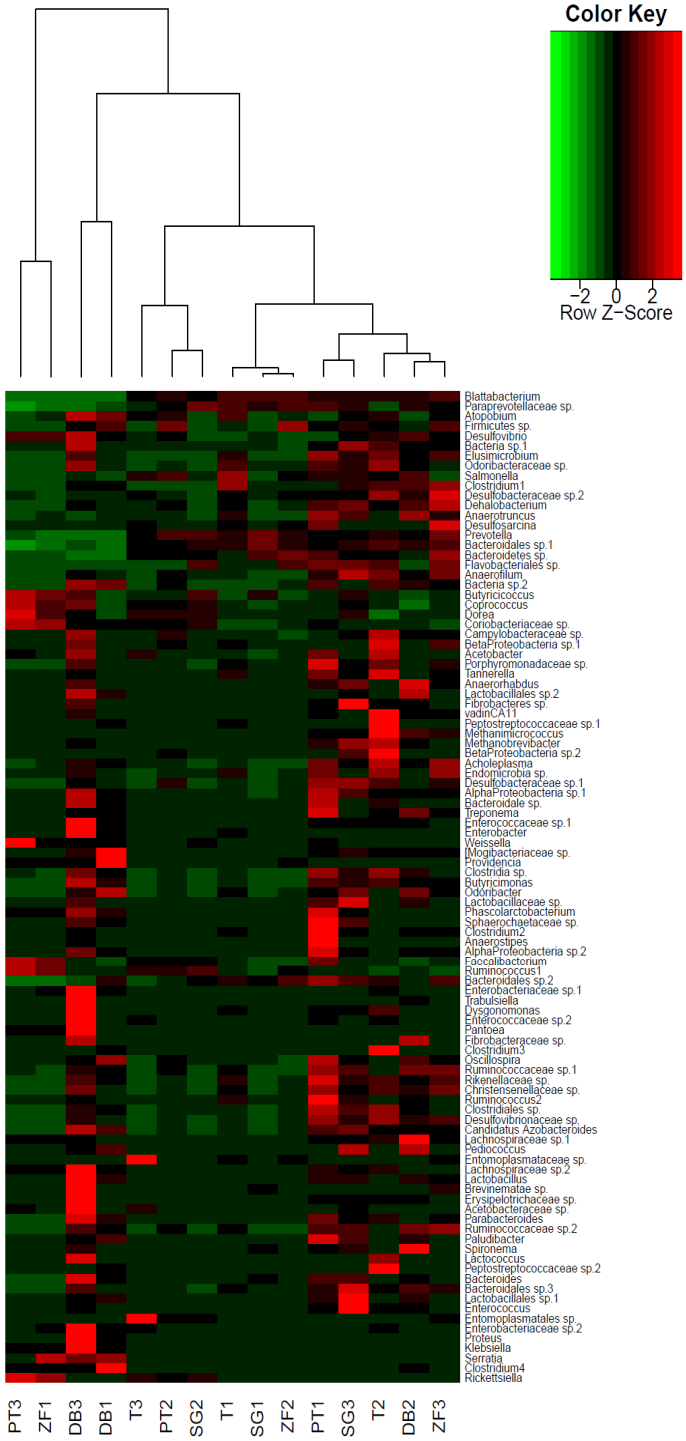

Figure 8. Heatmap of 5 Groups of *Periplaneta Americana* at the Genus Level

4. Discussion

Illumina HiSeq is one of the new high-throughput sequencing platforms. This sequencing method not only overcomes defects of traditional sequencing methods, such as short initial read and large error, but also obtains more accurate information about microbial communities (Frey et al., 2014; Weinstock et a.l, 2012). 16S rRNA exists in all creatures. Being highly conservative in structure and function (Zhao et al., 2013), it is often chosen as a molecular clock for microbial sequencing. At present, amplifying and sequencing of part of *16S rRNA* genes have been widely applied to the study of microbial community structure. Besides, among 9 hypervariable regions (V1-V9) of *16S rRNA*, V4 is the most accurate variable region the can identify up to generic-level (Ahn et al., 2011). Therefore, our study adopted Illumina Hiseq platform and explored microbes in *Periplaneta americana* under different breeding conditions, based on V3-V4 regions of *16S rRNA*. This method was applied to the study of microbial community structure in *Periplaneta americana* for the first time.

Periplaneta americana used in the breeding experiment were derived from Zuoyi Insect Breeder Center of Yunnan Provincial Key Laboratory of Entomological Biopharmaceutical R&D, Dali University and bred into adults using ordinary fodder. They had stable eating habits and this provided a good foundation for the microbial community structure in periplaneta americana. All groups of periplaneta americana were in bred in a dense cage. PT group fed on ordinary fodder. T group fed on pure sucrose. ZF group fed on cooking oil. DB group fed on pure pea powder. SG group fed on a mixed fodder of pure sucrose, cooking oil and pure pea powder. Periplaneta americana individuals in our study were rigorously and carefully selected and bred, to ensure the reliability of samples.

Shannon index of samples in SG group was lower than that of the other four groups, significantly lower than that of DB group ($P<0.05$), but not significantly different from other groups. This suggested that the intake of a mixed fodder with high sugar, high fat and high protein by *Periplaneta americana* can reduce the diversity of microbial communities.

Results of the present study showed that for intestinal microbial community structures of different *Periplaneta americana* individuals, there were three major dominant bacterial genera in all samples: Bacteroidetes, Proteobacteria and Firmicutes. This is basically identical with Fang et al.'s (Fang et al., 2013) findings about dominant bacterial communities in intestinal microbes of Periplaneta americana. The abundances of three dominant bacterial communities were slightly different, probably due to different sampling sites and test methods.

Our findings showed that breeding intervention with different fodders may cause differences in the contents of Bacteroidetes, Proteobacteria and Firmicutes in *Periplaneta americana*. Results showed that the relative abundance of Bacteroidetes in SG group was the highest (82.51%, mean), followed by T group and ZF group, while the relative abundance in DB group was low (42.47%, mean), suggesting that long-term intake of lots of sucrose and fat may increase the proportion of Bacteroidetes in *Periplaneta americana*. The relative abundance of Proteobacteria in PT group was the highest (39.09%, mean), followed by DB group and ZF group, while the relative abundance in SG group was low

(42.47%, mean), suggesting that long-term intake of lots of sucrose may reduce the proportion of Proteobacteria in *Periplaneta americana*. The relative abundance of Firmicutes in DB group was the highest (23.44%, mean), followed by T group and ZF group, while the relative abundance in ZF group was low (42.47%, mean), suggesting that long-term intake of lots of fat may reduce the proportion of Firmicutes in *Periplaneta americana*.

Two major dominant bacterial genera in all samples were *Blattabacterium* and *Rickettsiella*. But different feeding interventions can change the proportions of *Blattabacterium* and *Rickettsiella*. Results showed that the relative abundance of *blattabacterium* was the highest in SG group (79.49%, mean).

Sabree et al. (2009) discovered *Blattabacterium* in *Periplaneta americana* and performed whole genome sequencing on this bacteria. Results confirmed that *Blattabacterium* in *Periplaneta americana* may be involved in uric acid degradation, nitrogen assimilation and nutrient supply. Sabree et al. (2009) made a metabolic correlation analysis of *Blattabacterium* and suggested that *Blattabacterium* lacked identifiable uric acid decomposing enzyme, but it can use urease and glutamate dehydrogenase to recycle urea and ammonia nitrogen, degradation products of uric acid, and transformed them into glutamate. Then, *Blattabacterium* can make use of limited raw materials to produce all essential amino acids, multiple vitamins and other essential compounds for the survival of *Periplaneta americana*.

Therefrom, in microbial communities in the digestive tract of *Periplaneta americana* in SG group, T group and ZF group, the proportion of *Blattabacterium* was higher, suggesting that an intake of sucrose- and fat-rich fodder may facilitate the growth and reproduction of *Blattabacterium*. Therefore, in the breeding process of *Periplaneta americana*, sucrose and fat were added to the fodder to increase the content and proportion of *Blattabacterium* in microbial communities and facilitate the production of all essential amino acids, multiple vitamins and other essential compounds in *Periplaneta americana* and thus improve the quality of medical material.

5. Conclusion

Periplaneta americana has a complex microbial community structure. Different feeding conditions may change the microbial community structure of *Periplaneta americana*. An important bacterial genus in *Periplaneta americana*, *Blattabacterium* is positively correlated with the intake of sucrose- and fat-rich fodder. In the breeding process of *Periplaneta americana*, adding sucrose and fat to fodder may increase the content and proportion of *Blattabacterium* in microbial communities.

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