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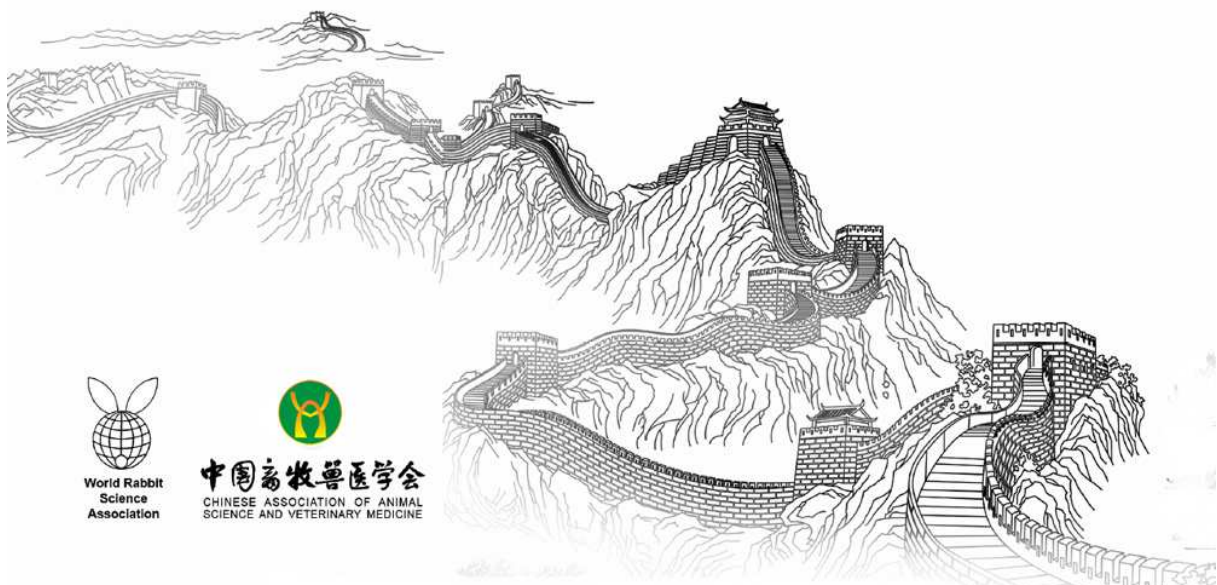
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PROFILING OF INTESTINAL MICROBIOME IN RABBIT

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ABSTRACT

It has been well acknowledged that the intestinal microbial community is positively involved in the regulation of biological functions under both healthy and disease conditions in animals. Among them, rabbit has the specific physiological characterization in relation to both intestinal anatomy and digestive process. In the present study, we employed the next generation sequencing technique for investigating the profiles of intestinal microbiome in growing rabbit via 16s rRNA marker. Based on the popular analysis pipeline, we revealed that the length distribution of clean tags from V3 region ranged from 66 bp to 220 bp in intestinal microbiome of rabbit. A total of 19, 621 OTUs were picked out and 42.24% of them were just shared by no more than three samples. Finally, we observed relatively high microbial richness (average Chao = 5971) and diversity (average Shannon = 4.19). Our results would be useful for guiding the further investigation of biological implications of intestinal microbiota in rabbit.

Key words: Microbiome, 16s rRNA, Rabbit, Intestine

INTRODUCTION

Because of the sensitivity of digestive system to both dietary composition and type, the intestinal digestive disorder is one of the most important diseases in rabbit and accounted for 70% incidences of death on average in commercial population (Gu, 2004). In addition to the well-known pathogens, the fibre level in diet has been believed to condition the incidence of digestive disorders. Meanwhile, the forth coming evidences also supported that the digestive disorders in rabbit would be also controlled by the genetic factors from host, to which a few candidate genes have been reported (Chen et al., 2013). Furthermore, it is clearly anticipated that the intestinal microbial community must be also involved in the biogenesis of digestive disorder similar to that observed in human and mouse. Unfortunately, the characterization of microbial composition in intestine of rabbit has rarely been studied (Combes et al., 2011).

In the present study, we employed the next generation sequencing technique for investigating the composition and diversity of intestinal microbiome in rabbit based on the molecular marker of 16s rRNA genes. The results would provide interesting clues for helping us to better understand the microbial components and abundances in rabbit.

MATERIALS AND METHODS

Animals and sampling of cecal contents

In the research farm of Sichuan Agricultural University, we selected a total of 43 healthy New Zealand White rabbits at ~60 days of age. All of these rabbits were housed in individual cages under same conditions and reared by our standard management protocol without any antimicrobial exposure. After slaughter, the cecal contents were immediately collected and blended for storage in liquid nitrogen.

PCR amplication and sequencing

Upon thawing, total DNA containing the microbial communities was extracted for individual sample using QIAamp DNA Stool Mini Kit(Qiagen, Shanghai, China) according to manufacturer's

instructions. The V3 region of bacterial 16S rRNA gene was amplified using HOTSTAR Taq Plus Master Mix Kit (Qiagen, Shanghai, China) and the universal primers (338F: 5'-ACTCCTACGGGAGGCAGCAG-3' and 533R: 5'-TTACCGCGGCTGCTGGCAC-3', Huse, 2008). The PCR condition involved an initial denaturation step at 95°C for 4 min and 20 cycles of 95°C for 1 min, 56 °C for 45sec, and 72°C for 1 min, and followed by an extension step at 72°C for 7 min using a Bio-Rad CFX96 thermal cycler (Bio-Rad, Hercules, USA). Amplicons were used to generate the sequencing libraries using Illumina DNA Sample Preparation Kit (Illumina, San Diego, USA). Finally, the libraries were sequenced on an Illumina HiSeq™ 2000 platform for generating the 150bp paired-end reads.

Data analysis

We employed the popular analysis pipeline which has been widely used in studies of intestinal microbiome (Schloss, 2009). Briefly, after the pre-processing of 16S rRNA sequenced reads, we got all the clean tags and sequentially subjected to chimera detection, OTU picking, and diversity estimation.

RESULTS AND DISCUSSION

A total of 81.65 million raw paired-end reads were generated among 43 individuals with an average of 1.90 million per sample (Table 1). We trimmed adaptor sequences using Cutadapt tool and merged them together using USEARCH tool (Edgar, 2010); the minimum length of overlapping region was 25 bp with 0 mismatch allowed. Finally, we got 59.13 million tags among 43 individuals with an average of 1.38 million per sample. The lengths ranged from 66 bp to 220 bp with the most predominant observations of 135 bp and 152 bp, respectively (Figure 1a). We further pooled the clean tags for all 43 samples and dereplicated them, which resulted in 1.40 million raw unique tags. After the processing of dereplication and chimera detection, the post-dereplication length distribution did not change (Figure 1b). These result supported that our sequencing library was successfully constructed (Oyola et al., 2012).

Table 1: The statistical results of sequenced reads before and after quality filtering

For all samples	Raw reads	Merged tags	Clean tags for OTUs picking	Number of OTUs
Mean	1, 898, 891	1, 375, 168	598, 578	3, 898
Minimum	808, 616	548, 706	244, 662	2, 211
Maximum	4, 298, 025	2, 956, 839	1, 167, 528	6, 024

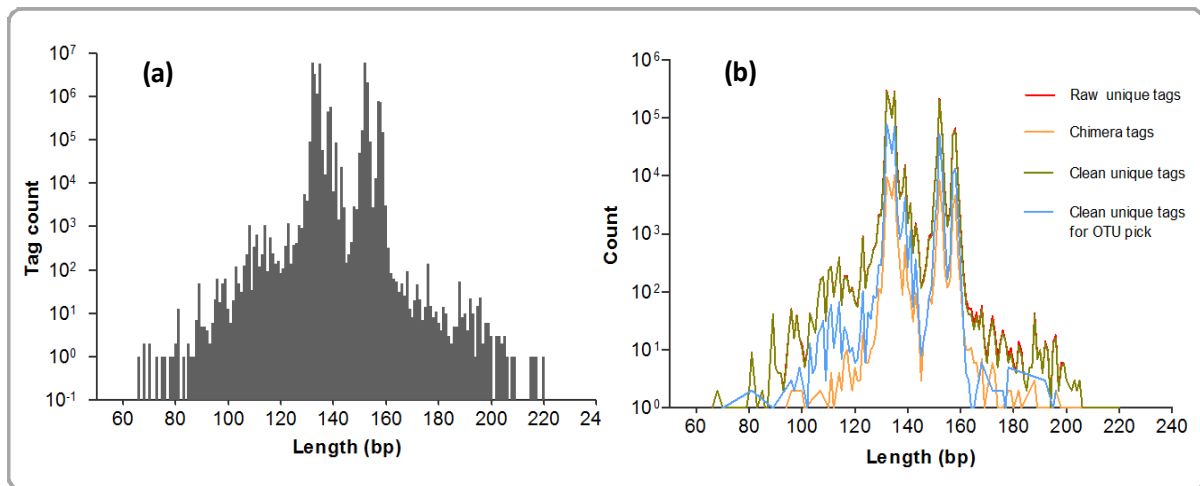


Figure 1: Length distribution of all clean tags (a) and unique tags (b).

We also investigated the abundance distribution among the clean unique tags (Figure 2). Among them, up to 74.86% tags were exclusively observed in one sample with an average abundance of 1.217 (92%

percentile: 1.0, maximum: 2455). Therefore, we further filtered out these clean unique tags exclusively observed in a single sample regardless of their abundances. Finally, a total of 335, 462 clean unique tags, which were observed at least in two samples, were subjected to OTUs picking. Finally, we successfully picked out 19, 621 OTUs and generated their abundances among all 43 samples; and among them, 8, 288 OTUs (42.24%) were shared by no more than three samples. Similar to former studies, we also employed the OUT-based method for profiling the microbial composition (Huse et al., 2012).

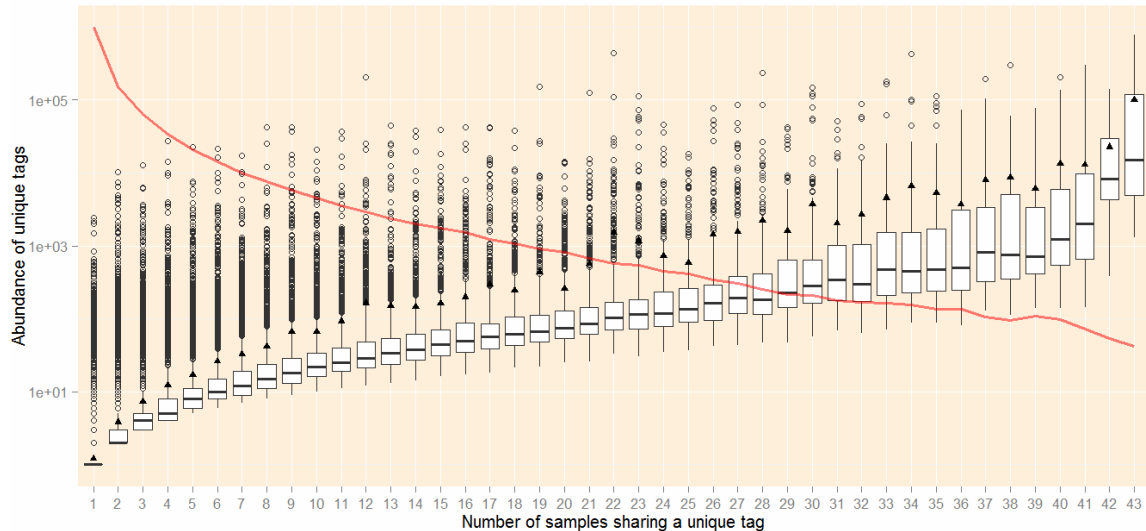


Figure 2. Box-and-WhiskerPlots show the abundance distribution of clean unique tags as observed in different numbers of samples. The box extends from the 25th to 75th percentiles. Furthermore, the absolute counts of tags are represented by the bold red line.

We further evaluated both microbial richness (Chao) and diversity (Shannon) at the OTU rank for each sample (Figure 3). The results revealed that all samples had the relatively high diversity according to both richness and diversity indexes. In fact, intestine has been widely acknowledged to be enormously settled by microbial species (Hattori et al., 2009), which also suggests the important participation of microbial components into biological functions of host.

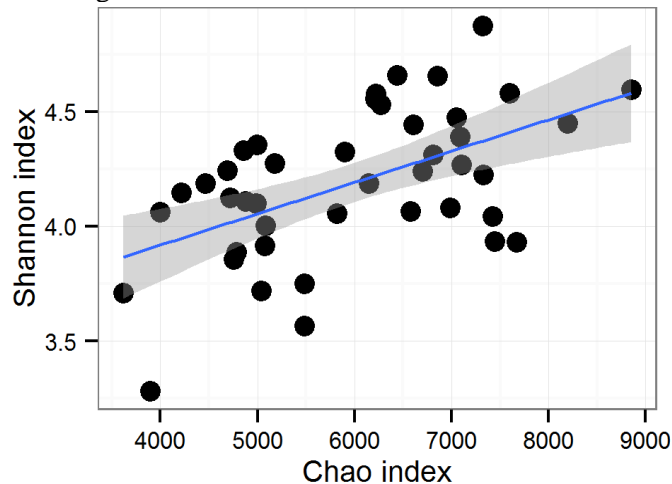


Figure 3: Both the Chao and Shannon indexes for each sample. The linear trend was also denoted according to linear model.

CONCLUSIONS

In the present study, we first provided an outline about intestinal microbiome in rabbit. Based on the high-throughput technique, the results would be comprehensive and shed more light to better understand the biological implications of intestinal microbes.

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