Postprint: Analysis of Rumen Bacterial Diversity in Jinjiang Cattle Using MiSeq Sequencing

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Abstract

This study aimed to utilize MiSeq high-throughput sequencing technology to reveal the bacterial composition and diversity in the rumen of Jinjiang cattle. Three adult Jinjiang bulls [body weight (400$±$20) kg] fitted with permanent rumen fistulas were selected, rumen fluid samples were collected, bacterial DNA was extracted, and the V4-V5 region of the 16S rDNA sequences of rumen bacteria was subjected to MiSeq sequencing to analyze species abundance, distribution, and α-diversity. The results showed that: a total of 56,763 high-quality 16S rDNA sequences and 946 operational taxonomic units (OTUs) were obtained from the three samples; the rumen bacterial richness indices Chao index and Ace index of Jinjiang cattle were 836 and 841, respectively, and the α-diversity indices Shannon index and Simpson index were 4.96 and 0.0215, respectively; through taxonomic annotation, 15 phyla, 23 classes, 26 families, and 44 genera were identified across all samples. At the phylum level, Bacteroidetes (64.37%), Firmicutes (21.20%), and Proteobacteria (7.59%) were the most abundant; at the class level, the community mainly comprised Bacteroidia (52.88%) and Clostridia (17.03%); at the family level, the community mainly comprised Prevotellaceae (36.26%), Ruminococcaceae (9.33%), and Lachnospiraceae (4.89%); at the genus level, the dominant bacteria mainly included Prevotella (28.97%), Paraprevotella (3.08%), Rikenella (2.28%), Clostridium IV (1.77%), Succiniclastium (1.62%), and Ruminococcus (1.53%). The results demonstrated that the rumen bacterial diversity of Jinjiang cattle was relatively low; the most dominant phylum in the rumen of Jinjiang cattle was Bacteroidetes, followed by Firmicutes; Prevotella was the most dominant bacterial genus in the rumen of Jinjiang cattle.

Full Text

Analysis of Rumen Bacterial Diversity in Jinjiang Cattle Using MiSeq Sequencing Technology

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Abstract

This study aimed to investigate the composition and diversity of rumen bacteria in Jinjiang cattle using MiSeq high-throughput sequencing technology. Rumen fluid samples were collected from three adult Jinjiang bulls (body weight 400$±$20 kg) fitted with permanent rumen fistulas. Bacterial DNA was extracted, and the V4-V5 region of the 16S rDNA gene was sequenced using MiSeq technology to analyze species abundance, distribution, and α-diversity. The results showed that a total of 56,763 high-quality 16S rDNA sequences and 946 operational taxonomic units (OTUs) were obtained across the three samples. The rumen bacterial richness indices (Chao and Ace) were 836 and 841, respectively, while the α-diversity indices (Shannon and Simpson) were 4.96 and 0.0215. Taxonomic annotation identified 15 phyla, 23 classes, 26 families, and 44 genera present in all samples. At the phylum level, Bacteroidetes (64.37%), Firmicutes (21.20%), and Proteobacteria (7.59%) were the most abundant. At the class level, Bacteroidia (52.88%) and Clostridia (17.03%) predominated. At the family level, Prevotellaceae (36.26%), Ruminococcaceae (9.33%), and Lachnospiraceae (4.89%) were dominant. At the genus level, the predominant bacteria included Prevotella (28.97%), Paraprevotella (3.08%), Rikenella (2.28%), Clostridium IV (1.77%), Succiniclastium (1.62%), and Ruminococcus (1.53%). These findings demonstrate that rumen bacterial diversity is relatively low in Jinjiang cattle. Bacteroidetes is the most dominant phylum, followed by Firmicutes, while Prevotella is the most abundant genus.

Keywords: Jinjiang cattle; rumen bacteria; diversity; MiSeq sequencing

Introduction

Rumen microorganisms, which inhabit the rumen of ruminants, play a crucial role in host growth, particularly in digestive metabolism [1-2]. The symbiotic relationship between rumen microbes and ruminants has existed for a long time, exhibiting co-evolutionary characteristics with the host [3]. Consequently, rumen microbial community structure is often used as an important indicator reflecting host genetic background [4-6]. Among these microbes, rumen bacteria are the most diverse and abundant, representing the primary functional group in the rumen microbiome. Moreover, compared to protozoa, inter-species variation in rumen bacterial populations far exceeds intra-species variation [6], making them better indicators of host breed differences.

China possesses the largest number of cattle breeds worldwide, yet current re-

search on rumen microbes in cattle has primarily focused on large breeds such as Holstein [7-8]. Limited knowledge exists regarding the rumen microbial community structure and diversity of small local breeds, particularly indigenous Chinese cattle breeds. Jinjiang cattle are a small yellow cattle breed native to southern China, characterized by excellent heat tolerance, roughage tolerance, and strong draft power. They represent an extremely valuable local genetic resource, mainly distributed in the low hills and plain areas along the middle reaches of the Jinjiang River in northwestern Jiangxi Province, with a population exceeding 1 million head according to a 2006 survey. Current research on Jinjiang cattle has primarily concentrated on resource surveys and nutritional regulation, with no reports on rumen microorganisms. This study employs MiSeq high-throughput sequencing technology to analyze the rumen microbiota structure and diversity of Jinjiang cattle, aiming to establish a foundation for the development and utilization of southern Chinese cattle genetic resources and to provide a reference for in-depth research on microecological nutrition in Jinjiang cattle.

Materials and Methods

1.1 Sample Collection

Three Jinjiang cattle fitted with permanent rumen fistulas (body weight 400$±$20 kg) were selected for rumen fluid collection. The experimental animals were housed individually with free access to water. The diet consisted of a corn-soybean meal concentrate and rice straw roughage at a concentrate-to-roughage ratio of 20:80, fed at 08:00 and 17:00 daily. On the sampling day, rumen fluid was collected before morning feeding, filtered through four layers of sterile gauze, aliquoted, immediately frozen in liquid nitrogen, and stored at -80°C until analysis.

1.2 Genomic DNA Extraction and MiSeq Sequencing

Genomic DNA was extracted from 0.5 mL of rumen fluid samples using the bacterial genomic DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd., following the manufacturer' s instructions. DNA concentration was measured using a NanoDrop-ND1000 spectrophotometer, and DNA quality was assessed by 1% agarose gel electrophoresis. Qualified DNA samples were sent to Shanghai Majorbio Bio-pharm Technology Co., Ltd. for sequencing of the bacterial 16S rDNA V4-V5 region (515F-907R) using the Illumina MiSeq PE250 platform.

1.3 Bioinformatics Analysis

Raw sequencing data underwent quality control, with low-quality sequences (tail base quality <20, post-quality control read length <50 bp) discarded. High-quality sequences were clustered using Usearch 7.1 software, with 97% similarity of 16S rDNA sequences used as the cutoff for operational taxonomic unit (OTU) delineation. The obtained OTUs were compared against the RDP database

(Release 11.1, http://rdp.cme.msu.edu/), and the RDP Classifier was used to identify the taxonomic status of representative OTU sequences. Richness indices (Chao, Ace) and diversity indices (Shannon, Simpson) were calculated using Mothur 1.30.1 software.

Results

2.1 Rumen Microbial α-Diversity in Jinjiang Cattle

Following data preprocessing, 56,763 high-quality 16S rDNA sequences with an average length of 393.74 bp were obtained from the three samples. A total of 946 OTUs were identified at the 97% sequence similarity level. At the OTU level, the average richness indices (Chao and Ace) for the three samples were 836 and 841, respectively, while the diversity indices (Shannon and Simpson) were 4.96 and 0.0215.

2.2 Rumen Microbial Community Structure in Jinjiang Cattle

In this study, 15 bacterial phyla were identified in Jinjiang cattle rumen, with Bacteroidetes (64.37%), Firmicutes (21.20%), and Proteobacteria (7.59%) being the most abundant (Table 1). Other phyla, including Fibrobacteres, Spirochaetes, Lentisphaerae, Tenericutes, Verrucomicrobia, Planctomycetes, Chloroflexi, Synergistetes, TM7, Elusimicrobia, Cyanobacteria, and Armatimonadetes, were present in all individuals but at relatively low abundances. At the class level, 23 classes were identified, with Bacteroidia and Clostridia being the most abundant, accounting for 52.88% and 17.03% of total bacteria, respectively. This was followed by Sphingobacteria, Gammaproteobacteria, and Negativicutes.

At finer taxonomic levels, only 70.29% and 54.26% of sequences could be classified to the family and genus levels, respectively. Twenty-six families were identified across all individuals, with Prevotellaceae (36.26%), Ruminococcaceae (9.33%), and Lachnospiraceae (4.89%) being the most abundant. Other dominant families included Sphingobacteriaceae, Rikenellaceae, Succinivibrionaceae, Porphyromonadaceae, and Acidaminococcaceae. At the genus level, 44 genera were identified in all samples. Prevotella was the most abundant genus, representing 28.97% of total bacteria, followed by Paraprevotella, Rikenella, Clostridium_{IV}, Succiniclastium, and Ruminococcus.

Discussion

Community ecology can reflect microbial community abundance and diversity through single-sample OTU and α-diversity analysis. In this study, MiSeq sequencing yielded 56,763 rumen bacterial 16S rDNA sequences from Jinjiang cattle, which were clustered into 946 OTUs—lower than reported values for Holstein cattle (1,800-2,939) [7-8] and yak (6,642) [9]. The Chao and Shannon indices were also relatively low compared to Holstein cattle and yak (836 vs. 1,311-4,242

and 4.96 vs. 6.23-8.28) [8-10], indicating lower richness and diversity of rumen bacteria in Jinjiang cattle. Rumen microbial diversity results from strong selection and co-evolution between host and rumen microbes, and differences in host evolutionary history may determine rumen differences [11]. Jinjiang cattle are primarily distributed in the subtropical humid climate zone along the middle reaches of the Jinjiang River in northwestern Jiangxi. Their narrow distribution range and mild climate may contribute to low rumen bacterial richness and diversity. Additionally, diet composition is an important factor affecting rumen microbes. Kobayashi et al. [12] found that wild and semi-domesticated animals had higher rumen microbial richness than domesticated animals, attributing this to greater dietary complexity in wild and semi-domesticated animals. Therefore, we speculate that a relatively simple diet structure (primarily natural pasture in spring, summer, and autumn; rice straw in winter; with minimal corn supplementation) may be another important reason for the low richness and diversity of rumen microbiota in Jinjiang cattle.

In most studies, Firmicutes is the dominant phylum in bovine rumen, followed by Bacteroidetes [9,13-15]. In contrast, this study found Bacteroidetes (64.37%) as the dominant phylum in Jinjiang cattle, with Firmicutes ranking second (21.20%). Some studies on Holstein dairy cattle have reported similar results [7,16], possibly due to dietary differences. Li [17] observed that after 18 months of continuous Miscanthus feeding, the proportion of Bacteroidetes in Xiangxi yellow cattle rumen increased from 16.33% to 28.15%, while Firmicutes decreased from 68.88% to 60.92%. Similar increases in Bacteroidetes were observed when Angus and Holstein cattle were fed high-fiber Bermudagrass [10]. A low Firmicutes-to-Bacteroidetes ratio may be more common with high-fiber diets. Compared to northern forages, southern forages are generally coarser and contain higher crude fiber content [18], and long-term high-fiber feeding may lead to increased Bacteroidetes abundance in Jinjiang cattle rumen.

Rumen microbes exhibit high diversity at species and subspecies levels but limited diversity at genus level. Jami et al. [7] collected rumen content samples from 16 lactating dairy cows and found that 51% of bacterial classifications were similar across individuals, with only 32 core genera. In this study, 44 genera were present in all individual rumens, with Prevotella being the most abundant (28.97%). Prevotella exhibits high genetic diversity [19-20] and plays important roles in the rumen as a primary starch-degrading bacterium, while also possessing the ability to degrade proteins, peptides, and polysaccharides [21-22]. Consequently, Prevotella has been identified as the most important rumen genus in yak [9], dairy cattle [10,15], and other ruminants such as reindeer [23] and goats [24-25].

Conclusion

This study analyzed the rumen bacterial community of Jinjiang cattle fed a low-concentrate diet using MiSeq high-throughput sequencing technology. The results demonstrate that, compared to other large cattle breeds, Jinjiang cattle

have relatively low rumen bacterial diversity. Bacteroidetes is the most dominant phylum, followed by Firmicutes, while Prevotella is the most abundant bacterial genus.

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Note: Figure translations are in progress. See original paper for figures.