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Characterization and comparison of the temporal dynamics of ruminal bacterial microbiota colonizing rice straw and alfalfa hay within ruminants

Junhua Liu, Mengling Zhang, Chunxu Xue, Weiyun Zhu, and Shengyong Mao[1]
Jiangsu Key Laboratory of Gastrointestinal Nutrition and Animal Health, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

ABSTRACT

Three ruminally cannulated Holstein cows were used to characterize the dynamics of bacterial colonization of rice straw and alfalfa hay and to assess the differences in the composition and inferred gene function of the colonized microbiota between these 2 forages. Nonincubated (0 h) rice straw and alfalfa hay samples and residues in nylon bags incubated for 0.5, 2, 6, 16, and 48 h were analyzed for dry matter and were used for DNA extraction and MiSeq (Illumina Inc., San Diego, CA) sequencing of the 16S rRNA gene. The microbial communities that colonized the air-dried and nonincubated (0 h) rice straw and alfalfa hay were both dominated by members of the *Proteobacteria* (contributing toward 70.47% of the 16S RNA reads generated). In situ incubation of the 2 forages revealed major shifts in the community composition: *Proteobacteria* were replaced within 30 min by members belonging to the *Bacteroidetes* and *Firmicutes*, contributing toward 51.9 and 36.6% of the 16S rRNA reads generated, respectively. A second significant shift was observed after 6 h of rumen incubation, when members of the *Spirochaetes* and *Fibrobacteria* phyla became abundant in the forage-adherent community. During the first 30 min of rumen incubation, ~20.7 and 36.1% of the rice straw and alfalfa hay, respectively, were degraded, whereas little biomass degradation occurred between 30 min and 2 h after the rice straw or alfalfa hay was placed in the rumen. Significant differences were noted in attached bacterial community structure between the 2 forage groups, and the abundances of dominant genera *Anaeroplasma*, *Butyrivibrio*, *Fibrobacter*, and *Prevotella* were affected by the forage types. Real-time PCR results showed that the 16S rRNA copies of total bacteria attached to these 2 forages were affected by the forage types and incubation time, and higher numbers of attached bacterial 16S rRNA were observed in the alfalfa hay samples than in the rice straw from 0.5 to 16 h of incubation. The metagenomes predicted by phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) revealed that the forage types significantly affected 21 metabolic pathways identified in the Kyoto Encyclopedia of Genes and Genomes, and 33 were significantly changed over time. Collectively, our results reveal a difference in the dynamics of bacterial colonization and the inferred gene function of microbiota associated with rice straw and alfalfa hay within the rumen. These findings are of great importance for the targeted improvement of forage nutrient use efficiency in ruminants.

Key words: rumen, rice straw, alfalfa hay, microbiome

INTRODUCTION

Ruminant animals and ruminal microorganisms have evolved together for millions of years, resulting in a rumen microbial population consisting of a highly diverse collection of obligately anaerobic microorganisms, including fungi, protozoa, bacteria, and archaea (Russell and Rychlik, 2001). Within this microbiome, bacteria are the dominant domain and make the greatest contribution to digestion and conversion of feeds to volatile fatty acids and microbial proteins (Kim et al., 2011). A previous study showed that bacterial attachment is instrumental in the process of microbial degradation of dietary plant material (McAllister et al., 1994), and particle-associated bacteria account for 70 to 80% of rumen microbial matter (Craig et al., 1987). Therefore, studies on the mode of bacterial attachment and the subsequent digestion of the plant biomass are essential for improving ruminant nutrient use efficiency.

Over the last decade, many experiments have demonstrated that rumen microorganisms rapidly associate with and colonize recently ingested feed particles (Edwards et al., 2007; Huws et al., 2013, 2016; Piao et al., 2014, 2015), and these studies have revealed that the composition of colonizing microbial communities is affected by the incubation time (Bowman and Firkins,

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[1]Corresponding author: maoshengyong@163.com

1993; Huws et al., 2013, 2016; Piao et al., 2014, 2015). Forage type is recognized as an important factor that affects the rumination time, rumen fermentation, and performance of the ruminant (Welch and Smith, 1969; Brask et al., 2013; Ding et al., 2015). One forage type, rice straw, is abundant and inexpensive, but is typically considered low-quality roughage. A second type, alfalfa, is well known for its high quality and is used worldwide as an important dietary forage in ruminant production; nevertheless, rice straw is the predominant forage source for ruminants in the tropical zones of the world. These 2 forages are known to differ in the digestibility of their DM in the rumen (Yang et al., 2011), and these differences could be attributed to differences in the physical structure and chemical characteristics between the 2 types of forage. However, the difference in the composition of degrading and colonizing ruminal bacterial communities of the 2 forages may also be an important factor.

In the current study, we hypothesized that forages with different quality will affect the composition of the microbiota that colonize and degrade the forage within the rumen, which will likely be accompanied by a difference in community function. Therefore, the primary objective of this study was to compare the effects of forage sources (rice straw vs. alfalfa hay) on the composition, structure, and inferred function of ruminal particle-associated microbiota in dairy cows. An additional aim was to investigate the temporal changes in the bacterial communities that colonize and degrade rice straw and alfalfa hay in the rumen.

MATERIALS AND METHODS

Animals and Sample Collection

Three mature, rumen-cannulated, nonlactating Holstein cows (average BW: 502 ± 25 kg) were used in this study. Cows were individually housed in 3 stalls in a barn with good ventilation and were fed 15 kg of DM (75% Chinese wild grass hay, 25% corn-based diet) per animal per day. The cattle were fed at 0700 and 1730 h, with one-half of the allowed daily ration given at each feeding. The cattle had free access to drinking water. All animal care procedures were approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University.

Air-dried rice straw and alfalfa hay were ground into 2-mm pieces using a Wiley mill, and the ground samples were weighed into individual in situ nylon bags (10 × 20 cm; pore size = 50 μm). Three bags containing 2 g of forage for each of the 2 forage treatments and 1 blank bag (i.e., a total of 7 bags for each of the 5 time periods, for a total of 35 bags per cow) were placed into the rumen of each cow and removed after 0.5, 2, 6, 16, and 48 h. After removal from the rumen, 4 bags containing forages (2 for rice straw and 2 for alfalfa hay) and the blank bag were rinsed and manipulated in cold water until the water ran clear, then squeezed by hand to remove excess water and used for nutritional ingredient degradation analysis. The remaining 2 bags (one filled with rice straw and one with alfalfa for each cow) were washed with sterile saline to remove loosely attached microbes and then immediately frozen on dry ice and transported to the laboratory for storage at −80°C until DNA extraction.

Chemical Analysis

Relative biomass degradation during rumen incubation was determined by DM, CP, NDF, and ADF analysis. Dry matter and CP were determined according to AOAC methods (AOAC, 1990), and NDF and ADF were determined using the procedures of Larter (1992).

DNA Extraction and Quantitative Real-Time PCR Analysis

Total microbial genomic DNA was extracted from 200 mg of the nonincubated control sample and from each rumen-incubated rice straw and alfalfa sample. The DNA was extracted by a bead-beating method using a mini-bead beater (Biospec Products, Bartlesville, OK), followed by phenol–chloroform extraction, as described by Mao et al. (2012). The concentration and purity of DNA were determined using a NanoDrop Spectrophotometer (Thermo Scientific, Wilmington, DE), and DNA samples were stored at −80°C until further processing.

Total attached bacterial 16S rDNA was quantified by real-time PCR. Primer pairs for the total bacteria were bacF (5′-CCATTGTAGCACGTGTGTAGCC-3′) and total bacR (5′-CGGCAACGAGCGCAACCC-3′), as reported by Hook et al. (2011). The PCR amplifications were performed in triplicate with SYBR Green PCR Mastermix (Applied Biosystems, Foster City, CA) using a CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hertfordshire, UK). The total volume of reaction solution (20 μL) contained 0.5 ng of DNA, 10 μL of Fast SYBR Green Master Mix (Applied Biosystems), 0.4 μL of each primer (5 μ*M*), 6.8 μL of nuclease-free water, 0.4 μL of ROX Reference Dye (50×), and 2 μL of the template, as described previously (Mao et al., 2015). Amplification was carried out using the following program: 95°C for 30 s for the initial denaturation and then 40 cycles of 95°C for 5 s followed by annealing/extension for 30 s at 60°C. A standard