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Temporal changes of the bacterial community colonizing wheat straw in the cow rumen

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ABSTRACT

This study used Miseq pyrosequencing and scanning electron microscopy to investigate the temporal changes in the bacterial community tightly attached to wheat straw in the cow rumen. The wheat straw was incubated in the rumens and samples were recovered at various times. The wheat straw degradation exhibited three phases: the first degradation phase occurred within 0.5 h, and the second degradation phase occurred after 6 h, with a stalling phase occurring between 0.5 and 6 h. Scanning electron microscopy revealed the colonization of the microorganisms on the wheat straw over time. The bacterial communities at 0.5, 6, 24, and 72 h were determined, corresponding to the degradation phases. Firmicutes and Bacteroidetes were the two most dominant phyla in the bacterial communities at the four time points. Principal coordinate analysis (PCoA) showed that the bacterial communities at the four time points were distinct from each other. The wheat straw-associated bacteria stabilized at the phylum level after 0.5 h of rumen incubation, and only modest phylum-level and family-level changes were observed for most taxa between 0.5 h and 72 h. The relative abundance of the dominant genera, *Butyrivibrio*, *Coprococcus*, *Ruminococcus*, *Succiniclasticum*, *Clostridium*, *Prevotella*, YRC22, CF231, and *Treponema,* changed significantly over time ($P < .05$). However, at the genus level, unclassified taxa accounted for 70.3% ± 6.1% of the relative abundance, indicating their probable importance in the degradation of wheat straw as well as in the temporal changes of the bacterial community. Thus, understanding the function of these unclassified taxa is of great importance for targeted improvement of forage use efficiency in ruminants. Collectively, our results revealed distinct degradation phases of wheat straw and corresponding changes in the colonized bacterial community.

1. Introduction

Dietary forage consumed by ruminants is degraded by the ruminal microbiota, which include bacteria, fungi, protozoa, and methanogens [1]. Attachment of the microbes to the consumed forages is a key step in the process of forage degradation [2]; more than 70% of the microbial organic matter in whole rumen contents was associated with the particulate phase [3]. Within the ruminal microbial community, bacteria are the most abundant domain and make the greatest contribution to the digestion and conversion of feeds into volatile fatty acids and microbial proteins [4]. Understanding the nature of the bacterial community attached to plant tissues is therefore essential for improving the efficiency of ruminal forage utilization.

The rumen microbiota can rapidly colonize freshly ingested plant tissues. For example, Edwards et al. [5] reported that bacteria numbers rapidly increased, within 5 min, on nonconserved perennial ryegrass and stabilized after 15 min of incubation in the rumen. Huws et al. [6] also reported colonization of bacteria onto freshly ingested perennial ryegrass within 15 min. In addition, temporal changes in bacterial populations were also reported following ruminal incubation of fresh perennial ryegrass [5,7], switchgrass [8], rice straw, and alfalfa [9]. The bacterial communities involved in the degradation of these different forages are also distinct [9], which may reflect differences in forage components. Therefore, the type of forage is also an important factor that affects rumen digestion and ruminant performance [10].

One key forage source for ruminants in temperate zones around the world is wheat straw. This type of forage is abundant and cheap, but low in quality. Efficient utilization of wheat straw in ruminants

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not only reduces feed cost, but it also has positive environmental benefits, especially in China, where wheat straw disposal normally consists of burning in the field. Little is known regarding the bacterial species that attach to wheat straw over time during rumen incubation, but increased knowledge of the attached microbes and their temporal variations could ultimately aid in the development of novel strategies for improving the feed use efficiency in ruminants.

In the present study, we hypothesized that the rumen bacterial communities colonizing wheat straw would show shifts over time and would be distinct from those attached to other forages. We therefore used Miseq sequencing to determine the bacterial communities colonizing wheat straw at different incubation times in the cow rumen, and we compared these with the bacterial communities colonizing other forages.

2. Materials and methods

2.1. Animals

Three ruminally cannulated Holstein cows were used in this study. The diet contained (on a dry matter basis): 20% corn silage, 30% Chinese wildrye, 20% alfalfa hay, 15% corn, 2% wheat bran, 10% soybean meal, 0.7% calcium carbonate, 0.8% calcium orthophosphate, 0.5% sodium chloride, and 1% mineral and vitamin premix (Supplementary Table S1). The cows were fed three times per day, at 4:00 am, 10:30 am, and 4:00 pm. The animals had received the ration more than one month to equilibrate the ruminal populations before the experiment was conducted. During the trial, the management of cows remained unchanged. All animal use procedures were approved by the Animal Care and Use Committee of Nanjing Agricultural University.

2.2. Experimental design and sample collection

The wheat straw was dried at 65 °C and then milled through a 0.5 mm screen, before being weighed into nylon bags with 38.5 μm pores (2.5 g/bag). Eighteen bags were placed in the rumen of each of the three cows before the 4:00 a.m. feeding. Two bags per cow were then recovered at 0.5, 1, 2, 4, 6, 12, 24, 48, and 72 h, for a total of six bags at each time point. All bags were washed gently with PBS buffer (pH 7.4). Samples of the residues of digested wheat straw in each bag were removed and fixed with 2.5% glutaraldehyde for scanning electron microscopy (SEM). Other residue samples were used for determination of tightly attached bacteria, using the protocol described by Larue et al. [11]. The remaining digested wheat straw was washed and dried at 65 °C for analysis of the dry matter.

2.3. Degradation analysis of wheat straw

The dry matter (DM) of wheat straw was determined according to AOAC (2000) [12]. NDF and ADF were determined by the method of van Soest et al. [13].

2.4. DNA extraction, real-time PCR quantification

DNA was extracted by the bead-beating and phenol-chloroform-isoamyl alcohol extraction method of Zoetendal et al. [14]. A FastPrep®-24 Instrument (MP Biomedicals, South Florida, USA) was run at a setting of 5 for 2 min for the bead-beating step. The DNA was suspended in 50 μL Tris-EDTA buffer and quantified using a NanoDrop ND-1000 Spectrophotometer (Nyxor Biotech, Paris, France).

Real-time PCR was performed to quantify the amounts of bacteria using an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems, California, USA) and a SYBR® Premix Ex Tag TM kit (TaKaRa, Dalian, China). The universal bacterial primers used in this study were the following: forward primer: 5′- CCT ACG GGA GGC AGC AG - 3′, and reverse primer: 5′- ATT ACC GCG GCT GCT GG - 3′) [15]. The amount of DNA was determined in triplicate. External standards were prepared by making 10-fold serial dilutions of purified plasmid DNA of bacterial clones. Results were expressed as the number of marker gene copies per gram dry weight of the digested wheat straw residue.

2.5. Scanning electron microscopy

The samples were washed three times with phosphate buffered saline (pH 7.4) and then dehydrated using 50, 70, 80, 90, and 100% ethanol. The ethanol was replaced with tertiary butyl alcohol, prior to drying using an ES-2030 freeze dryer (Hitachi, Tokyo, Japan). The dried samples were then sputter coated with 10 nm of Au/Pd using an E-1010 Ion sputter (Hitachi, Tokyo, Japan). SEM images were obtained with an S-3000 N microscope (Hitachi, Tokyo, Japan).

2.6. Illumina miseq sequencing and data processing

PCR was run with the bacterial universal primers (341F 5′-CCTAYGGGRBGCASCAG - 3′ and 806R 5′- GGACTACNNGGGTATCTAAT - 3′), according to Behrendt et al. [16]. The PCR amplicons were sequenced by paired-end sequencing on an Illumina MiSeq

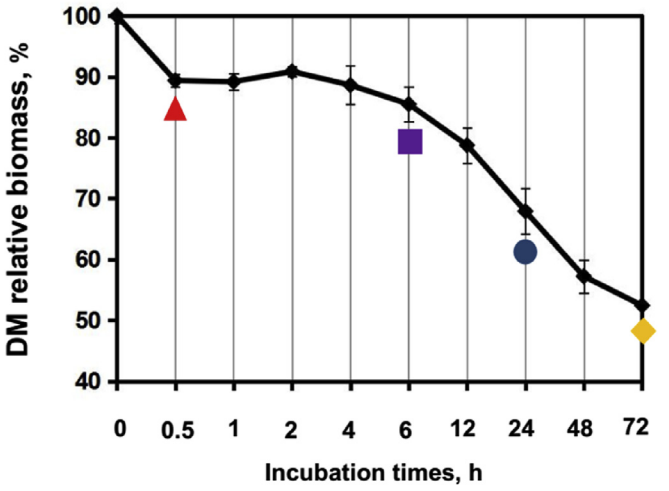

Fig. 1. Biomass degradation during rumen *in situ* incubation. The line graph shows the relative DM change during incubation.

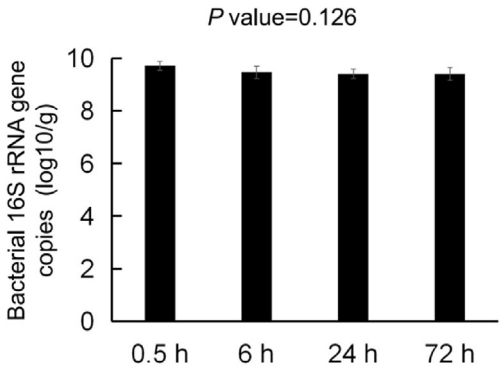

Fig. 2. The 16S rRNA genes copy numbers of rumen bacteria colonizing wheat straw at 0.5, 6, 24, and 72 h after incubation.