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**Bacterial community composition and fermentation in the rumen of Xinjiang
brown cattle (*Bos taurus*), Tarim red deer (*Cervus elaphus yarkandensis*), and
Karakul sheep (*Ovis aries*)**

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Abstract

The rumen microbiota plays a major role in the metabolism and absorption of indigestible food sources. Xinjiang brown cattle (*Bos taurus*), Tarim red deer (*Cervus elaphus yarkandensis*), and Karakul sheep (*Ovis aries*) are important ruminant species for animal husbandry in the Tarim Basin. However, the microbiota and rumen fermentation of these animals are poorly understood. Here, we apply high-throughput sequencing to examine the bacterial community in the rumen of cattle, red deer and sheep and measured rumen fermentation products. Overall, 548,218 high-quality sequences were obtained and then classified into 6,034 operational taxonomic units (OTUs). *Prevotella* spp., *Succinivibrionaceae* spp., and unclassified bacteria within the families *Succinivibrionaceae*, *Lachnospiraceae*, and *Veillonellaceae* were the dominant bacteria in the rumen across the three hosts. Principal coordinate analysis (PCoA) identified significant differences in the bacterial communities across the three hosts. *Pseudobutyrvibrio* spp., *Oscillospira* spp., and *Prevotella* spp. were more prevalent in the rumen of the cattle, red deer and sheep, respectively. Among the three hosts, the red deer rumen had the greatest amounts of acetate and butyrate and the lowest pH value. These results showed that *Prevotella* spp. are the dominant bacteria in the rumen of the cattle, red deer and sheep, providing new insight into the rumen fermentation of ruminants distributed in the Tarim Basin.

Key words: ruminants; microbiota; *Prevotella* spp.; fermentation products

Introduction

The suborder Ruminantia, which includes the families Cervidae and Bovidae, is the most important group of large terrestrial herbivorous mammals (Fernández and Vrba 2005). Among these mammals, ruminants are particularly interesting because they are ecologically and physiologically diverse and have the ability to ingest large amounts of fibrous plants (Clauss et al. 2010). However, ruminants do not produce fiber-degrading enzymes to digest fibrous plant materials; instead, they depend on a diverse and dense array of microorganisms, including bacteria, fungi, archaea and protozoa (Wright and Klieve 2011). Among these microorganisms, bacteria play a major role in degrading plant biomass, and the subsequent rumen fermentation produces ammonia and volatile fatty acids (VFAs), such as acetate, propionate and butyrate (Puniya et al. 2015). These end products supply the host with essential nutrients and metabolic energy (i.e., VFAs) (Bergman 1990). Therefore, the rumen microbiota directly affects the host's physiology, health and production.

Xinjiang brown cattle (*Bos taurus*), Tarim red deer (*Cervus elaphus yarkandensis*), and Karakul sheep (*Ovis aries*) are important ruminant species in the animal husbandry industry of the Xinjiang Uygur Autonomous Region, China. These animals are distributed along the southeastern edge of the Tarim Basin, which is surrounded by the Taklimakan Desert, and they have adapted to the extremely harsh living conditions by incorporating *Phragmites communis* and *Populus diversifolia* as the main forage in their diet (Qiao et al. 2006). However, the rumen microbiota of these animals fed typical diets has not been characterized to date. Red deer are

considered an endangered species by the International Union for the Conservation of Nature (IUCN) (Halk et al. 2004), and examining the bacterial community could improve our understanding of the rumen ecology of endangered species and provide valuable information for species conservation strategies.

The host-associated microbiota is significantly affected by heritable factors, such as host genetics (Goodrich et al. 2014), environmental factors such as the diet composition (Henderson et al. 2015; Smith et al. 2015), and the geographic location (Moeller et al. 2013). Therefore, the rumen microbiota may differ among Xinjiang brown cattle, Karakul sheep and Tarim red deer. In addition, previous studies have demonstrated that diet plays a role in shaping the gastrointestinal microbiota (Carmody et al. 2015). Pérez-Barberia *et al.* (2004) revealed the rate of evolution of fiber digestibility to be related to climatically driven environmental changes over the past several million years. Moeller *et al.* (2013) showed that geographic isolation among host species promoted evolutionary differentiation in the gut bacterial community of great apes. Considering the main forages of the three studied hosts, including *Phragmites communis* and *Populus diversifolia* (Qiao et al. 2006), examining rumen fermentation will improve our understanding the roles of the bacterial community.

In the present study, we aimed to investigate the bacterial community of the rumens of Xinjiang brown cattle, Karakul sheep and Tarim red deer distributed in a desert region, and examine and compare the rumen fermentation parameters across the three hosts.

Materials and methods

Animals and sample collection

Three rumen-cannulated brown cattle [mean body weight (MBW) = 400 ± 5.5 kg], three Tarim red deer (MBW = 140 ± 1.6 kg), and three karakul sheep (MBW = 36 ± 1.2 kg) were used in this study and maintained at the experimental animal center of Tarim University. All of the animals were assigned to individual pens under the following conditions: 30°C mean temperature and 55 mm annual rainfall, from August to September of 2015. The animals were fed twice each day at 8:00 AM and 7:00 PM and provided free access to water. The brown cattle, Tarim red deer and Karakul sheep were fed the same typical diet of forage and concentrate (forage:concentrate = 70:30, dry matter basis, **Table 1**) for 20 days. All of the animal procedures were approved and authorized by the animal ethics committee of Tarim University. Over the last three days of the feeding period, the whole rumen contents were collected at 2 h after the morning feeding. The rumen samples were stored at -80°C for further analysis.

DNA extraction, sequencing and bioinformatics analyses

The total genomic DNA of the rumen microorganisms was extracted from the whole rumen contents of each animal using the QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. The universal primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the V4 region of the bacterial 16S rRNA gene (Caporaso et al. 2012). The amplicons were quantified using

a QuantiFluor[®]-P Fluorometer (Promega, CA, USA) and sequenced on an Illumina HiSeq 2500 platform to generate 250 bp paired-end reads.

The sequences were processed and analyzed using QIIME ver. 1.7.0 (Caporaso et al. 2010). Chimeric sequences were detected and removed using the software Uchime (Edgar et al. 2011). The remaining sequences were classified into operational taxonomic units (OTUs) based on a sequence identity of 97% at the species level using UPARSE (Edgar 2013). The representative sequences of each OTU were searched against the GreenGene database using the Ribosomal Database Project (RDP) classifier with a 0.80 confidence threshold (DeSantis et al. 2006; Wang et al. 2007). Good's coverage estimator and the Shannon-Wiener, Simpson, and Chao 1 alpha diversity indices were calculated for all samples using QIIME ver. 1.7.0 (Caporaso et al. 2010). To compare the bacterial communities in the rumen across all three hosts, the unweighted UniFrac distances (which examine the presence and absence of bacterial lineages) and weighted UniFrac distances (which consider the relative abundance of bacterial lineages) were used in the principal coordinate analysis (PCoA) (Lozupone et al. 2011). The sequences were deposited in the Sequence Read Archive under accession number SRP090262.

Measurement of ruminal fermentation parameters

The pH value of the ruminal contents was immediately determined using a pH meter (FE20, Mettler-Toledo, Switzerland). The amount of ammonia was measured according to the phenol-sodium hypochlorite method as previously described (Li et al. 2015). The concentrations of VFAs in the rumen were analyzed via gas

chromatography (TP-2060F, Beijing BeifenTianpu Instrument Technology Co. Ltd., Beijing, China) with a flame ionization detector and a PEG-20M H₃PO₄ glass column. The flow rate of the carrier gas was 30 ml/min with the column temperature at 120 °C, and the detector temperature was 220 °C.

Statistical analysis

Values for the bacterial composition and fermentation parameters were expressed as the mean $\pm$ S.D. (standard deviation). Multiple sample comparisons were performed using a one-way analysis of variance (ANOVA). $P < 0.05$ was regarded as a significant difference.

Results

Summary of the sequencing data

A total of 548,218 high-quality sequences were obtained for the bacterial 16S rRNA gene. Based on a 97% sequence identity, these bacterial sequences were assigned to 6,034 OTUs (**Table 2**). Good's coverage estimate, which reflects sequencing efforts, showed that 98.0 ± 0.1 (Mean $\pm$ S.D.) % of the total bacterial species were captured in any given sample. The comparisons across all three hosts showed that the richness (Chao1) and diversity (Shannon) indices were not significantly different among the three hosts ($P > 0.05$).

Bacterial composition in the rumen of three hosts

Overall, 53 phyla were identified in the rumen across the three hosts, and they were dominated by Bacteroidetes (cattle = $42.7 \pm 3.6\%$, red deer = $36.2 \pm 3.2\%$, and sheep = $46.6 \pm 6.6\%$), Proteobacteria (cattle = $19.6 \pm 2.0\%$, red deer = $24.7 \pm 3.3\%$,

and sheep = $23.2 \pm 5.7\%$), and Firmicutes (cattle = $20.4 \pm 2.7\%$, red deer = $17.0 \pm 2.8\%$, and sheep = $15.5 \pm 2.6\%$) (**Figure 1A**).

At the genus level, *Prevotella* spp. ($26.8 \pm 3.3\%$) were the most dominant bacteria in the rumen of the cattle, and it was followed by *Succiniclasticum* spp. ($3.5 \pm 1.2\%$), unclassified bacteria within the families Lachnospiraceae ($2.0 \pm 0.3\%$) and Veillonellaceae ($1.7 \pm 0.7\%$), and *Butyrivibrio* spp. ($1.3 \pm 0.4\%$). In the rumen of the red deer, *Prevotella* spp. ($23.1 \pm 3.5\%$) were the dominant bacteria, followed by *Succiniclasticum* spp. ($2.8 \pm 1.2\%$) and unclassified bacteria within the families Succinivibrionaceae ($2.4 \pm 0.8\%$), Lachnospiraceae ($1.6 \pm 0.4\%$), and Veillonellaceae ($1.6 \pm 0.7\%$). In the rumen of the sheep, *Prevotella* spp. ($34.2 \pm 3.8\%$) were the predominant bacteria, followed by *Selenomonas* spp. ($2.3 \pm 1.5\%$) and unclassified bacteria within the families Veillonellaceae ($2.2 \pm 0.5\%$), Lachnospiraceae ($1.4 \pm 0.4\%$), and Succinivibrionaceae ($1.1 \pm 0.03\%$) (**Figure 1B**).

Comparison of the rumen bacterial composition across all three hosts

The unweighted UniFrac distance (presence/absence of bacterial taxa) showed that the bacterial community composition in the rumen of the cattle, red deer, and sheep was different, and it explained 37.92% of the variance (**Figure 2A**). Moreover, the weighted UniFrac distance (based on the presence-absence and the relative abundance of bacterial taxa) for the differences in the bacterial community structure across all three hosts explained 58.46% of the variance (**Figure 2B**). We further compared the relative abundance of bacteria in the rumen across the three hosts (**Figure 2C**), and found that the abundance of *Pseudobutyrvibrio* spp. ($0.21 \pm 0.01\%$)

was significantly higher in the rumen of the cattle than the sheep ($0.03 \pm 0.001\%$, $P = 0.0008$) and red deer ($0.08 \pm 0.002\%$, $P = 0.002$). The proportion of *Oscillospira* spp. in the rumen of the cattle ($0.06 \pm 0.002\%$, $P = 0.02$) and sheep ($0.06 \pm 0.003\%$, $P = 0.02$) was significantly lower than that of the red deer ($0.14 \pm 0.02\%$). The genus *Prevotella* was more abundant in the rumen of the sheep ($34.2 \pm 3.8\%$) than in the rumen of the red deer ($23.1 \pm 3.5\%$, $P = 0.03$), whereas the relative abundance of *Streptococcus* spp. in the rumen of the sheep ($0.007 \pm 0.004\%$) was lower than that in the rumen of the red deer ($0.02 \pm 0.003\%$, $P = 0.04$).

Comparison of fermentation parameters among different animal species

The pH in the rumen of cattle (6.09 ± 0.09) and sheep (6.10 ± 0.12) was significantly higher than that of red deer (5.94 ± 0.03 , $P < 0.05$). The concentration of ammonia in the rumen across the three hosts was not significantly different ($P > 0.05$). The concentrations of acetate (43.4 ± 1.4) and butyrate (27.5 ± 4.7) in the rumen of the red deer were significantly higher than those of the cattle (acetate: 38.1 ± 3.5 ; butyrate: 17.0 ± 3.2) and sheep (acetate: 38.9 ± 3.6 ; butyrate: 23.5 ± 3.6). The amounts of propionate, isobutyrate and isovalerate in the rumen of the cattle tended to be greater than that in the rumen of the red deer and sheep, whereas the concentration of valerate in the rumen of the sheep tended to be greater than that in the rumen of the cattle and red deer, although the differences were not significant (**Figure 3**).

Discussion

In this study, we examined and compared the rumen bacterial community and fermentation parameters in the rumens of Xinjiang brown cattle, Tarim red deer and

Karakul sheep. The results indicated that *Prevotella* spp. were the dominant bacteria in the rumen across the three hosts. Moreover, the relative abundance of bacteria and the amounts of fermentation products also differed across the three hosts.

The results presented here indicate that *Prevotella* spp. are the dominant bacteria in the rumen of the cattle, red deer, and sheep. This result was consistent with the findings for other ruminant species, including Bovinae, Cervidae, Caprinae, Camelidae and Giraffidae (Henderson et al. 2015). Moreover, the predominant bacteria in the rumen of red deer were similar to those in elk, white-tailed deer, roe deer, sika deer, reindeer and moose (Gruninger et al. 2014; Ishaq and Wright 2014; Li et al. 2013; Li et al. 2014; Sundset et al. 2007). This result suggest an effect on rumen microbiota by host genetics (Moeller et al. 2013). *Prevotella* spp. are a bacterial group that presents genetic and metabolic diversity in rumen microbial communities (Bekele et al. 2010; Purushe et al. 2010). Previous studies demonstrated that *Prevotella* spp. contained active hemicellulolytic and proteolytic enzymes (Matsui et al. 2000), which played roles in degrading starch, xylan, lignans and pectin (Cotta 1992; Gardner et al. 1995). Additionally, metagenomic analyses in the rumen of Svalbard reindeer and sika deer also suggested that *Prevotella* spp. plays a potential role in cellulose degradation (Li et al. 2013; Pope et al. 2012). Indeed, Huws *et al.* (2016) demonstrated that a high abundance of *Prevotella* spp. was observed during the successional colonization of fresh perennial ryegrass in the rumen of cows, regardless of temporal changes. Taken together, these results suggest the potential roles of *Prevotella* spp. in the decomposition of plant fiber materials.

The results also showed that *Succiniclasticum* spp. were the second most predominant bacteria in the rumen of the cattle and red deer, whereas unclassified bacteria within the families Succinivibrionaceae and Lachnospiraceae were abundant in the rumen across the three hosts. The ecological roles or physiological properties of *Succiniclasticum* spp. in the rumen are poorly understood because only one isolate has been cultured *in vitro*. Van Gylswyk (1995) reported that *Succiniclasticum ruminis* plays a major role in converting succinate to propionate as the sole energy-yielding mechanism. Certain bacterial members within the family Succinivibrionaceae produce succinate as the principal fermentation product, and a number of exogenous sources of hydrogen could stimulate the formation of succinate (Pope et al. 2011; Stackebrandt and Hespell 2006), which was dominant in the rumen of sika deer (Li et al. 2015). Bacteria within the family Lachnospiraceae produced butyrate (Cotta and Forster 2006), which was also dominant in the rumens of moose and various arctic ruminants (Ishaq and Wright 2012; Ishaq and Wright 2014) as well as in the foregut of dromedary camels in Australia (Samsudin et al. 2011). Taken together, these results suggested that these bacteria may contribute to the converting plant materials to propionate and butyrate to meet the host's energy demands under harsh conditions.

The present study also showed a significant difference in the bacterial community composition across the three hosts. The difference among the three hosts was increased with the weighted UniFrac distance (based on presence-absence and relative abundance of bacterial taxa) compared with the unweighted UniFrac distance (presence/absence of bacterial taxa, **Figures 2A and 2B**), indicating that the relative

abundance of certain bacteria contributed to the bacterial community difference. *Pseudobutyrvibrio* spp., *Oscillospira* spp., and *Prevotella* spp. were more prevalent in the rumen of the cattle, red deer and sheep, respectively (**Figure 2C**). *Pseudobutyrvibrio* spp. play fibrolytic roles during rumen fermentation because they contain xylanases that randomly cleave the β -1,4-backbone of the complex plant cell wall polysaccharide xylan (Grilli et al. 2012; Krause et al. 2003). In addition, amylase can be produced by the members of *Pseudobutyrvibrio* spp. in the rumen (Moon et al. 2008). These results suggested an important role of these bacteria during rumen fermentation in cattle. *Oscillospira* spp. are broadly observed in various rumen ecosystems, such as those of cattle, sheep and reindeer, and the level is dependent on the dietary composition (Mackie et al. 2003). However, a pure isolate within *Oscillospira* spp. has never been cultured (Konikoff and Gophna 2016). Previous studies showed that members of the *Oscillospira* genus were enriched in lean subjects and unlikely to represent complex fiber degraders in the human gut (David et al. 2014; Goodrich et al. 2014). Moreover, a previous study demonstrated that venison has a high protein and low fat content (Asano et al. 2005), indicating that *Oscillospira* spp. may play a role in rumen fermentation and host metabolism.

In the present study, we observed that the amount of acetate in the rumen of the red deer was significantly greater than that of the cattle and sheep, whereas the pH value in the rumen of the red deer was significantly lower than that of the cattle and sheep (**Figure 3**). Moreover, the concentrations of isobutyrate and isovalerate in the rumen of the studied cattle tended to be greater than that in the rumen of the sheep

and red deer (**Figure 3**). Isobutyrate and isovalerate are degradation products derived from branched-chain amino acids, such as leucine, valine, and isoleucine (Allison 1978). Thus, our results indicated an increased level of protein degradation in the rumen of the cattle. The changed amounts of isobutyrate and isovalerate may be related to particle retention in the rumen, which is associated with the body of the ruminant and represents a heritable trait (Goopy et al. 2014). Asano *et al.* (2005) reported that the rate of ruminal passage was higher in sika deer than in cattle. The longer particle passage rate of the particulate and liquid fractions resulted in changes to the protozoal composition because the generation of protozoa was observed to adapt to changes in the substrates as well as the turnover rate in the rumen (Sylvester et al. 2009), thereby contributing to the increased degradation of amino acids from protozoa (Newbold et al. 2015). In future studies, the relationship between protozoa and nutrient digestibility in the rumen across the three hosts must be further documented.

In the present study, we demonstrated that *Prevotella* spp. were the dominant bacteria in the rumen of the studied cattle, red deer, and sheep. In addition, the amounts of rumen fermentation products suggested that the rumen metabolism across the three hosts differed, which may have been caused by changes in the rumen microbiota. These results expand our knowledge of the rumen ecology of ruminants distributed in the Tarim Basin, which presents harsh environmental conditions.

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Figure legends

Figure 1. Rumen bacterial compositions at the phylum (A) and genus (B) levels across the three hosts.

Figure 2. Comparisons of the bacterial communities in the rumen of the cattle, red deer and sheep. Principal coordinate analyses based on unweighted UniFrac distance (A) and weighted UniFrac distance among the groups (B). Significant differences were observed at the genus level across the three hosts (C). * $P < 0.05$, * * $P < 0.01$, *** $P < 0.001$.

Figure 3. Comparison of the concentrations of volatile fatty acids and $\text{NH}_3\text{-N}$ in the rumen of the cattle, red deer and sheep. * $P < 0.05$, * * $P < 0.01$, *** $P < 0.001$.

Table 1. Ingredient composition of the diet fed to the cattle, red deer and sheep.

Formula	Ingredient	% (Dry matter)
Forage	Reed	40.00
	Dried poplar leaf	30.00
Concentrate	Corn starch	18.63
	Wheat hull	4.00
	Soybean meal	4.20
	Bone powder	0.16
	Salt	0.50
	Additive	1.00
	Linseed meal	1.50

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Table 2. Summary of the sequencing data and alpha diversity across the three hosts

Samples	Sequences	OTUs	Good's coverage	Shannon	Simpson	Chao1
Cattle1	60,076	3181	0.98	8.73	0.99	3637.19
Cattle2	60,066	2997	0.98	8.81	0.99	3427.09
Cattle3	66,689	3549	0.98	9.23	0.99	4052.88
Red deer1	56,787	3028	0.98	8.84	0.99	3502.01
Red deer2	66,412	3593	0.98	9.19	0.99	4180.43
Red deer3	56,937	3110	0.99	9.35	0.99	3428.90
Sheep1	51,348	3050	0.97	7.84	0.97	5620.82
Sheep2	62,229	3195	0.98	8.13	0.98	3643.49
Sheep3	67,674	3060	0.98	7.96	0.96	3560.85

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A

B

C