Molecular diversity of rumen bacterial communities from tannin-rich and fiber-rich forage fed domestic Sika deer (Cervus nippon) in China

Zhi Peng Li1, Han Lu Liu1, Guang Yu Li1, Kun Bao1, Kai Ying Wang1, Chao Xu1, Yi Feng Yang1, Fu He Yang1 and André-Denis G Wright2

Abstract

Background: Sika deer (Cervus nippon) have different dietary preferences to other ruminants and are tolerant to tannin-rich plants. Because the rumen bacteria in domestic Sika deer have not been comprehensively studied, it is important to investigate its rumen bacterial population in order to understand its gut health and to improve the productivity of domestic Sika deer.

Results: The rumen bacterial diversity in domestic Sika deer (Cervus nippon) fed oak leaves- (OL group) and corn stalks-based diets (CS group) were elucidated using 16S rRNA gene libraries and denaturing gradient gel electrophoresis (DGGE). Overall, 239 sequences were examined from the two groups, 139 clones from the OL group were assigned to 57 operational taxonomic units (OTUs) and 100 sequences from the CS group were divided into 50 OTUs. Prevotella-like sequences belonging to the phylum Bacteroidetes were the dominant bacteria in both groups (97.2% OL and 77% CS), and sequences related to Prevotella brevis were present in both groups. However, Prevotella shahii-like, Prevotella veroralis-like, Prevotella albensis-like, and Prevotella salivae-like sequences were abundant in the OL group compared to those in the CS group, while Succinivibrio dextrinosolvens-like and Prevotella ruminicola-like sequences were prevalent in the CS group. PCR-DGGE showed that bacterial communities clustered with respect to diets and the genus Prevotella was the dominant bacteria in the rumen of domestic Sika deer. However, the distribution of genus Prevotella from two groups was apparent. In addition, other fibrolytic bacteria, such as Clostridium populeti and Eubacterium cellulosolvens were found in the rumen of domestic Sika deer.

Conclusions: The rumen of domestic Sika deer harbored unique bacteria which may represent novel species. The bacterial composition appeared to be affected by diet, and sequences related to Prevotella spp. may represent new species that may be related to the degradation of fiber biomass or tannins. Moreover, the mechanism and biological functions of Prevotella spp. in the rumen ecosystem, and synergistic interactions with other microorganisms should be noticed.

Keywords: Ecology, Prevotella, Fiber, Tannin
Background

Sika deer (*Cervus nippon*) represent the most ancient and primitive members of the genus *Cervus* because of the simple structure of their antlers, which is very distinct from those of reindeer. Velvet antlers are one of the main products from Sika deer, and are used in traditional Chinese medicine. In addition, Sika deer yield high quality meat and skin. Domestication of Sika deer began much later than for other ruminants. At present, the number of domesticated Sika deer in China is approximately 550,000 head, most of which are distributed in northwestern China.

In nature, Sika deer graze a wide range of forage types, such as Amur grape, elm, maple, bamboo and some toxic species including Chinese Stellera roots and large flowered larkspurs. Moreover, grazing Sika deer have been observed to prefer tannin-rich plants, such as oak leaves. Similar behavior has also been observed in wild Sika deer (*Cervus nippon yesoensis*) inhabiting the Shiretoko Peninsula of Hokkaido Island in Japan, and in the roe deer (*Capreolus capreolus*) [1,2]. However, domesticated Sika deer held in captivity are commonly fed corn stalks containing a much higher fibrous content. Like other ruminants, Sika deer depend on the rumen for fermentation that involves the conversion of plant fiber to volatile fatty acids. This involves a diverse and dense array of microorganisms, including bacteria, fungi, archaea and protozoa [3]. Among these microorganisms bacterial populations have been extensively studied for many years since rumen bacteria have important roles in the efficient degradation of plant biomass and detoxification of secondary compounds in plants [1,4-7]. This has led to a variety of studies investigating rumen bacterial structure have been conducted on domestic cows, sheep, yak, Reindeer in Norway and wild Sika deer in Japan [4,5,8-10]. Moreover, rumen bacterial communities are affected by the host and diet [11,12]. To our knowledge, very little is known about the rumen bacterial community of domesticated Sika deer in China. A comprehensive understanding of bacterial ecology in the rumen of domesticated Sika deer is necessary to increase the efficiency of fiber digestion and to improve the productivity of velvet antlers.

Thus, we hypothesize the bacterial communities in the rumen of domesticated Sika deer may be unique. And the objectives of the present study were: (1) to describe the bacterial diversity in the rumen from domesticated Sika deer ingesting different diets based on 16S rRNA gene sequence libraries and PCR-DGGE; and (2) to compare the unique rumen bacterial populations of domesticated Sika deer ingesting tannin-rich and fiber-rich materials.

Results

Comparative analysis of 16S rRNA gene libraries from two groups

A total of 239 non-chimeric sequences were analyzed, 139 sequences from the OL 16S rRNA clone library and 100 sequences from the CS clone library. The two rumen bacterial populations were distinct according to the RDP classifier tool at a confidence threshold of 80% (Figure 1). Within the two groups, members of the phylum Bacteroidetes were the predominant bacteria (99.3% and 85% of clones in the OL and CS groups, respectively). Domesticated Sika deer consuming corn stalks has Firmicutes present whereas they were not found in oak leaves fed domesticated Sika deer. Similarly, Proteobacteria were more expressed in corn stalks than oak leaves diets. The Chao1 (114.2 vs 143.5) and Shannon-Wiener (3.5 vs 3.7) indices of domesticated Sika deer consuming oak leaves were decreased compared to those feeding on corn stalks (Table 1). Moreover, the Libshuff analysis also showed that the bacterial communities between two diets were significantly differed ($p<0.0001$). Rarefaction curves at 3% distance levels revealed 74% and 66% coverage for the OL and CS groups, respectively (Figure 2).

Using the software program MOTHUR and a sequence identity criterion cut off of 97%, the 139 OL clone sequences were assigned to 57 OTUs and the 100 CS clone sequences were assigned to 50 OTUs (Table 1). To determine the nearest valid related species, the 16S rRNA gene sequences were compared using GenBank’s Basic Local Alignment Search Tool (BLAST). Within the OL library, 53 of the 57 OTUs (i.e. 97.2% of clones) had 85% or greater sequence identities to genus *Prevotella* (Table 2). Within these OTUs, 23 OTUs (38.1% of clones) showed 87-92% sequence identities to *P. brevis*, 11 OTUs (16.5% of clones) had 86-90% sequence identities to *P. shahii*, 3 OTUs (23.8% of clones) had 91-92% sequence identities to *P. veroralis*, 6 OTUs (12.3% of clones) had distant sequence identities to *P. salivae*, and the remaining 9 OTUs (6.5% of clones) showed sequence identities to several *Prevotella* species including *P. albensis*, *P. dentalis*, *P. ruminicola*, *P. multiformis*, *P. sterecorea*, *P. bryantii* and *P. copri* (Table 2). Of the remaining 4 OTUs (of the 57 total OTUs), 2 OTUs (1.4% of clones) were distantly related (85%) to *Alistipes shahii*, 1 OTU (0.7% of clones) had 84% identity to
Barnesiella intestinohominis, and 1 OTU (0.7% of clones) had 97% sequence identity to *S. dextrinosolvens*.

Within the CS clone library, 36 of the 50 OTUs were 85-98% related to species belonging to genus *Prevotella*. Within these 36 OTUs, only one OTU (2% of clones) had >97% sequence identity to *P. brevis*, 14 OTUs (36% of clones) had 90-93% identity to *P. brevis* and 11 OTUs (27% of clones) had 91-95% identity to *P. ruminicola* making them the dominant bacterial species, whereas the remaining 10 OTUs (12% of clones) exhibited distant sequence identity to *P. shahii*, *P. veroralis*, *P. albensis*, *P. saliva* and *P. dentalis*. Of the remaining 14 OTUs (of the 50 total), 3 OTUs (3% of clones) were distantly related (89%) to *Paraprevotella clara*, 1 OTU (9% of clones) showed 97% identity to *S. dextrinosolvens*, 3 OTUs (3% of clones) had 90-95% identity to *Ruminococcus bromii*, 2 OTUs (2% of clones) had 84% identity to *Parabacteroides merdae*, 1 OTU (1% of clones) was 86% related to *Clostridium aldrichii*, and 1 OTU (1% of clones) was 91% related to *Clostridium bolteae*, 4 other OTUs (4% of clones) showed distant sequence identities to *Roseburia hominis*, *Proteiniphilum acetatigenes*, *A. shahii* and *Sporanaerobacter acetigenes*, respectively.

Overall, phylogenetic analysis revealed that the 107 OTUs were divided into six distinct phylogenetic groups (Figure 3). In addition, the comparison between Norwegian reindeer, Svalbard reindeer and domesticated Sika deer at community level with Fast UniFrac [13], which analyze phylogenetic lineages, showed that the bacterial composition in the rumen of domesticated Sika deer fed oak leaves based diets was more similar to that of domesticated Sika deer fed corn stalks based diets, and differed from Svalbard reindeer and Norwegian reindeer (Figure 4). However, there were also shared bacterial communities between domestic Sika deer and Reindeer.

### Rumen bacterial diversity based on the PCR-DGGE profile

PCR-DGGE banding profiles showed that the bacterial communities clustered with respect to diets (Figure 5). However, considerable animal-to-animal variation was also observed. A distinct difference in the bacterial structure was observed between two diets. By comparing the PCR-DGGE profiles between the two diets, the number of DGGE bands from CS group was considerably abundant compared to those from OL group (Figure 5). There were also several bands that were common for all domestic Sika deer.

In total, 47 dominant bands were excised from the PCR-DGGE profile and sequenced, of which 20 and 27 bands obtained from the OL and CS groups, respectively (see Additional file 1). Sequences from the excised bands from the OL group belonged to the phyla Firmicutes, Bacteroidetes and Proteobacteria, whereas DGGE sequences from the CS group belonged to the phyla Firmicutes, Bacteroidetes, Proteobacteria and Synergistetes. Among the 47 bands, 13 bands in two groups were identified as known species based on ≥ 97% sequence similarity (Table 3). Bands O-1, C-3 and C-5 showed ≥ 98% similarity with known species of *C. populeti* 743A. Bands O-3 and O-18 were identified as *Streptococcus pasteurianus* CIP 107122, while bands O-9 and C-14 showed 98% similarity with of *Eubacterium cellulosolvens* 6. Band O-12 displayed 97% similarity with known species of *Moryella indoligenes* AIP 220.04, and band O-13 showed species-level sequence similarity to *Pseudobutyribrio ruminis* DSM9787. Bands O-10 and C-10 displayed 98% similarity to *Succinivibrio dextrinosolvens* 0554, while bands C-18 and C-1 had 98% sequence similarity to *Coprococcus eutactus* ATCC 27759 and *Prevotella ruminicola* ATCC 19189, respectively. Moreover, band C-21 had the 93% similarity with known species of *Eubacterium ruminantium* GA 195. Bands C-13 and C-22 were distantly related to *Galibacter mesophilus* Mok-17 with 88% and 91% similarity, respectively. Band C-24 displayed 88% similarity with *Caproctophaga cynodegmi* CIP 103937, and band C-27 showed 94% similarity with known species of *Bacteroides uniformis* JCM 5828. Bands C-19 and C-20 had 92% similarity with known species of *Dethiosulfovibrio acidaminovorans* sr15. The remaining 30 bands from two

---

**Table 1 Number of OTUs, diversity and coverage at 3% distance level using the MOTHUR platform**

| Groups | Clones | OTUs | Chao 1a | Shannon-Wienerb | Coverage |
|--------|--------|------|---------|-----------------|----------|
| OL     | 139    | 57   | 114.2   | (81,192.8)      | 3.5      | 0.74    |
| CS     | 100    | 50   | 143.5   | (85,829.1)      | 3.7      | 0.66    |

a Chao 1 is a nonparametric estimator of the richness in a sample. It is based on the number of rare ribotypes (singletons and doublets) and used to predict the species richness.

b The Shannon-Wiener index is a nonparametric diversity index that combines estimates of richness (total numbers of ribotypes) and evenness (relative abundance of each ribotype) suggesting diversity. It takes into account the abundance of individual taxa and can be used as an overall indicator of the level of diversity in a sample.
Table 2 Comparison of 16S rRNA gene libraries between the OL and CS groups

| Phylotype | Clones | OTU# | Nearest Taxon | % | Phylotype | Clones | OTU# | Nearest Taxon | % |
|-----------|--------|------|---------------|---|-----------|--------|------|---------------|---|
| SDMOL10   | 1      | 1    | P. brevis     | 89| SDCS52    | 1      | 1    | P. brevis     | 90|
| SDMOL20   | 1      | 2    | P. brevis     | 89| SDCS61    | 1      | 2    | P. brevis     | 90|
| SDMOL48   | 1      | 3    | P. brevis     | 89| SDCS69    | 6      | 3    | P. brevis     | 90|
| SDMOL69   | 1      | 4    | P. brevis     | 89| SDCS71    | 2      | 4    | P. brevis     | 90|
| SDMOL96   | 10     | 5    | P. brevis     | 89| SDCS1     | 3      | 5    | P. brevis     | 91|
| SDMOL29   | 2      | 6    | P. brevis     | 89| SDCS74    | 1      | 6    | P. brevis     | 91|
| SDMOL33   | 1      | 7    | P. brevis     | 90| SDCS80    | 1      | 7    | P. brevis     | 91|
| SDMOL38   | 1      | 8    | P. brevis     | 90| SDCS14    | 1      | 8    | P. brevis     | 92|
| SDMOL80   | 1      | 9    | P. brevis     | 90| SDCS54    | 4      | 9    | P. brevis     | 92|
| SDMOL91   | 1      | 10   | P. brevis     | 90| SDCS49    | 3      | 10   | P. brevis     | 92|
| SDMOL107  | 1      | 11   | P. brevis     | 90| SDCS51    | 5      | 11   | P. brevis     | 92|
| SDMOL108  | 1      | 12   | P. brevis     | 90| SDCS5     | 1      | 12   | P. brevis     | 93|
| SDMOL115  | 2      | 13   | P. brevis     | 90| SDCS8     | 1      | 13   | P. brevis     | 93|
| SDMOL120  | 2      | 14   | P. brevis     | 90| SDCS93    | 6      | 14   | P. brevis     | 93|
| SDMOL4    | 1      | 15   | P. brevis     | 91| SDCS16    | 2      | 15   | P. brevis     | 98|
| SDMOL27   | 2      | 16   | P. brevis     | 91| SDCS85    | 1      | 16   | P. salivae    | 90|
| SDMOL32   | 1      | 17   | P. brevis     | 91| SDCS48    | 2      | 17   | P. salivae    | 91|
| SDMOL84   | 2      | 18   | P. brevis     | 91| SDCS2     | 1      | 18   | P. salivae    | 92|
| SDMOL92   | 2      | 19   | P. brevis     | 91| SDCS90    | 1      | 19   | P. ruminicola | 91|
| SDMOL17   | 5      | 20   | P. brevis     | 92| SDCS98    | 5      | 20   | P. ruminicola | 92|
| SDMOL55   | 1      | 21   | P. brevis     | 92| SDCS53    | 1      | 21   | P. ruminicola | 93|
| SDMOL68   | 8      | 22   | P. brevis     | 92| SDCS54    | 3      | 22   | P. ruminicola | 93|
| SDMOL110  | 4      | 23   | P. brevis     | 92| SDCS78    | 1      | 23   | P. ruminicola | 93|
| SDMOL70   | 1      | 24   | B. intestihominis | 86| SDCS37    | 7      | 24   | P. ruminicola | 93|
| SDMOL5    | 1      | 25   | P. shahii     | 86| SDCS44    | 1      | 25   | P. ruminicola | 94|
| SDMOL21   | 1      | 26   | P. shahii     | 88| SDCS47    | 1      | 26   | P. ruminicola | 94|
| SDMOL71   | 2      | 27   | P. shahii     | 89| SDCS94    | 1      | 27   | P. ruminicola | 94|
| SDMOL18   | 1      | 28   | P. shahii     | 90| SDCS11    | 1      | 28   | P. ruminicola | 95|
| SDMOL30   | 1      | 29   | P. shahii     | 90| SDCS9     | 5      | 29   | P. ruminicola | 95|
| SDMOL75   | 10     | 30   | P. shahii     | 90| SDCS87    | 2      | 30   | Par. clara    | 88|
| SDMOL76   | 1      | 31   | P. shahii     | 90| SDCS7     | 1      | 31   | Par. clara    | 89|
| SDMOL82   | 2      | 32   | P. shahii     | 90| SDCS60    | 1      | 32   | P. shahii     | 85|
| SDMOL88   | 1      | 33   | P. shahii     | 90| SDCS76    | 2      | 33   | P. shahii     | 85|
| SDMOL109  | 1      | 34   | P. shahii     | 90| SDCS13    | 1      | 34   | P. shahii     | 90|
| SDMOL118  | 1      | 35   | P. shahii     | 90| SDCS86    | 1      | 35   | P. veroralis  | 91|
| SDMOL7    | 1      | 36   | P. bryantii   | 90| SDCS77    | 1      | 36   | P. veroralis  | 92|
| SDMOL28   | 1      | 37   | P. copri      | 87| SDCS104   | 1      | 37   | P. dentalis   | 91|
| SDMOL26   | 1      | 38   | P. copri      | 89| SDCS88    | 1      | 38   | P. albensis   | 87|
| SDMOL135  | 1      | 39   | P. copri      | 91| SDCS21    | 1      | 39   | Ros. hominis   | 90|
| SDMOL34   | 1      | 40   | P. salivae    | 89| SDCS28    | 1      | 40   | Pab. merdae   | 84|
| SDMOL47   | 2      | 41   | P. salivae    | 90| SDCS20    | 8      | 41   | S. dextrinosolvens | 97|
| SDMOL64   | 3      | 42   | P. salivae    | 91| SDCS89    | 1      | 42   | Rum. bromii   | 90|
Table 2 Comparison of 16S rRNA gene libraries between the OL and CS groups (Continued)

| OTU No. | OTU Name | OTU #   | Species Name        | Sequence Identity | OTU Name | OTU #   | Species Name        | Sequence Identity |
|---------|----------|---------|---------------------|-------------------|----------|---------|---------------------|-------------------|
| SDMOL74 | 3        | 43      | P. salivae          | 91                | SDCS36   | 1       | 43                  | Rum. bromii       | 95               |
| SDMOL98 | 3        | 44      | P. salivae          | 91                | SDCS97   | 1       | 44                  | Rum. bromii       | 95               |
| SDMOL139| 3        | 45      | P. salivae          | 92                | SDCS38   | 1       | 45                  | Pab. mordax       | 84               |
| SDMOL63 | 1        | 46      | P. veroralis        | 91                | SDCS50   | 1       | 46                  | Pro. acetigenes   | 83               |
| SDMOL44 | 16       | 47      | P. veroralis        | 92                | SDCS83   | 1       | 47                  | A. shahii         | 85               |
| SDMOL136| 16       | 48      | P. veroralis        | 92                | SDCS96   | 1       | 48                  | Sp. acetigenes    | 84               |
| SDMOL53 | 1        | 49      | P. albensis         | 91                | SDCS102  | 1       | 49                  | C. aldrichii      | 86               |
| SDMOL58 | 2        | 50      | P. stercorea        | 87                | SDCS105  | 1       | 50                  | C. bolteae        | 91               |
| SDMOL100| 1        | 51      | P. multiformis      | 91                |          |         |                     |                   |                  |
| SDMOL117| 1        | 52      | P. ruminicola       | 92                |          |         |                     |                   |                  |
| SDMOL143| 1        | 53      | P. dentalis         | 91                |          |         |                     |                   |                  |
| SDMOL31 | 1        | 54      | A. shahii           | 85                |          |         |                     |                   |                  |
| SDMOL37 | 1        | 55      | A. shahii           | 88                |          |         |                     |                   |                  |
| SDMOL127| 1        | 56      | S. dextrinosolvens  | 97                |          |         |                     |                   |                  |
| SDMOL66 | 1        | 57      | P. brevis           | 87                |          |         |                     |                   |                  |

P Prevotella, S Succinivibrio, A Alistipes, Par Paraprevotella, Ros Roseburia, Rum Ruminococcus, Sp Sporanaerobacter, C Clostridium, Pab Parabacteroides, Pro Proteiniphilum, B Barnesiella, a number of clones, b sequence identity, OTU # OTU No.

groups had 92-96% sequence similarities with several species belonging to genus Prevotella including P. loescheii, P. pleuritidis, P. corporis, P. buccalis, P. dentalis, P. melanogenica, P. salivae, P. copri, P. denticola, P. oulorum and P. histicola.

Discussion

In the present study, two 16S rRNA gene libraries and PCR-DGGE were used to study the rumen bacteria in the rumen of domesticated Sika deer feeding on oak leaves-based (OL) and corn stalks-based (CS) diets. Sequences from the two clone libraries and PCR-DGGE bands indicated that the majority of sequences belonged to phylum Bacteroidetes. The findings from the current study are similar to previous findings for other ruminants, such as Reindeer, yaks, cattle and goats [14-18]. The predominance of sequences belonging to the phylum Bacteroidetes highlights their important role in the rumen fermentation of domesticated Sika deer. While, the phylum Firmicutes being prevalent in other ruminants, such as Reindeer, yaks, cattle and goats [14-18]. Therefore, the dominant genus Prevotella in the rumen of domesticated Sika deer was likely because they utilize a wide variety of polysaccharides, and are thought to be important contributors to xylan degradation in the rumen [29-32]. Although other studies found that concentrate diets increased the numbers of clones related to Prevotella spp. [33,34], however, in comparison with other ruminants, there was an apparent difference in the proportion of Prevotella spp. [6,25,27,28]. Prevotella spp. belonged to the hydrogen-consuming bacteria, which could produce propionate via succinate or acrylate pathways though fermentation of sugars and lactate, respectively [35-37]. Therefore, the dominant genus Prevotella in the rumen of domesticated Sika deer suggested that the propionate pathway may be relatively vital in the rumen fermentation of domesticated Sika deer, which, in turn, may lead to the decreased production of methane, since the succinate-propionate pathway could compete with methanogens for hydrogen [38]. The relationship between Prevotella spp. and methanogens in the rumen of domesticated Sika deer was worth of further investigating. In addition, the bacterial communities in the rumen between domesticated Sika deer, Svalbard reindeer and Norwegian reindeer, all cervids, were compared using Fast UniFrac, which can be used to determine whether communities are significantly different [13]. The results of Principal coordinate analysis (PCoA) between domesticated Sika deer and Reindeer using the Fast
Figure 3 Phylogenetic tree of bacterial 16S rRNA sequences from two groups using the Neighbor-Joining method and Kimura two-parameter model in MEGA. Clones from Sika deer fed oak leaves beginning with SDMOL, followed by clone number, and from corn stalks beginning with SDCS, followed by clone number. *Aquifex pyrophilus* was used as the outgroup. Statistical significance was verified by bootstrapping 1000 replicates.
Unifrac platform clearly showed that the rumen bacterial communities were distinct, which can be attributed to the host-species (Figure 5) [13,26,39].

It is important to note, that fibrolytic bacteria, such as *C. populeti*, *E. cellulosolvens* and *Ps. ruminis* were discovered in our analysis based on PCR-DGGE, rather than the predominant fibrolytic bacteria, *B. fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *R. albus*. This may suggest that the rumen of domesticated Sika deer depend on unique bacterial communities in rumen fermentation. In contrast, the absence of *R. flavefaciens*, *B. fibrisolvens*, *F. succinogenes* and *R. albus* in the present work may be attributed to the small number of clones may have missed some other members of the bacterial community, and the weak or unidentifiable bands in DGGE. Future work will employ next generation sequencing to effectively elucidate the bacterial diversity present in the rumen of domesticated Sika deer and other livestock. Collectively, these data indicated that the rumen of domesticated Sika deer harbored unique bacterial populations for the fermentation of plant biomass and concentrate diet.

Interestingly, in both clone libraries, none of the sequences were 100% identical. Rather, most clones were in the range of 83-98% identify to known species in both libraries. These results suggested that the rumen bacteria of domesticated Sika deer were not previously characterized and that these clones related to *Prevotella* spp. in the rumen represented new species. This agrees with previous findings suggesting that most of the bacterial species in rumen of other cervids (96% for Hokkaido Sika deer and 100% for Svalbard reindeer) are unknown [26,40]. Despite the diets and geographic location are important factors affecting bacterial diversity in the rumen, however, the presence of these unknown or unidentified species may be the result of co-evolution between microbial communities and the host.

PCR-DGGE analysis showed that the bacterial diversity in domesticated Sika deer fed corn stalks differed from the domesticated Sika deer consuming oak leaves (Figure 5), indicating forage affected the relative abundance and composition of the bacteria. Moreover, the difference in the *Prevotella* species between the two groups was very apparent (Table 3). For instance, the results of clone library showed that the proportion of *P. ruminicola*-like clones (27%) was abundant in the CS group comparing with those in the OL group, and sequences analysis of PCR-DGGE also indicated that...
**Table 3 Sequences analysis of V3 region of 16S rRNA gene from PCR-DGGE**

| Band No | Nearest cultured relative (GenBank accession No) | % a | Band No | Nearest cultured relative (GenBank accession No) | % a |
|---------|-----------------------------------------------|-----|---------|-----------------------------------------------|-----|
| O-1     | C. populeti (NR026103)                         | 99  | C-1     | P. ruminicola (NR044632)                        | 98  |
| O-2     | P. salivae (NR024816)                          | 93  | C-2     | P. loescheii (NR043216)                         | 96  |
| O-3     | St. pasteurianus (NR043660)                    | 100 | C-3     | C. populeti (NR026103)                         | 98  |
| O-4     | P. dentalis (NR029284)                         | 94  | C-4     | P. pleuritidis (NR041541)                      | 94  |
| O-5     | P. salivae (NR024816)                          | 96  | C-5     | C. populeti (NR026103)                         | 98  |
| O-6     | P. denticola (NR042842)                        | 95  | C-6     | P. pleuritidis (NR041541)                      | 94  |
| O-7     | P. olearum (NR029147)                          | 94  | C-7     | P. corporis (NR044627)                         | 94  |
| O-8     | P. buccalis (NR044630)                         | 94  | C-8     | P. buccalis (NR044630)                         | 94  |
| O-9     | E. cellulosolvens (NR026106)                   | 98  | C-9     | P. dentalis (NR029284)                         | 95  |
| O-10    | S. dextinosolvens (NR026476)                   | 98  | C-10    | S. dextinosolvens (NR026476)                   | 98  |
| O-11    | P. salivae (NR024816)                          | 95  | C-11    | P. dentalis (NR029284)                         | 93  |
| O-12    | M. indoligenes (NR043775)                      | 97  | C-12    | P. melaninogenica (NR042843)                   | 95  |
| O-13    | Ps. ruminis (NR026315)                         | 99  | C-13    | G. mesophilus (NR041450)                        | 88  |
| O-14    | P. olearum (NR029147)                          | 94  | C-14    | E. cellulosolvens (NR026106)                   | 98  |
| O-15    | P. dentalis (NR029284)                         | 94  | C-15    | P. dentalis (NR029284)                         | 95  |
| O-16    | P. histicola (NR044407)                        | 95  | C-16    | P. loescheii (NR043216)                        | 93  |
| O-17    | P. dentalis (NR029284)                         | 95  | C-17    | P. salivae (NR024816)                         | 88  |
| O-18    | St. pasteurianus (NR043660)                    | 100 | C-18    | Cp. utactus (NR044049)                         | 98  |
| O-19    | P. dentalis (NR029284)                         | 96  | C-19    | D. acidaminovorans (NR029034)                  | 92  |
| O-20    | P. dentalis (NR029284)                         | 96  | C-20    | D. acidaminovorans (NR029034)                  | 92  |
| O-21    | E. ruminantium (NR024661)                      | 93  | C-21    | G. esophilius (NR041450)                       | 91  |
| O-22    | G. esophilius (NR041450)                       | 91  | C-22    | P. copri (NR040877)                            | 92  |
| O-23    | Ca. cynodegmi (NR043063)                       | 88  | C-24    | P. copri (NR040877)                            | 93  |
| O-25    | P. salivae (NR024816)                         | 94  | C-26    | P. dentalis (NR029284)                         | 94  |
| O-27    | B. uniformis (NR040866)                       | 94  | C-27    |                                                   |    |

C Clostridium, E Eubacterium, P Prevotella, S Succinivibrio, St Streptococcus, M Moryella, Ps Pseudobutyrivibrio, Cp Coprococcus, G Gallibacter, Ca Capnocytophaga, B Bacteroides, D Dethiosulfovibrio, a sequence similarity.

*P. ruminicola* was only presented in CS group. Interestingly, *Prevotella* species in the rumen could contribute to cell wall degradation through synergistic interactions with species of cellulolytic bacteria [41]. Therefore, considering the relatively high fiber content (about 36%) in corn stalks, these *P. ruminicola*-like clones in the CS group may play a role in the degradation of cellulose. This explanation is partly supported by recent metagenomics data from the Svalbard reindeer rumen microbiome, where the presence of polysaccharide utilizing glycoside hydrolase and other carbohydrate-active enzyme families target various polysaccharides including cellulose, xylan and pectin [18].

In the OL group, the distribution of *P. shahii*-like clones (16.5%), *P. veroralis*-like clones (23.8%) and *P. salivae*-like clones (12.3%) were several times higher in the OL library than in the CS library, and several bands in the PCR-DGGE analysis showed sequence similarities to *P. salivae* (Table 3). Previous study reported that *P. ruminicola* may tolerate condensed tannins [22]. Considering the genetic diversity of *Prevotella* spp. [27,42], it is assumed that the tolerance to tannins of domestic Sika deer may be related to the abundance of *Prevotella* spp. in the OL group. In addition, we found two bands (O-3 and O-18) were identified as *St. pasteurianus* using PCR-DGGE. Thus this species may also be important in the process of degrading tannins in diets, because tannin-degrading capability of *Streptococcus* sp. have been demonstrated in other studies [43-46]. However, these assumptions need to be investigated in future studies.

Phylogenetic analysis indicated the presence of diet-specific subpopulations of *Prevotella*. *Prevotella* clusters 1 and 2 not only demonstrated the genetic diversity of *Prevotella* spp., but also confirmed the above assumption that clones grouped within clusters 1 or 2 may be related to
the degradation of fiber (cluster 1) or tannins (cluster 2), whereas, the clones in cluster 3 may have common features of degrading starch and proteins contained in concentrate diets (Figure 3). However, clones related to the bacterial genera *Sporanaerobacter, Parabacteroides* and *Proteiniphilum* were found in the rumen of domesticated Sika deer fed corn stalks that were not previously reported in the rumen from other ruminants. *Sporanaerobacter acetigenes* is an acetogenic and a sulfur-reducing bacterium that was isolated from an anaerobic sludge blanket reactor in Mexico [47,48]. The rumen has considerable capacity to convert sulfate into sulfur-containing amino acids. Similarly, little is known about *Proteiniphilum acetigenes*, which was originally isolated from a UASB reactor treating brewery wastewater in China [49]. These bacteria in rumen of domesticated Sika deer may have other biological functions and is worthy of further investigation.

**Conclusions**

In conclusion, this study is the first to report the rumen bacteria in Chinese domesticated Sika deer, consuming either oak leaves-based or corn stalks-based diets. Sequences analysis from 16S rRNA clone libraries and PCR-DGGE revealed that the domesticated Sika deer harbored unique rumen bacterial populations, most of which may present novel species, and that the bacterial compositions were affected by forage. It is speculated that the possible new species of *Prevotella* may be related to the degradation of tannins or fiber biomass. Moreover, the species diversity of *Prevotella* sp. in the rumen combined with their synergistic interactions with other microorganisms requires further in depth investigation.

**Methods**

**Animals and sampling**

Four male rumen-cannulated domestic Sika deer (*Cervus nippon*) maintained at the research farm (44.04° N, 129.09° E) of the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, in Jilin Province, were used in this study. From September to October, four domestic Sika deer were offered the same concentrated diets (64.5% corn, 19.7% soybean meal, 12.8% distiller dried grains with solubles and a 3% mixture of vitamins and mineral salts) and mixed with either oak leaves (OL) or corn stalks (CS). All domestic Sika deer were fed twice each day at 8:00 AM and 4:00 PM and had free access to water. The whole rumen contents, which included solid and fluid fractions, were collected via rumen cannula before the morning feeding, and stored at −20°C for analysis. All domestic Sika deer used in present experiment must be performed according to the animal health and well-being regulations, all animal procedures were approved and authorized by the Chinese Academy of Agricultural Sciences Animal Care and Use Committee, and by the Wild Animal and Plant Subcommittee, Institute of Special Animal and Plant Sciences.

**DNA extraction**

Total DNA was directly extracted from rumen contents containing solid and liquid fraction according to methods described by LaMontagne [50] with few modifications. In brief, 800 μl lysis buffer (0.15 M NaCl, 0.2 M EDTA, 10 mg.ml⁻¹ lysozyme, pH8.0), 20 μl of 20 mg.ml⁻¹ proteinase K (Sigma, Germany), and 0.3 g glass beads (0.1 mm, Sigma, Germany) were added to 0.5 g of whole rumen contents. After shaking at 37°C for 1 h, 300 μl heated lysis buffer (10% SDS, 0.1 M NaCl, 0.5 M Tris–HCl, pH8.0) at 65°C, 300 μl phosphate buffer (pH8.0) and 600 μl chloroform-isoamyl alcohol (24:1, V/V) were added, and the mixture was incubated at 65°C in a water bath for 30 min with intense shaking 30 s at 10 min intervals. After centrifugation at 5,000 rpm for 6 min, the supernatant was transferred to a clean tube. DNA was then precipitated with a 0.6 volume of isopropanol at −80°C for 15 min, and the pellet was washed several times with 75% ethanol. The DNA was dried and dissolved in TE buffer (pH 8.0). The DNA quality was assessed by 0.8% agarose gel electrophoresis, and the purity was determined by spectrophotometry (SPECTORD 50, analytikjena, Germany), after which it was purified using a QIAEX II Gel Extraction Kit (QIAGEN, Germany).

**Construction of 16S rRNA gene clone libraries and sequences analyses**

Universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) were used to amplify the 16S rRNA gene (approximately 1.5 kb) [51]. Each 50 ul reaction contained 50 ng template DNA, 0.25 mM of each primer, 250 mM dNTPs, 1.25 U of Ex Taq and 5 μl Ex Taq buffer (TaKaRa, Dalian). PCR was performed on a 2720 Thermal Cycler (Applied Biosystems, USA) with hot start at 94°C for 5 min, followed by 20 cycles of 30 s at 94°C, 1 min at 55°C and 2 min at 72°C; and a final extension at 72°C for 10 min. The PCR product was assessed using 2% agarose gel electrophoresis (approximately 1.5 kb), and were purified using a TaKaRa MiniBEST DNA Fragment Purification Kit (TaKaRa, Dalian) and then pooled within each group. Two 16S rRNA gene clone libraries were constructed from the pooled PCR products using the TOPO® TA Cloning® Kit (Invitrogen, USA). Positive (white) clones were screened by colony PCR with the M13 Forward and M13 Reverse primers, and sequenced using an ABI 3730XL DNA Analyzer.

The chimera check program Bellerophon was used to identify chimeric sequences [52]. The remaining sequences were assigned using the Classifier tool available
at Ribosomal Database Project (RDP) Release 10 at a confidence threshold of 80% [53]. OTUs based on 97% sequence identity, and the Shannon-Wiener index-based diversity estimator and the Chao1 based index of richness were calculated using MOTHUR platform to determine the diversity and richness of bacterial communities in each group based on the 16S rRNA gene libraries [54]. Libshuff analysis was performed to estimate the similarity between libraries from two diets based on evolutionary distance of all sequences. Coverage and rarefaction curves were also determined using the MOTHUR platform [54]. The 16S rRNA gene sequences were screened using GenBank's BLAST program [55]. The closest related sequences were retrieved and aligned with sequences from the present study using the CLUSTALW 1.83 program in MEGA 5.05 software [56]. A phylogenetic tree was constructed using the Kimura two-parameter model and the Neighbor-Joining method as part of the MEGA 5.05 software. The statistical significance was verified by 1000 bootstrapped replicates. The sequences obtained from this study were submitted to GenBank under the accession numbers JX889268 to JX89378. Furthermore, an unweighted UniFrac distance matrix was constructed from the phylogenetic tree of clone libraries of Norwegian reindeer, Svalbard reindeer and Sika deer, and was visualized using uCOA [13,26,39].

**PCR-DGGE banding profiles and statistical analysis**

The variable region (V3) of the bacterial 16S rRNA gene was amplified using the primers of F341GC and R534, and PCR condition was described previously [57]. A 40 bp GC-clamp (5′-CGCCCGGGGGCGCCCGGCCCCCCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG

**Recombinant plasmids of positive clones (white)** were sequenced using ABI 3730XL DNA Analyzer. The sequences were compared with those sequences deposited in NCBI web site using BLAST program [55].

**Additional file**

**Additional file 1: Dominant bands of PCR-DGGE banding patterns of bacteria 16SrRNA gene (V3 region).** In the text, bands from OL group were defined as O and followed by bands number, bands from CS group begin with C and followed by bands numbers.

**Abbreviations**

DGGE: Denaturing gradient gel electrophoresis; OTUs: Operational taxonomic units; VFA: Volatile fatty acids; BLAST: Basic local alignment search tool; RDP: Ribosomal database project; PCOA: Principal coordinates analysis.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

ZPL sampled rumen contents, extracted DNA, constructed the clone library, data analysis and drafted the manuscript. ADGW was involved with interpretation of data and with preparing the manuscript. HLL designed the study and drafted the paper. KB, YFY, CX and KYW contributed to sample rumen contents and all of lab works. GYL and FHY conceived the study. All authors read and approved the final manuscript.

**Acknowledgements**

Special thanks to Dr. Yanfeng Cheng in the analysis of 16S rDNA gene sequences and Dr. Zhi Gang Cui in the analysis of PCR-DGGE. This work is supported by a Young Researcher funded project (201101086), Science and Technology Development project (20090238) and a Leading Talent and Creative Team project (20121810), all from Jilin province, the Ministry of Agriculture Public Sector (Agriculture) Special Research Project (200903014) and Key Projects in the National Science & Technology Pillar Program (2011BA03B02).

**Author details**

1. Department of Economical Animal Nutrition and Feed Science, Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, Jilin, China. 2. Department of Animal Science, University of Vermont, 570 Main Street, Burlington, VT 05405-0148, USA.

Received: 4 March 2013 Accepted: 1 July 2013 Published: 8 July 2013

**References**

1. Hiura T, Hashidoko Y, Kobayashi Y, Tahara S: Effective degradation of tannic acid by immobilized rumen microbes of a sika deer (Cervus nippon yezoensis) in winter. Anim Feed Sci Technol 2010, 155(1-1):1-8.

2. Clauss M, Lason K, Gehrke J, Lechner-Doll M, Fickel J, Grune T, Jurgen Streich W: Captive roe deer (Capreolus capreolus) select for low amounts of tannic acid but not quebracho: fluctuation of preferences and potential benefits. Comp Biochem Physiol B Biochem Mol Biol 2003, 136(2):369-382.

3. Wight A-DG, Kleve AK: Does the complexity of the rumen microbial ecology preclude methane mitigation? Animal Feed Sci Technol. 2011, 166-167:248–253.

4. Tajima K, Aral S, Ogata K, Nagamine T, Matsui H, Nakamura M, Aminov RI, Benno Y: Rumen bacterial community transition during adaptation to high-grain diet. Anaerobe 2000, 6(5):275–284.

5. An DD, Dong XZ, Dong ZY: Prokaryote diversity in the rumen of yak (Bos grunniens) and Jinnan cattle (Bos taurus) estimated by 16S rDNA homology analyses. Anaerobe 2005, 11(4):207–215.

6. Pei CX, Liu QA, Dong CS, Li HQ, Jiang JB, Gao WJ: Diversity and abundance of the bacterial 16S rRNA gene sequences in forestomach of alpacas (Lama pacos) and sheep (Ovis aries). Anaerobe 2010, 16(4):426–432.
7. Yang LY, Chen J, Cheng XL, Xi DM, Yang SL, Deng WD, Mao HM: Phylogenetic analysis of 16S rRNA gene sequences reveals rumen bacterial diversity in Yaks (Bos grunniens). Mol Biol Rep 2010, 37(1):1553–562.

8. Aagnes TH, Sormo W, Mathiesen SD: Ruminal microbial digestion in free-living, in captive lichen-fed, and in Starved Reindeer (Rangifer tarandus tarandus) in winter. Appl Environ Microbiol 1995, 61(2):553–591.

9. Edwards JE, McKean NR, Travis AJ, Wallace RJ: 16S rDNA library-based analysis of ruminal bacterial diversity. Antonie Leeuwenhoek Int J Gen Mol Microbiol 2004, 86(3):263–281.

10. Ichimura Y, Yamano H, Takano T, Koike S, Kobayashi Y, Tanaka K, Ozaki N, Suzuki M, Okada H, Yamana M: Rumen microbes and fermentation of wild sika deer on the Shiretoko peninsula of Hokkaido Island, Japan. EcoL Res 2004, 19(6):389–395.

11. Kocherginskaya SA, Aminov RI, White BA: Bacterial diversity in the rumen of Gayals (Bubalus bubalis). Microbes Environ 2001, 16(1):69–101.

12. Shi PJ, Meng K, Zhou ZG, Wang YR, Diao QY, Yao B: Phenotypic characterization of polysaccharidases produced by four Prevotella type strains. Curr Microbiol 2000, 41(1):45–49.

13. Lozupone C, Knight R: UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 2005, 71(12):828–835.

14. Cho SJ, Cho KM, Shin EC, Hong SY, Choi BR, Hong SY, Choi BR, Kang JM, Lee SM, Kim YH, Kim H, et al: 16S rDNA analysis of bacterial diversity in three fractions of cow rumen. J Microbiol Biotechnol 2006, 16(1):92–101.

15. Yang SL, Ma SC, Chen J, Mao HM, He YD, Xi DM, Yang LY, He TB, Deng WD: The bacterial community in the rumen of Yaks (Bos grunniens) in winter. Appl Environ Microbiol 1992, 58(9):3288–3292.

16. Cunha IS, Barreto CC, Costa OYA, Bomfim MA, Castro AP, Kruger RH, Quirino JF: Bacteria and archaea community structure in the rumen microbiome of goats (Capra hircus) from the semi-arid region of Brazil. Anaerobe 2011, 17(3):118–124.

17. Li MJ, Zhou M, Adamowicz E, Basarab JA, Guan LL: Characterization of bovine ruminal epithelial bacterial communities using 16S rRNA sequencing, PCR-DGGE, and qRT-PCR analysis. Vet Microbiol 2012, 155(1):72–80.

18. Pope PB, Mackenzie AK, Gregor I, Mathiesen SD, Mackie RI: Effects of sainfoin (Onobrychis vicifolia Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Appl Environ Microbiol 1994, 60(4):1374–1378.

19. Min BR, Atwood GT, McNabb WC, Molan AL, Bary TN: The effect of condensed tannins from Lotus corniculatus on the proteolytic activities and growth of rumen bacteria. Anim Feed Sci Technol 2005, 121(1–2):49–58.

20. Kolke S, Yoshitani S, Kobayashi Y, Tanaka K: Phylogenetic analysis of fiber-associated rumen bacterial community and PCR detection of uncultured bacteria. FEMS Microbiol Lett 2003, 229(1):23–30.

21. Stevenson DM, Weiner PJ: Dominance of Prevotella and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. Appl Microbiol Biotechnol 2007, 75(1):165–174.

22. Sundset MA, Preesteng KE, Cann IK, Mathiesen SD, Mackie RI: Novel rumen bacterial diversity in two geographically separated sub-species of reindeer. Microb Ecol 2007, 54(3):424–438.

23. Bekele AZ, Kolke S, Kobayashi Y: Genetic diversity and diet specificity of ruminal Prevotella revealed by 16S rRNA gene-based analysis. FEMS Microbiol Lett 2010, 305(1):49–57.

24. Wu S, Baldwin RL, Li W, Li C, Conner EE, Li RW: The bacterial community composition of the bovine rumen detected using pyrosequencing of 16S rRNA genes. Metagenomics 2012, 1:1–11.

25. Cotta MA: Interaction of ruminal bacteria in the production and utilization of maltooligosaccharides from starch. Appl Environ Microbiol 1992, 58(1):469–54.

26. Gardner RG, Wells JE, Russell JB, Wilson DB: The cellular location of Prevotella ruminicola beta-1,4-D-endoglucanase and its occurrence in other strains of ruminal bacteria. Appl Environ Microbiol 1995, 61(9):2828–3292.

27. Matsu H, Ogata K, Tajima K, Nakamura M, Nagamine T, Aminov RI, Benno Y: Phenotypic characterization of polysaccharidases produced by four Prevotella type strains. Curr Microbiol 2000, 41(1):45–49.

28. Krause DO, Dempman SE, Mackie RJ, Morrison M, Rae AL, Atwood GT, McSweeney CS: Opportunities to improve fiber degradation in the rumin: microbiology, ecology, and genomics. FEBS Microbiol Rev 2003, 27(3):663–693.

29. Tanno FC, Puviv HT, Najar FZ, Sukhamon LO, Krehbel CR, Nagaraja TG, Roe BA, deSilva U: Rumen microbial population dynamics during adaptation to a high-grain diet. Appl Environ Microbiol 2010, 76(2):7482–7490.

30. Sader-Bourgeteau S, Martin C, Morgavi DP: Bacterial diversity dynamics in rumin epithelium of wethers fed forage and mixed concentrate forage diets. Vet Microbiol 2010, 146(1–2):298–104.

31. Strobel HJ: Vitamin B12-dependent propionibacterial production by the ruminal bacterium Prevotella ruminicola. Appl Environ Microbiol 1992, 58(7):2331–2333.

32. Pursue J, Fouts DE, Morrison M, White BA, Mackie RJ, North American Consortium for Rumen B, Coutinho PM, Henrisset B, Nelson KE: Comparative genome analysis of Prevotella ruminicola and Prevotella bryantii: insights into their environmental niche. Microb Ecol 2010, 60(6):721–729.

33. Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA: Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. Nat Rev Microbiol 2008, 6(2):121–131.

34. Newbold CJ, Lopez S, Nelson N, Ouda JO, Wallace RJ, Moss AR: Propionate precursors and other metabolic intermediates as possible alternative electron acceptors to methanogenesis in ruminal fermentation in vitro. Br J Nutr 2005, 94(1):27–35.

35. Sundset M, Edwards J, Cheng Y, Senosiain R, Fraile M, Northwood K, Praesteng KE, Gladt T, Mathiesen S, Wright AD: Molecular diversity of the rumen microbiome of Norwegian Reindeer on natural summer pasture. Microb Ecol 2009, 57(3):335–348.

36. Kobayashi Y: Inclusion of novel rumen microbiology: need for basic and applied science. Anim Sci J 2006, 77(4):375–385.

37. Whitehead TR: Analysis of the rumen bacterial 16S rDNA library-based diversity under two different diet conditions using denaturing gradient gel electrophoresis, random sequencing, and statistical ecology approaches. Anaerobe 2001, 7(3):119–134.

38. Shi PJ, Meng K, Zhou ZG, Wang YR, Diao QY, Yao B: Phenotypic characterization of polysaccharidases produced by four Prevotella type strains. Curr Microbiol 2000, 41(1):45–49.
novel acetogenic, facultatively sulfur-reducing bacterium. Int J Syst Evol Microbiol 2002, 52(Pt 4):1217–1223.

49. Chen S, Dong X: Proteiniphilum acetatigenes gen. nov., sp. nov., from a UASB reactor treating brewery wastewater. Int J Syst Evol Microbiol 2005, 55(Pt 6):2257–2261.

50. LaMontagne MG, Michel FC, Holden PA, Reddy CA: Evaluation of extraction and purification methods for obtaining PCR-amplifiable DNA from compost for microbial community analysis. J Microbiol Methods 2002, 49(3):255–264.

51. Lane DJ: 16S/23S rRNA sequencing. In Nucleic acid techniques in bacteria systematics. Edited by Stackebrandt EGM. New York: Wiley; 1991:115–175.

52. Huber T, Faulkner G, Hugenholtz P: Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics 2004, 20(14):2317–2319.

53. Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM, et al: The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 2009, 37:D141–D145.

54. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, et al: Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 2009, 75(23):7527–7531.

55. Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997, 25(17):3389–3402.

56. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S: MEGAS: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011, 28(10):2731–2739.

57. Muyzer G, de Waal EC, Utterlinden AG: Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 1993, 59(3):695–700.

doi:10.1186/1471-2180-13-151
Cite this article as: Li et al.: Molecular diversity of rumen bacterial communities from tannin-rich and fiber-rich forage fed domestic Sika deer (Cervus nippon) in China. BMC Microbiology 2013 13:151.