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Characterization of Bacterial Microbial Diversity in Wild Yak and Domestic Yak in Qiangtang Region of Tibet

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SZ, KQ, LJ, LS and WH contributed to the initial design of this project. SZ, KQ, TZ and SP collected samples from different animals. SZ and KQ conducted the experiment, conducted bioinformatics analyses and prepared the manuscript of this publication.

Key words
Wild yak, Domestic yak, Faecal, Bacteria, The high-throughput sequencing technology

ABSTRACT

After several years of domestication, domestic yak is inferior to wild yak in many aspects. Gut microbes play an important role in the digestion, absorption and health of animals. Studying the intestinal flora of yak has significance to improving its productivity and immunity. In this study, we analysed the bacterial diversity in fresh faeces of wild yak and domestic yak. Results showed that the structure of the bacterial flora in wild yak and domestic yak was significantly different. Firmicutes, Actinobacteria, Bacteroidetes and TM7 were the dominant phyla, and Micrococcaceae, Ruminococcaceae, Planococcaceae, Peptostreptococcaceae, Clostridiaceae, Lachnospiraceae, Christensenellaceae, Coriobacteriaceae and Bacillaceae were the dominant families. Comparing the relative abundance of different levels of bacteria, a total of 78 bacteria significantly differed between wild yak and domestic yak. Amongst these bacteria, Chloroflexi, Clostridiaceae, *Microbispora*, *Blautia*, *Carnobacterium* and *Salinibacterium* were obviously higher in wild yak than in domestic yak. Proteobacteria, *Epulopiscium*, *Amycolatopsis*, Brucellaceae, *Sediminibacterium* and *Rhodococcus* were significantly higher in domestic yak than in wild yak. The present study reported the microbial diversity of bacteria between wild yak and domestic yak. The findings can help improve the production performance and disease resistance of domestic yak.

INTRODUCTION

Intestinal microbiota is composed of complex, dense and metabolically active microbial groups (Macfarlane and Macfarlane *et al*., 2007; Ley *et al*., 2008). The intestine is the main organ where the animal digests and absorbs food, and intestinal microbiota play a vital role in this process (Looft *et al*., 2014; Kim *et al*., 2012; Yatsunenko *et al*., 2012). A close relationship exists between the host and the flora in the intestine. Breeds (Kohl *et al*., 2011), Food (Chen *et al*., 2011) and living environment (Wu *et al*., 2012) can affect the composition and function of intestinal flora. Moreover, intestinal flora changes can affect the nutrition of the organism (Turnbaugh *et al*., 2006), organ function (Benson *et al*., 2010) and immune status (Danielsen *et al*., 2007). Therefore, studying and analysing the structure of intestinal flora in animals are important for animal feeding management and treatment of epidemic diseases.

Wild yak (*Bos mutus*) is a precious wild animal resource on the Qinghai–Tibet Plateau and is one of the national-level protected animals in China. It is the only cattle breed that has survived and been reproduced so far in the high-cold region. Wild yak is mainly distributed in the intermountain basins, lake basins and gentle slopes above 4000–5000 m altitude, covering national nature reserves (such as Aerjin Mountains, Qiangtang and Hoh Xil) and surrounding areas in no man's land (Li *et al*., 2014). It has a strong adaptability to the harsh natural environment of the Qinghai-Tibet Plateau. Domestic yak (*Bos grunions*) is a domesticated species from wild yak, and it is an important livestock in Tibet. Yaks have a high content of milk fat, good meat quality and excellent plush quality. They can provide milk, meat, skin, hair and other necessities for people on the plateau and have irreplaceable ecological, social and economic status (Yin *et al*., 2009; Negishi *et*

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al., 2011).

After years of domestication, obvious differences exist between domestic yak and wild yak in many aspects. For example, the size of wild yak is obviously larger than that of domestic yak, and wild yak has stronger rough feeding resistance and disease resistance traits than domestic yak. Studies have found obvious differences in the composition of rumen flora between plateau yak and plain cattle (Zhang *et al*., 2016; Koh *et al*., 2016). Thus, exploring the differences in the composition of intestinal flora between wild yak and domestic yak is vital to improve the production performance and meat quality of domestic yak. On this basis, the present study detected the composition of intestinal flora in wild yaks and domestic yaks and compared their differences to discover the relationship between intestinal bacteria of yak and its body type, roughage resistance and disease resistance and provide a theoretical basis for improving the growth performance and disease resistance of domestic yak.

MATERIALS AND METHODS

Research location and sample collection

The samples of wild yak and grazing domestic yak were collected from the Qiangtang National Nature Reserve and areas around Shuanghu County, Naqu, Tibet. The vegetation type in this area is alpine steppe, which mainly includes zonal vegetation such as *"Austrostipa pubescens"*, with an average elevation above 5000 m. This region is characterised by high temperatures, low precipitation and high wind speeds in July to August. House-feeding domestic yaks were selected from Tibet Naqu Tianmu Animal Husbandry Development Co., Ltd. The domestic yaks were mainly fed with green hay.

From July 6, 2019 to July 8, 2019, a group of grazing domestic yaks (about 60) was found in the grasslands near Shuanghu County (31°61′N, 89°55′E, elevation: 4617 m). Two groups of wild yaks (about 9) were found at two sites in Qiangtang reserve (33°82′N, 89°02′E, elevation: 5345 m; 33°67′N, 88°83′E, elevation: 5383 m). Using a telescope to observe for defecation, and then five fresh faecal samples were collected from grazing domestic yak and wild yak when the animals left. The outer layer of the faeces was removed using a sterilised toothpick. Then, the non-contaminated fresh faeces was collected and placed in 20 mL cryopreservation tubes, and the surface of the test tube was marked as follows: A group (marked as A1, A2, A3, A4 and A5) was selected from wild yaks, whereas C group (marked as C1, C2, C3, C4 and C5) was selected from grazing domestic yaks. Five fresh faecal samples were collected from house-feeding domestic yak (31°56′N, 91°88′E, elevation: 4644 m). The non-contaminated parts were selected, placed in a cryotube and marked as B group (B1, B2, B3, B4 and B5). All samples were stored at -20 °C and used to determine the fungal microbial diversity.

Total DNA extraction

The total microbial genomic DNA was extracted from 15 fecal samples of different animals by using QIAamp Fast DNA Stool Mini Kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions. The concentration and quality of DNA were detected with a nucleic acid detector and 1.2% agarose gel electrophoresis, respectively.

ITS hypervariable region gene amplification

The standard bacteria V3–V4 hypervariable region gene PCR primers (forward primer: ACTCCTACGGGAGGCAGCA; reverse primer: GGACTACHVGGGTWTCTAAT) were used. The total microbial genomic DNA was uniformly diluted to 20ng/μL sample and used as a template. The PCR amplification system contained 5 μL 5×reaction buffer, 5 μL 5×GC buffer, 2 μL dNTP (2.5 mM), 1 μL forward primer (10 μM), 1 μL reverse primer (10 μM), 0.25 μL Q5 DNA polymerase, 2 μL DNA template, and 8.75 μL ddH_2O with a total volume of 25 μL. PCR amplification was performed under the following conditions: initial denaturation at 98°C for 2 min; 30 cycles of denaturation at 98°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s; a final extension at 72°C for 5 min. The PCR amplification products were detected via 2% agarose gel electrophoresis, and target fragments were recovered using AXYGEN gel recovery kit. The recovered PCR products were detected by Quant-iT PicoGreen dsDNA Assay Kit.

Library preparation and sequencing

The sequencing library was constructed using aTruSeq Nano DNA LT Library Prep kit (Illumina). The sequencing library was selected and purified by 2% agarose gel electrophoresis, and the quality was tested by Agilent High-Sensitivity DNA Kit. Then, paired-end sequencing of the qualified sequencing library was performed using Illumina MiSeqe quipment (MiSeq Reagent Kit V3; Personalbio, Shanghai, China).

Sequence data processing and statistical analysis

The sequences were established as operational taxonomic units (OTUs) via Uclust withover 97% similarity (Bokulich *et al*., 2013), and the highest abundant sequence in each OUT was selected as the representative sequence (Caporaso *et al*., 2010). Then, OTUs were taxonomically classified and grouped by comparing with those in the Greengenes database (Koljalg *et al*., 2013).

The richness and evenness index of microbial flora were calculated using the measurement indexes (Chao1, ACE, Shannon, and Simpson); Beta diversity was analyzed by utilizing the similarity of microbial community structures among different groups through principal component analysis (PCA) (Ramette, 2007). The Linear discriminant analysis was used to analyze the discrepancy in microbial communities between groups at different levels (Segata *et al*., 2011). Data were evaluated statistically by one-way analysis of variance.

RESULTS

Sequencing results and OTUs information

The V3–V4 region (internal transcribed spacer) of the faecal microflora of wild yaks and domestic yaks was detected using paired-end sequencing by Illumina MiSeq. A total of 528,581 high-quality sequences were obtained from faecal samples of the three groups by filtering, extracting and double-end splicing the sequences. The average effective sequences in each sample exceeded 35,238. The length of these sequences in all samples ranged from 400 bp to 450 bp. The sequences were established as operational taxonomic units (OTUs) via UCLUST with over 97% similarity. The OTUs in phylum, class, order, family, genus and species levels of each yak are shown in Figure 1. The Venn diagram showed that 3342 OTUs were obtained from all samples (Fig. 2). As shown in the Figure 2, 2144 OTUs were identified in group A, whereas 2043 and 2423 OTUs were found in groups B and C, respectively. The unique OTUs of groups A, B and C reached 276, 423 and 431, respectively, with 1056 common OTUs amongst the three groups. More common OTUs were observed than unique OTUs in all samples.

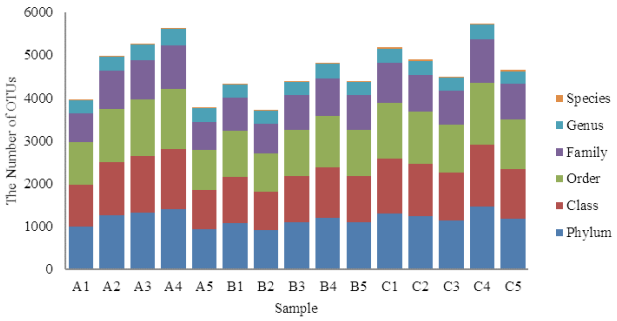

Fig. 1. The quantity of OTUs in different yak samples.

The microbial community diversity of yaks in three groups

The Simpson and Shannon indices were 0.91 and 6.29, 0.87 and 6.00, and 0.93 and 6.95 in groups A, B and C, respectively, with obvious differences amongst the three groups. The Simpson and Shannon indices demonstrated a striking difference in the flora evenness between different groups. The Chao1 and ACE indices were 1302.10 and 1349.72, 1205.16 and 1242.01, and 1342.01 and 1368.41 in groups A, B and C, respectively, with no visible difference between the different groups. The Chao1 and ACE indices revealed no significant difference in the microflora abundance between different groups (Table I). The principal component of microbial community structure in different yak groups, as obtained by principal component analysis (PCA, Fig. 3), indicated that the microbial community structures in the three groups were clustered in different regions, especially between the wild yak group and the house-feeding domestic yak group.

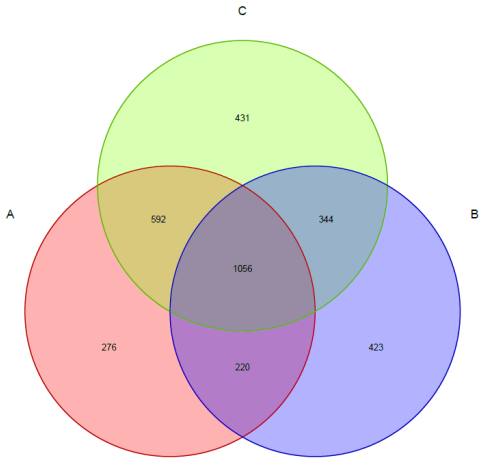

Fig. 2. Venn map of comparison of OUTs distribution in three groups (A, Wild yaks; B, Housing domestic yaks; C, Grazing domestic yaks).

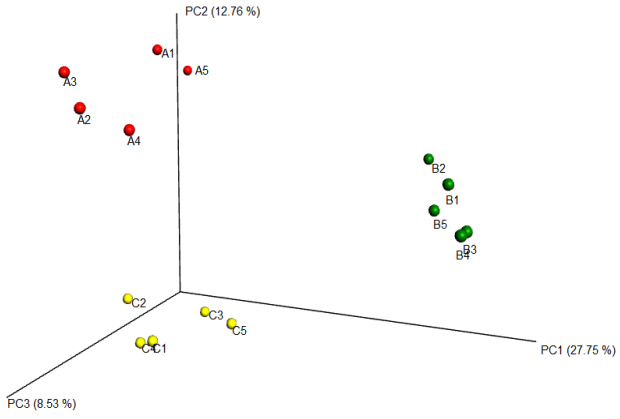

Fig. 3. PCA analysis of the principal component of the similarity of microbial community structure in different samples (A, Wild yaks; B, Housing domestic yaks; C, Grazing domestic yaks).

Table I. Diversity results of the experimental group.

Group	Simpson	Chao1	ACE	Shannon
A	0.91±0.02^{ab}	1302.10±228.35^{a}	1349.72±252.48^{a}	6.29±0.56^{ab}
B	0.87±0.05^{a}	1205.16±64.25^{a}	1242.01±92.99^{a}	6.00±0.63^{a}
C	0.93±0.01^{b}	1342.01±241.53^{a}	1368.41±226.15^{a}	6.95±0.30^{b}

Note: The same letters on the column indicate nosignificant difference (*P>0.05*), the different letters on the column indicate significant difference (*P<0.05).*

Table II. The microbial community structure in Wild yaks and Domestic yaks (%).

Classification level	Bacterial	Group (%)		
		A	B	C
Phylum	Firmicutes	40.26±14.82^{a}	48.80±5.73^{a}	54.28±6.24^{a}
	Actinobacteria	52.62±16.21^{a}	44.18±6.76^{a}	39.56±7.28^{a}
	Bacteroidetes	2.26±1.35^{a}	3.12±1.90^{a}	1.50±0.41^{a}
	TM7	1.86±0.33^{a}	1.54±0.58^{a}	2.68±1.26^{a}
Class	Actinobacteria	51.94±16.29^{a}	42.98±7.09^{a}	38.36±7.49^{a}
	Clostridia	33.16±8.29^{a}	39.12±5.31^{a}	38.24±4.69^{a}
	Bacilli	6.86±4.05^{a}	9.28±4.46^{a}	15.82±8.89^{a}
	Bacteroidia	2.26±1.35^{a}	3.00±1.86^{a}	1.48±0.39^{a}
	TM7-3	1.86±0.33^{a}	1.54±0.58^{a}	2.68±1.26^{a}
	Coriobacteriia	0.68±0.19^{a}	1.20±0.40^{a}	1.20±1.13^{a}
Order	Actinomycetales	51.94±16.29^{a}	42.94±7.14^{a}	38.36±7.49^{a}
	Clostridiales	33.16±8.29^{a}	39.12±5.31^{a}	38.24±4.69^{a}
	Bacillales	6.40±4.00^{a}	6.44±3.22^{a}	15.42±8.67^{b}
	Bacteroidales	2.26±1.35^{a}	3.00±1.86^{a}	1.48±0.39^{a}
	CW040	1.86±0.33^{a}	1.54±0.58^{a}	2.68±1.26^{a}
	Coriobacteriales	0.68±0.19^{a}	1.20±0.40^{a}	1.20±1.13^{a}
	Lactobacillales	0.42±0.32^{a}	2.12±0.94^{b}	0.26±0.15^{a}
Family	Micrococcaceae	51.44±16.34^{a}	41.98±7.47^{a}	37.68±7.25^{a}
	Ruminococcaceae	9.08±3.61^{a}	10.94±3.07^{ab}	13.62±2.18^{b}
	Planococcaceae	5.98±3.52^{a}	5.54±2.73^{a}	13.60±8.60^{a}
	Peptostreptococcaceae	0.64±0.24^{a}	11.32±1.09^{c}	2.68±0.58^{b}
	Clostridiaceae	5.68±3.33^{b}	3.62±0.99^{ab}	1.12±0.24^{a}
	Lachnospiraceae	1.86±0.32^{a}	3.68±0.70^{b}	1.40±0.30^{a}
	F16	1.86±0.33^{a}	1.54±0.58^{a}	2.68±1.26^{a}
	Christensenellaceae	0.70±0.14^{a}	0.94±0.55^{a}	1.52±0.42^{b}
	Coriobacteriaceae	0.68±0.19^{a}	1.20±0.40^{a}	1.20±1.13^{a}
	Bacillaceae	0.38±0.22^{a}	0.88±0.79^{ab}	1.66±0.80^{b}
Genus	Arthrobacter	51.32±16.31^{a}	41.64±7.56^{a}	37.54±7.21^{a}
	Solibacillus	3.92±3.35^{a}	2.06±0.81^{a}	5.02±3.09^{a}
	Sporosarcina	0.38±0.27^{a}	0.06±0.05^{a}	7.46±4.05^{b}
	Bacillus	0.38±0.22^{a}	0.88±0.79^{ab}	1.64±0.82^{b}

Note: The same letters on the column indicate no significant difference (*P>0.05*), the different letters on the column indicate significant difference (*P<0.05*).

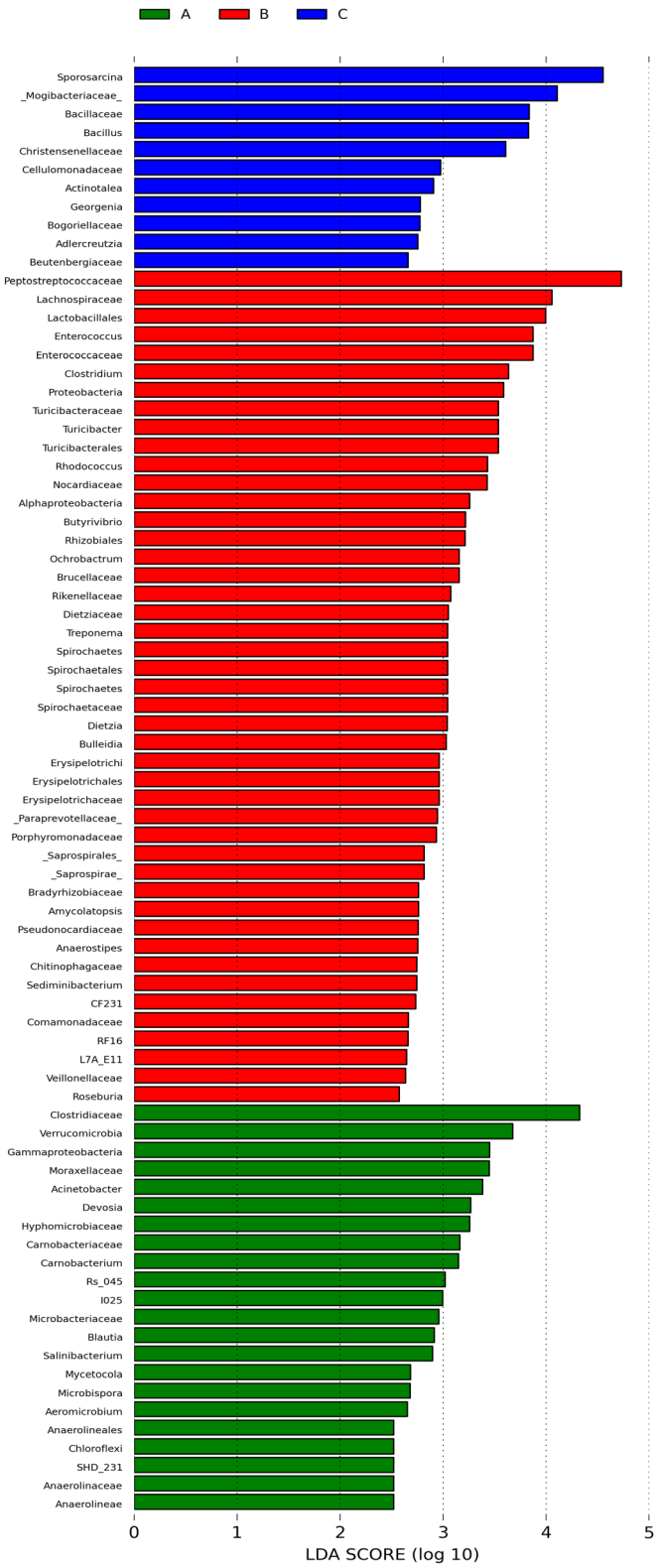

Fig. 4. The taxon with significant differences between the three groups (A, Wild yaks; B, Housing domestic yaks; C, Grazing domestic yaks).

Microbial community structure in different levels in the three groups

In the phylum level (Table II), Firmicutes, Actinobacteria, Bacteroidetes and TM7 contribute to most of the microbial community in wild yaks, house-feeding domestic yaks and grazing domestic yaks with no significant difference between different groups ($P > 0.05$). In the class level, Actinobacteria, Clostridia, Bacilli, Bacteroidia, TM7-3 and Coriobacteriia were the dominant microbial community in the three groups. However, Clostridia and Bacilli in wild yak were lower than those in domestic yak with no obvious difference ($P > 0.05$). Actinobacteria was higher in in wild yak than in grazing domestic yak and house-feeding domestic yak with no visible difference ($P > 0.05$). In the order and family levels, Christensenellaceae and Bacillales in grazing domestic yak were significantly higher than those in wild yak and house-feeding domestic yak ($P < 0.05$). Lachnospiraceae, Peptostreptococcaceae and Lactobacillales in house-feeding domestic yak were obviously higher than those in wild yak and grazing domestic yak ($P < 0.05$). Ruminococcaceae and Bacillaceae in house-feeding domestic yak were obviously higher than those in wild yak and obviously lower than those in grazing domestic yak ($P < 0.05$). Clostridiaceae in house-feeding domestic yak was obviously lower than that in wild yak and obviously higher than that in grazing domestic yak ($P < 0.05$). In the genus level, *Arthrobacter* and *Solibacillus* showed no significant difference in the three groups ($P > 0.05$). *Sporosarcina* and *Bacillus* in grazing domestic yak were significantly higher than those in wild yak and house-feeding domestic yak ($P < 0.05$).

Comparison of the microbial community structure in the three groups

To analyse the differences in the flora structure between different animals, the differences in faecal microorganisms amongst wild yak, house-feeding domestic yak and grazing domestic yak were further analysed using LEfSe (Fig. 4). As shown in the figure, 78 different microbiota had a linear discriminant analysis value exceeding 2.0 amongst the three groups (with significant differences between groups). The average abundance of 22 floras was the highest in group A. *Mycetocola*, *Microbispora* and *Salinibacterium* showed a significant difference at $P < 0.01$, whereas I025, Rs_045, Anaerolinaceae, Chloroflexi, Anaerolineales, SHD_231, Anaerolineae, Carnobacteriaceae, *Carnobacterium*, Hyphomicrobiaceae, *Aeromicrobium*, Microbacteriaceae, *Acinetobacter*, Gammaproteobacteria, *Blautia*, Moraxellaceae, *Devosia*, Clostridiaceae and Verrucomicrobia showed a significant difference at $P < 0.05$. The average abundance of 45 floras was the highest in group B. Peptostreptococcaceae, *Bulleidia*, Turicibacterales, *Turicibacter*, Turicibacteraceae, Lachnospiraceae, Dietziaceae, *Dietzia*, *Amycolatopsis*, Pseudonocardiaceae, *Treponema*, Spirochaetales, Spirochaetaceae, Spirochaetes, *Rhodococcus*, *Butyrivibrio*, Nocardiaceae and Rhizobiales showed a significant difference at $P < 0.01$, whereas *Ochrobactrum*, Brucellaceae, *Anaerostipes*, Saprospirales, Saprospirae, *Clostridium*, Porphyromonadaceae, Chitinophagaceae, *Sediminibacterium*, Alphaproteobacteria, *L7A_E11*, *Roseburia*, *CF231*, Lactobacillales, Rikenellaceae, Paraprevotellaceae, Veillonellaceae, Bradyrhizobiaceae, Enterococcaceae, *Enterococcus*, Comamonadaceae, RF16, Proteobacteria, Erysipelotrichaceae, Erysipelotrichales and Erysipelotrichia showed a significant difference at $P < 0.05$. The average abundance of 11 floras was the highest in group C. *Sporosarcina* and Mogibacteriaceae showed a significant difference at $P < 0.01$, whereas *Actinotalea*, Beutenbergiaceae, Bogoriellaceae, *Georgenia*, *Bacillus*, Bacillaceae, Cellulomonadaceae, Christensenellaceae and *Adlercreutzia* showed a significant difference at $P < 0.05$.

DISCUSSION

Intestinal microbiota are closely related to the body's immunity, physiological metabolism, nutrient absorption, growth and development. Many studies show that wild yak is superior to domestic yak in many aspects. Therefore, studying the structure of intestinal bacterial flora in wild yaks and domestic yaks is important for improving the production performance of domestic yaks.

In the present study, we characterised the microbial diversity of bacteria in wild yak and domestic yak in Qiangtang Region of Tibet by high-throughput sequencing. A significant difference was found in the principal component of microbial community structure in different yak groups by PCA. This finding indicated that the living environment and feeding methods may affect the intestinal bacterial flora structure of yak. Studies have shown that a variety of bacteria are distributed in the faeces of wild yak and domestic yak, with Firmicutes, Actinobacteria and Bacteroidetes being the most dominant bacteria. This finding is similar to the results of studies on the intestinal microbes of cattle (Nie *et al*., 2017), horses (Proudman *et al*., 2015), sheep (Wang *et al*., 2016) and Tibetan pigs (Shang *et al*., 2019). This phenomenon also indirectly shows that the intestinal flora of the animal is affected by eating habits. At the genus level, more than 160 genera of wild yak and domestic yak intestinal bacteria were identified. Most of them were unclassified bacteria.

Chloroflexi was significantly higher in wild yaks compared with house-feeding domestic yaks and grazing domestic yaks. Therefore, Chloroflexi may be related to the

excellent traits of wild yaks. However, little information is available on Chloroflexi, and the specific physiological function of this bacterium needs further studies. Proteobacteria was found to be significantly higher in house-feeding domestic yaks than in wild yaks and grazing domestic yaks. Proteobacteria, including many pathogenic bacteria such as *Escherichia coli*, *Salmonella*, *Vibrio cholerae* and other species, has important significance in the clinical diagnosis of animal gastrointestinal diseases (Walujkar *et al*., 2014; Evans *et al*., 2011). The strong disease resistance of wild yaks may be related to the low abundance of Proteobacteria.

Clostridiaceae comprises a variety of butyrate-producing strains. These strains degrade fructose and maltose in food, and their products such as organic acids and alcohols help regulate the balance of intestinal microflora. Clostridiaceae also regulates T cell-mediated immune response in the intestine, which is related to colitis resistance (Louis and Flint, 2009; Atarashi *et al*., 2011). As a probiotic, the metabolites of *Microbispora* have antibacterial activity (Coombs and Franco, 2003). *Blautia* produces short-chain fatty acids, which is beneficial to intestinal health (Ozato *et al*., 2019). Clostridiaceae, *Microbispora* and *Blautia* were obviously higher in wild yaks compared with domestic yaks, indicating that the strong immunity and antibacterial ability of wild yaks may be related to the high content of these three bacteria. Studies have shown that *Carnobacterium* gradually increases with the growth of *Yili* horses. The abundance of *Carnobacterium* in the intestine of 1 month old, 2 months old and 3 months old *Yili* horses is 0, 0.89 and 1.82, respectively. By inference, *Carnobacterium* may be related to the digestive performance of animals (Li *et al*., 2017). *Carnobacterium* was significantly higher in wild yaks than in domestic yaks, and the rough feeding resistance of wild yaks was stronger than that of domestic yaks, which may be related to the higher abundance of *Carnobacterium*. *Salinibacterium* was significantly higher in wild yaks than in domestic yaks, which may indicate that *Salinibacterium* is related to the excellent traits of wild yaks. However, its specific mechanism of action needs further studies.

Epulopiscium can reduce the activity of the host's intestinal amylase, protease and lipase at physiologically relevant pH (Miyake *et al*., 2016). As *Epulopiscium* was significantly lower in wild yaks than in domestic yaks, the digestive ability of the former may be stronger than that of the latter. Brucellosis is a zoonotic disease caused by Brucellaceae, which is widely distributed all over the world and seriously endangers human health (O'Callaghan *et al*., 1999). A significant amount of Brucellaceae was found in domestic yaks than in wild yaks, which may indicate that domestic yaks have a higher risk of infecting Brucellosis than wild yaks. This may be related to feeding methods and living environments.

In this study, *Turicibacter*, *Ochrobactrum*, *Clostridium*, *SHD-231*, *Adlercreutzia*, *Lysinibacillus*, *Bulleidia*, *Paludibacter*, *Dietzia*, *Roseburia*, *CF231*, *L7A_E11*, *Syntrophococcus*, *Alistipes*, *Serratia*, *Anaerostipes*, *Planomicrobium*, *Perlucidibaca*, *Stenotrophomonas*, *Treponema* and *Isobaculum* showed significant differences in wild yaks and domestic yaks. However, the specific function of these bacteria in yaks remains to be further studied.

In conclusion, the present study first reported the changes of microbial diversity of bacteria in wild yaks and domestic yaks in the Qiangtang Region of Tibet. The findings may help in the improvement of the production performance of yaks.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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