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Microbial diversity in raw milk and traditional fermented dairy products (Hurood cheese and Jueke) from Inner Mongolia, China

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ABSTRACT. Hurood cheese (HC) and Jueke (Jk) are 2 traditional fermented dairy products produced from raw milk (RM) in the Inner Mongolia region of China. They have a long history of production and consumption. The microbial compositions of RM, HC, and Jk vary greatly, and are influenced by their geographical origins and unique processing methods. In this study, 2 batches of RM, HC, and Jk samples were collected (April and August 2015) from the Zhenglan Banner, a region located in the southern part of Inner Mongolian belonging to the Xilingol league prefecture. The bacterial and fungal diversities of the samples were determined by 16S rRNA and 18S rRNA gene sequence analysis, respectively. A total of 112 bacterial and 30 fungal sequences were identified, with Firmicutes and Ascomycota being the predominant phyla for bacteria and fungi, respectively. *Lactococcus* and *Lactobacillus* were identified as the main bacterial genera, whereas *Kluyveromyces* was the predominant fungus identified in the 3 dairy products. Different bacterial and fungal compositions were observed

in RM, HC, and Jk samples collected at different times. These results suggested that time of production may be an important factor influencing the microbial diversity present in RM, HC, and Jk.

Key words: Microbial diversity; Raw milk; Hurood cheese; Jueke; 16s rRNA; 18s rRNA

INTRODUCTION

Hurood cheese (HC) and Jueke (Jk) are the 2 most famous traditional fermented dairy products produced and consumed by the people of Inner Mongolia, China. These dairy products have had a long history in the Zhenglan Banner, a county located in the southern part of Inner Mongolia, which belongs to the Xilingol league prefecture. The manufacture of HC and Jk involves curdling a mixture of raw milk (RM) at a cool place in the absence of starter cultures or mold spores for natural fermentation and condensation. This process takes 1-2 days in summer or 3-7 days during other seasons. The RM used in the making of traditional fermented products comes from goat, sheep, cow, mare, and camel (Degen, 2007; Konuspayeva et al., 2009). Following fermentation, the uppermost layer of the cream is collected as Jk, and is consumed in salads. while the remaining curd is poured into a pot to separate out the whey. After that, the curd is transferred to molds, pressed, and slowly dried in the shade to make HC. HC is a type of extra-hard cheese with a moisture content of approximately 25% (Zhang et al., 2014). HC and Jk both have a white to light yellow color with distinctive flavors. They are the most common dairy products in Mongolian herdsmen families, and are deeply loved by the Mongolian people and tourists from other regions of China and abroad.

The quality of RM (unpasteurized milk) plays an important role in the manufacturing process of dairy products. Lactic acid bacteria (LAB) and yeasts are normally used in milk fermentation to make traditional cheese in Mongolia (Yu et al., 2011; Liu et al., 2012). However, during the preparation of HC and Jk, various other microorganisms are also introduced into the mixture via contamination, and participate in the fermentation process. Autochthonous fermenting microfloras present in RM have an important function in the fermentation process due to their contribution in flavoring of HC and Jk. Therefore, the organoleptic properties of HC and Jk are largely influenced by the local environment. The natural microflora present in RM can affect the making of HC and Jk as well as their shelf-life. They can be either beneficial microorganisms that confer health benefits or harmful bacteria that may cause diseases in consumers. HC and Jk provide a complex habitat for different microorganisms (both prokayrotes and eukaryotes) that interact and develop throughout the cheese-making process, such as during the ripening process (Flórez and Mayo, 2006). When cheese is being produced, many non-starter LAB may find their way into the dairy products through the milk, the cheese-making equipment, and the immediate environment, and thus contribute to the cheese ripening process (Van Hoorde et al., 2008). Microorganisms from the environment, some of which are pathogens, and others potential probiotic microorganisms, also exist in HC and Jk. Many researchers have reported the successful isolation and identification of some probiotics from traditional spontaneously fermented foods (Kuda et al., 2014; Kawahara et al., 2015).

The analysis of microbial composition present in fermented dairy products has been carried out by many investigators over the past few years. For example, An et al. (2004) found

that the predominant species in fermented mares' milk from Inner Mongolia are *Lactobacillus pentosus*, *Lactobacillus plantarum*, *Lactococcus lactis* subsp. *cremoris*, *Leuconostoc mesenteroides*, *Leuconostoc pseudomesenteroides*, and *Streptococcus parauberis*. On the other hand, Shuangquan et al. (2006) identified *Lactococcus raffinolactis* as the predominant lactocci species, and *Lactobacillus plantarum* and *Lactobacillus casei* as the predominant lactobacilli species in the traditional starter cultures used for the fermented milk, hurunge, in Inner Mongolia. Furthermore, Wu et al. (2009) showed that *Lactobacillus casei*, *Lactobacillus helveticus*, and *Lactobacillus plantarum* are the main lactobacilli species in koumiss. Zhang et al. (2014) identified a total of 121 LAB in homemade extra hard Mongolian HC, and 7 of these are *Lactobacillus plantarum*. Kuda et al. (2016) have also confirmed the presence of LAB and yeast co-fermentation in traditional fermented dairy foods from Inner Mongolia. Autochthonous microbiota plays an important role in enhancing the quality of fermented dairy products. However, there is no report on the identity and characterization of the natural microflora in RM, HC, and Jk from Inner Mongolia. Therefore, it is necessary to identify the natural microflora of RM, HC, and Jk in order to improve the quality and flavors in these products.

In this study, we investigated the microbial diversity of RM, HC, and Jk produced in Inner Mongolia using culture-independent 16S rRNA and 18S rRNA gene analysis. Our objective was to identify the LAB and yeast species present in RM, HC, and Jk. The result of this study could have important implication for improving the quality of dairy products in Inner Mongolia.

MATERIAL AND METHODS

Sample collection

Two batches of RM, HC, and Jk samples were obtained from 6 local dairy farms in the Zhenglan Banner (approx 42°38'N, 115°67'E), a county located in the southern part of the Inner Mongolian autonomous region of China. One batch was obtained in April 2015, and the other batch was obtained in August 2015. Each batch was composed of 6 RM, 4 HC, and 5 Jk samples. All samples were transferred to the laboratory under refrigerated conditions (4°C), and were stored at -80°C until use.

DNA extraction and polymerase chain reaction (PCR) amplification

Aliquots of RM (10 mL), crushed HC (10 g), and thawed Jk (10 g) were separately suspended in 50 mL sterile PBS (Sangon Biotech, Shanghai, China) containing glass beads (3 mm in diameter, Sangon Biotech, Shanghai, China). The mixtures were fully homogenized at 4°C in a lab blender (Stomacher 3500 Seward, UK) for 15 min. The homogenized samples were centrifuged at 700 *g* for 5 min. Supernatants were transferred to sterile tubes and further centrifuged at 13000 *g* for 10 min at 4°C. The top fat layer of each sample was then removed with sterile cotton tips, and the supernatant was discarded. Genomic DNA was extracted from the remaining microbial pellets using the Qiagen DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The extracted DNA was quantified using a Thermo Nanodrop 2000 spectrophotometer (Thermo Scientific, USA), and was analyzed via 0.8% agarose gel electrophoresis. All extracted DNA samples were stored at -20°C until further analysis.

DNA samples for each product collected during the same month were pooled and used as templates for amplification of the bacterial 16S rRNA gene and the V4 region of the fungal 18S rRNA gene by PCR. There were 6 pooled PCR samples in total; 2 samples for each dairy product, collected in April and August. The pooled sample for each dairy product will henceforth be referred to as a group. The bacterial 16S rRNA gene was amplified using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The V4 region of fungal 18S rRNA gene was amplified with the forward 3NDF (5'-GGCAAGTCTGGTGCCAG-3') and the reverse V4_euk_R2 (5'-ACGGTATCT(AG)ATC(AG)TCTTCG-3') primers (Bråte et al., 2010). Each 50 µL PCR mixture contained approximately 50 ng template DNA, 1.0 µM of each primer, 2.5 mM of each deoxynucleoside triphosphate, 2.5 mM $MgCl_2$, 5 µL 10X *LA Taq* Buffer II (Mg^{2+} free), and 2.5 U *TaKara LA Taq* polymerase (TaKaRa, Dalian, China). The following PCR conditions were used: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 5 min, and a final extension step at 72°C for 10 min. Following agarose gel electrophoresis, all PCR products from each template DNA were purified using the TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver. 3.0 (TaKaRa).

Construction of clone libraries for restriction fragment length polymorphism (RFLP) analysis

Purified PCR products were ligated to the pMD™19-T vector using a pMD™19-T Vector Cloning Kit (TaKaRa). The ligation products were transformed into *Escherichia coli* JM 109 (TaKaRa) according to manufacturer's instructions. Positive clones were selected and used as DNA templates in subsequent PCR amplifications with *Bca*BEST sequencing primers RV-M and M13-47. The PCR products were analyzed by 1.0% agarose gel electrophoresis to confirm fragment sizes. The confirmed fragments were used as DNA templates to amplify the bacterial 16S rRNA gene and the V4 region of the fungal 18S rRNA gene. All experiments were repeated 3 times.

The resulting purified PCR products were digested with the restriction endonucleases *Hae* III and *Hinf* I (TaKara) according to manufacturer's instructions, and were then analyzed by 3% agarose gel electrophoresis to generate RFLP profiles. In addition, individual purified PCR products of the 16S rRNA gene and 18S rRNA gene from each group were fully and bi-directionally sequenced by Beijing Genomic Institute (BGI, China) using an ABI 3730 DNA automatic sequencer (Applied Bio Systems, USA). Identities of sequences from each group searched against the National Center for Biotechnology Information (NCBI) database (http://www. ncbi.nlm.nih. gov/BLAST) via BLAST. Sequences with a percentage identity of 97% or greater were considered to belong to the same species (Flórez and Mayo, 2006) (Flórez & Mayo 2006). The estimated coverage of the 16S rRNA and 18S rRNA gene sequences were calculated as

$$C = [1 - (n_1 / N)] \, x \, 100\%$$

where n_1 is the number of singleton sequences, and N is the total number of sequences.

Nucleotide sequence accession numbers

The bacterial 16S rRNA gene sequences and fungal 18S rRNA gene sequences identified in this study have been submitted to the GenBank database.

RESULTS

Analysis of bacterial 16S rRNA and fungal 18S rRNA sequences

Following restriction enzyme digestion and RFLP analysis, 253 purified PCR products were obtained for the 16S rRNA and 18S rRNA genes, which were bidirectionally sequenced (Table 1). Sixteen sequences were found to be chimeras, and were omitted from further analysis. A total of 169 near full-length bacterial 16S rRNA sequences (continuous stretches of approximately 1450 bp) were generated and compared with sequences from the NCBI database. The species were initially determined by the BLAST program on NCBI (http://www. ncbi.nlm.nih.gov/). Interestingly, only 68 fungal 18S rRNA gene sequences (continuous stretches of approximately 500 bp) were generated. Two clone libraries were obtained from the available sequences, one for 16S rRNA and the other for 18S rRNA. The coverage of each clone library was calculated, and both coverage values met the requirements (> 70%) for constructing a clone library (Table 1). Through ClustalX alignment and 100% sequence identity clustering, the unique representative sequences were delimited for further analysis, which corresponded to 112 bacterial and 30 fungal sequences. These sequences were deposited into the GenBank with the following assigned accession numbers: KT985394-KT985452, KT971140-KT971156, KU363856-KU363908, KU361789-KU361801. It was found that 142 sequences showed 89-100% identity to their nearest sequences, as revealed by BLAST search of the NCBI database. Results revealed that 12 16S rRNA gene sequences (10.7%) from 5 samples showed <98% sequence identity to their nearest sequences (**Table S1**), and may represent bacterial species that have not yet been characterized. In addition, 2 of the 18S rRNA sequences obtained from the HC and Jk groups collected in August showed <98% sequence identity to their nearest sequences (**Table S2**). These results suggested that RM, HC, and Jk may exhibit higher bacterial diversity as compared to fungal diversity.

Table 1. Sequence analysis of 16S rRNA and 18S rRNA clone libraries in raw milk, Hurood cheese, and Jueke.

Sample	Number of clones		Number of sequences (after RFLP analysis)		Coverage (%)		Number of sequences (after sequencing)		Number of chimera sequences		Number of sequences (submit to GenBank database)	
	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
RM April	334	100	59	21	82.3	79.0	36	6	1	1	17	2
RM August	302	98	46	17	84.8	82.7	34	4	1	0	19	2
HC April	120	105	19	24	84.2	77.1	7	19	0	2	6	6
HC August	113	102	21	26	81.4	74.5	12	10	0	2	10	5
Jk April	304	187	69	35	77.3	81.3	54	20	5	0	36	9
Jk August	276	197	48	40	82.6	79.7	34	17	1	3	24	6
Total							177	76	8	8	112	30

Bacterial composition of RM, HC, and Jk

Fifteen bacterial genera were identified in the 6 samples of RM, HC, and Jk (Figure 1A). *Lactococcus* was found to be the dominant genus, and was identified in 41 sequences: 10 sequences from RM collected in April, 6 sequences from RM collected in August, 4 sequences from HC collected in April, 2 sequences from HC collected in August, 13 sequences from Jk collected in April, and 6 sequences from Jk collected in August. *Lactobacillus* was the subdominant genus in 6 samples, and was found in 27 sequences. The other 13 genera, namely, *Leuconostoc*, *Streptococcus*, *Bacterium*, *Enterococcus*, *Chryseobacterium*, *Providencia*,

Acetobacter, *Weissella*, *Dysgonomonas*, *Macrococcus*, *Xenophilus*, *Pseudoclavibacter*, and *Corynebacterium*, together comprised 44 of the total 112 bacterial sequences. In addition, the percentages of sequences from 15 genera varied among the 6 groups. *Lactococcus* contributed to more than 50% of total bacterial sequences in RM and HC samples taken in April. *Lactobacillus* was dominant in 5 samples (RM April, RM August, HC August, Jk April, and Jk August), and contributed to more than 24% of the total bacterial sequences; however, it was absent in the HC April group. A large difference was observed in the bacterial compositions between HC taken in April and HC taken in August, whereas only a few genera of bacteria were significantly different among the same types of samples collected during different times. We detected significant differences in bacterial compositions among RM, HC, and Jk (Figure 1A). Although 6 RM, 4 HC, and 5 Jk samples were collected from different dairy farms, total DNA from all samples were pooled and used for PCR analysis. Therefore, the results obtained should be a relatively accurate representation of the distribution of bacterial compositions in these 3 dairy products from this particular region of Inner Mongolia. Since not all of the selected farms produced all 3 products, instead of 6 samples, only 4 HC and 5 Jk samples were available across the 6 farms.

The bacteria in RM, HC, and Jk belonged to 5 phyla: Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Bacteriophyta (Figure 1B). The predominant phylum was Firmicutes, which included 84.82% of the 112 bacterial sequences, and was found in 65.22, 81.25, and 86.67% of the bacterial sequence reads of RM, HC, and Jk, respectively. Bacteroidetes was the subdominant phylum, which accounted for 6.25% of the total bacterial sequences. Proteobacteria, Actinobacteria, and Bacteriophyta together made up 8.93% of the total bacterial sequences. Only bacteria in the phylum Acidobacteria, but not the other 4 phyla, were found in the Jk April sample. Bacteria isolated from RM, HC, and Jk mainly belonged to the phyla Firmicutes [*Lactococcus* (36.61%), *Lactobacillus* (24.12%), *Leuconostoc* (12.5%), *Streptococcus* (7.14%), *Enterococcus* (2.68%), *Macrococcus* (0.89%), *Weissella* (0.89%)] and Bacteroidetes [*Chryseobacterium* (5.36%) and *Dysgonomonas* (0.89%)] (Figure 1B).

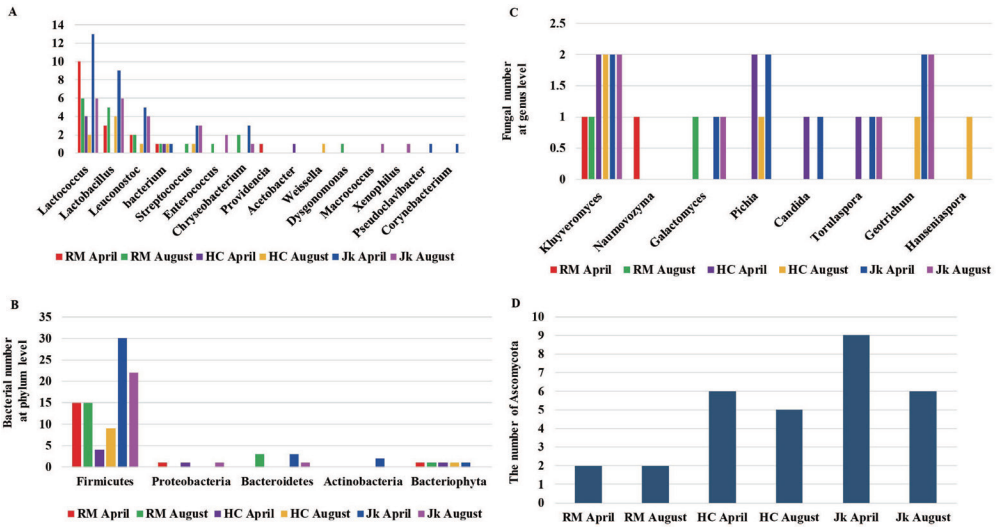

Figure 1. Number of bacteria (**A**, **B**) and fungi (**C**, **D**) at the genus and phylum levels.

Fungal composition of RM, HC, and Jk

Eight fungal genera were identified from a total of 30 fungal sequences obtained from RM, HC and Jk. At the genus level, *Kluyveromyees*, *Pichia*, *Geotrichum*, *Galactomyces*, *Torulaspora*, *Candida*, *Naumovozyma,* and *Hanseniaspora* accounted for 10, 5, 5, 3, 3, 2, 1, and 1 fungal sequences, respectively (Figure 1C). Among them, *Kluyveromyees*, *Pichia,* and *Geotrichum* (containing 33.33, 16.67, and 16.67% of total fungal sequences, respectively) were the dominant fungal genera. *Kluyveromyees* was prevalent in all 3 dairy products, whereas *Pichia* and *Geotrichum* were found only in HC April and Jk April samples. *Naumovozyma* was only found in the RM August sample, and *Hanseniaspora* was only found in the HC August sample. The fungal phylum dominant in all 3 dairy products was Ascomycota, accounting for 100% of total fungal sequences (Figure 1D). Ascomycota was found to be most abundant in the Jk April sample, followed by the HC April and Jk August samples.

Comparative analysis of bacterial composition in RM, HC, and Jk sampled at 2 different times

Analysis of the 16S rRNA gene sequences at the species level revealed a much more diverse bacterial population in RM (17 species) and Jk (24 Species) as compared to that in HC (11 species) (Figure 2). Furthermore, aside from Jk, higher bacterial diversity was observed in samples taken in August as compared to those taken in April. Among the 32 bacterial species, *Lactococcus lactis* was the most prevalent, but greater variation in the distribution of of this species was found in RM, HC, and Jk samples taken in April (29.41, 50 and 19.44% for RM, HC, and Jk, respectively) as compared to those taken in August (21.05, 10.00, and 12.50%). *Lactobacillus helveticus* was found in the RM April (11.76%), RM August (21.05%), HC August (30.00%), Jk April (16.67%), and Jk August (16.67%) samples. Uncultured *Lactococcus* sp. was only found in the April samples; *Streptococcus thermophiles* and *Leuconostoc citreum* were only found in Jk, while *Acetobacter tropicalis* was only present in the April HC sample. Other minor genera, such as *Chryseobacterium*, *Streptococcus*, *Providencia*, *Enterococcus*, *Weissella*, and *Macrococcus* were also present, but constituted only a small portion of the total bacterial sequences in some samples.

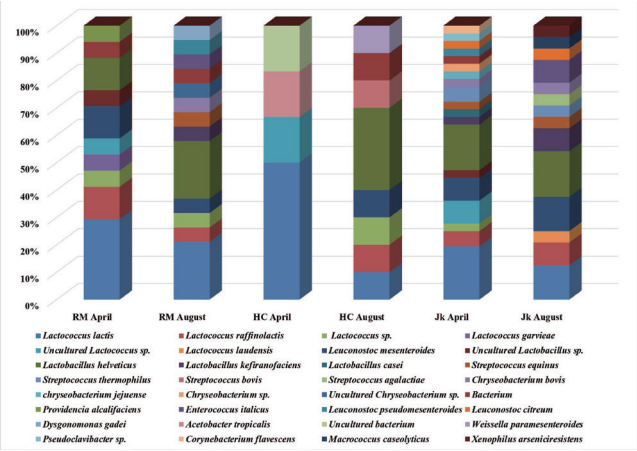

Figure 2. Prevalence of bacteria in raw milk, Horood cheese, and Jueke based on 16S rRNA gene sequences analysis.

Comparative analysis of fungal composition in RM, HC, and Jk samples from two different times

Ten fungal species were identified in the 3 dairy products by 18S rRNA gene sequences analysis (Figure 3). Four strains belonging to 3 fungal species (*Kluyveromyces marxianus*, *Galactomyces geotrichum*, and *Naumovozyma* sp.), were found in RM samples, which accounted for 13.33% of the total (30) number of fungal sequences. HC and Jk samples demonstrated enriched population of fungal species, which mainly consisted of the genera *Kluyveromyces*, *Pichia*, and *Geotrichum*. *Kluyveromyces marxianu*s was the dominant fungal species in all 3 dairy products, accounting for 26.67% of the total fungal sequences. *Kluyveromyces lactis* was only found in Jk, and *Pichia fermenntans* was only present in the HC and Jk samples taken in April. *Pichia kudriavzevii* was present only in HC samples. *Geotrichum candidum* contributed to 16.67% of the total fungal sequences found in the HC sample taken in August, and was also present in Jk samples taken in both April and August. *Galactomyces geotrichum*, *Torulaspora delbrueckii*, *Candida zeylanoides*, *Hanseniaspora guilliermondii* and *Naumovozyma* sp. were rarely observed in the 3 dairy products, and were occasionally even absent.

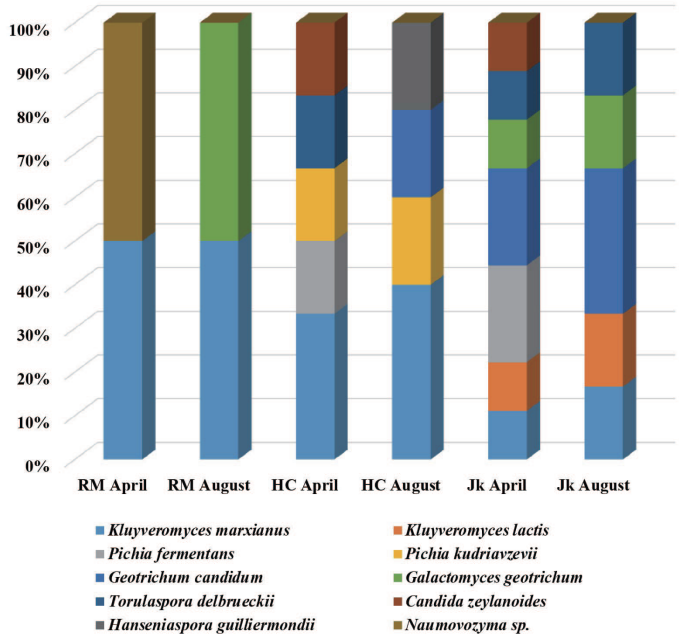

Figure 3. Prevalence of fungi in raw milk, Hurood cheese, and Jueke based on 18S rRNA gene sequences analysis.

DISCUSSION

The analysis of microbial diversity in RM, HC, and Jk from Inner Monglia in China was conducted using culture-independent 16S rRNA and 18S rRNA gene sequence analysis. This method provided a thorough description of the microbiota community using bioinformatics

analysis. The estimated coverage of 16S rRNA and 18S rRNA clone libraries were both over 74% (Table 1), fulfilling the requirements (>70%) for constructing a clone library. The results indicated that RM, HC, and Jk samples collected from the same region (Zhenglan Banner), but at different times (April and August) have diverse microbiota community structures. With respect to bacterial population, the initial bacteria in RM used for making HC and Jk (the two traditional fermented dairy products) were remarkably different from those of HC and Jk. It was thought that RM from the udder proper contained very few bacteria. These bacteria may have originated from the environment of the animals, their surroundings, and the milking equipment (Quigley et al., 2013). We also found that traditional fermented dairy products have higher fungal diversity than RM. As HC contained more lactic acid and less water, its microbiota community was relatively less diverse as compared with those of RM and Jk (Table 1). Firmicutes was the dominant bacteria at the phylum level, but differed considerably in abundance among the 6 groups (Figure 1B). This finding was in line with other studies investigating the microbial diversity of naturally fermented dairy products, such as raw cow milk (Raats et al., 2011; Zhang et al., 2015), kefir (Chen et al., 2008), yond bap (Liu et al., 2015b), and artisanal cheeses (Quigley et al., 2012). The fungal community identified in RM, HC, and Jk all belonged to the phylum Ascomycota; this phylum is also prevalent in some RM used to make French cheeses (Callon et al., 2006), Korean alcoholic beverages (Jung et al., 2012), and Chinese liquors (Li et al., 2011). Furthermore, it was found that the bacterial community present in the samples was more diverse than the fungal community, which was similar to the results shown in a previous study (Liu et al., 2015a).

Lactococcus lactis was the major species found in all 3 types of dairy products. *Lactococcus* and *Lactobacillus* were the dominant genera in RM, HC, and Jk samples from both April and August (Figure 1A). These 2 genera are commonly associated with naturally fermented dairy products (Watanabe et al., 2008; Alegría et al., 2012; Bao et al., 2012; Zhang et al., 2015). *Lactococcus* was the predominant genus in the RM and Jk samples taken in April. It has been shown that certain species of *Lactococcus* can be isolated from chigee and mare milk in Inner Mongolia as well as from traditional Polish cheese during the early stage of fermentation (An et al., 2004; Alegría et al., 2012). *Lactococcus* is a common curd cheese-associated LAB and a homofermentative species (Dzieciol et al., 2016). In addition to *Lactococcus*, another genus found to be prevalent in RM was *Leuconostoc* (Figure 1A), which has also been reported to be present in raw goat milk (Callon et al., 2007). *Leuconostoc mesenteroides* was detected in RM and Jk samples, but was present at very low level (1/10 species) in HC sample taken in August. Studies have shown that *Leuconostoc mesenteroides* is often found in a variety of cheeses (Cibik et al., 2000; Pérez et al., 2002).

Streptococcus thermophilus is a thermophilic bacterium, and was found only in Jk samples. Several species of the genus *Chryseobacterium* were found in Jk and RM samples collected in August. This genus has previously been detected in raw dairy products and dairy environments (Quigley et al., 2013; Weber et al., 2014). The genus *Bacterium* was detected in all 3 dairy products, but not in the Jk sample taken in August (Figure 1A). Its presence in HC and Jk samples may have been the result of bacterial contamination in RM and/or during the fermentation process.

The analysis of fungal 18S rRNA gene sequences showed that all fungi found in RM, HC, and Jk belonged to the phylum of Ascomycota. Among them, *Kluyveromyces marxianus* was the dominant species in all 3 dairy products. This species is also a major species in airag, which is another traditional Mongolian fermented product (Liu et al., 2015a). In addition,

Kluyveromyces marxianus is also ubiquitous in some RM French cheeses (Callon et al., 2006) and milk kefir grains from different regions in Italy (Garofalo et al., 2015). *Kluyveromyces lactis* is one of the most important non-*Saccharomyces* yeasts capable of growing on lactose as the sole carbon source, and is commonly used in various food and feed applications, genetic studies, and industrial applications (Gorietti et al., 2015). In this study, *Kluyveromyces lactis* was only found in Jk. *Pichia* was detected in HC and Jk, but was not found in RM. *Pichia* has been reported to participate in fermentative metabolic processes of traditional Mongolian fermented dairy products (Watanabe et al., 2008; Miyamoto et al., 2010). It can hydrolyze protein and fat in milk, assimilate lactic acid, and produce ethanol (Sudun et al., 2013). *Geotrichum candidum* has been detected in dairy products such as Reblochon-type cheeses (Castellote et al., 2015) and Mongolian naturally fermented cow's milk (Liu et al., 2015a). It is involved in lactic acid utilization, proteolysis, lipolysis, and flavor development (Wyder et al., 1999). We also detected *Torulaspora delbrueckii*, *Candida zeylanoides*, *Hanseniaspora guilliermondii*, and *Naumovozyma* sp. in RM, HC, and Jk from both April and August samples.

Interestingly, the bacterial community of RM, HC, and Jk samples taken in August was more diversified than that of samples taken in April. This could be due to differences in temperature, as April is warmer than August in Inner Mongolia, and most LAB species tend to grow better in warmer environments. The fewer LAB species present in HC could be due the different properties of HC as compared to RM and Jk. Only a few fungal species were detected in RM; it is possible that some of the yeasts identified in HC and Jk tend to prefer the environments of these 2 dairy products, which contain relatively more sugar than RM. The microbiota structure of RM, HC, and Jk may be affected by geographical locations as well as several other factors, such as the surrounding environment, the manufacturing process, the fermentation time, and temperature. As a result, these factors all play a role in the special flavor and texture of these dairy products.

In this study, we used culture-independent approaches to analyze the bacterial and fungal communities in the 3 dairy products, RM, HC, and Jk. A major limitation of this method is that PCR-based assays cannot discriminate between DNA derived from viable and dead microbial cells. Therefore, the results would have been enhanced if microbial culture methods have also been used. However, some of the bacteria and fungi present in the dairy products may not easily cultured in artificial media due to technical difficulties. The diversities of bacterial and fungal communities in RM, HC, and Jk collected at two different times were determined using 16S rRNA gene and18S rRNA gene analysis. Different microbiota structures were found in these dairy products, with *Lactococcus* and *Lactobacillus* being the dominant bacteria and *Kluyveromyces* being the predominant fungus. The difference in manufacture time may be an important determinant of microbial diversity in the products. Future research should aim to combine culture-dependent approach for studying the microbial diversity in RM, HC, and Jk. This may improve screening and isolation of LAB and fungal strains that can be used for making other dairy products.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material

Table S1. Sequence identities between the putative bacteria and its closest relative via BLAST search in the GenBank database.

Table S2. Sequence identities between the putative fungi and its closest relative for the species via BLAST search in the GenBank database.