Analyses of physicochemical properties, bacterial microbiota, and lactic acid bacteria of fresh camel milk collected in Inner Mongolia

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ABSTRACT

Camel milk has significant economic value and is an important food in the region of Alxa Left Banner of Inner Mongolia. Fifteen fresh camel milk samples were collected from domesticated camels in a pasture of Alxa Left Banner. The physicochemical properties and bacterial diversity of camel milk samples were analyzed. The average values of fat, total protein, nonfat milk solids, acidity, and density were 4.40%, 3.87%, 9.50%, 16.95°T, and 1.02 g/cm³, respectively. The bacterial microbiota of the collected fresh camel milk was investigated using PacBio single-molecule real-time (Pacific Biosciences, Menlo Park, CA) sequencing. The camel milk microbiota was highly diverse and comprised 8,513 operational taxonomic units belonging to 32 phyla, 377 genera, and 652 species. The major phyla included *Proteobacteria*, *Firmicutes*, *Deinococcus-Thermus*, *Bacteroidetes*, and *Actinobacteria*. A small number of lactic acid bacteria sequences were detected, representing the species *Streptococcus thermophilus*, *Lactobacillus helveticus*, *Lactococcus lactis*, and *Leuconostoc mesenteroides*. A total of 72 strains of lactic acid bacteria were isolated and identified from 15 samples, including *Lactobacillus paracasei*, *Enterococcus italicus*, *Enterococcus durans*, *Lactococcus lactis* ssp. *lactis*, *Weissella confusa*, and *Enterococcus faecium*. These results confirm that fresh camel milk has a high bacterial diversity and is a valuable natural resource for isolation of novel lactic acid bacteria.

Key words: camel milk, bacterial microbiota, lactic acid bacteria, single-molecule real-time sequencing, Alxa Left Banner

INTRODUCTION

Bactrian camels are found in the desert zone of central Asia and China (Kadim et al., 2008). There are approximately 316,000 Bactrian camels in China (China Statistical Yearbook, 2013), mainly domesticated in Inner Mongolia, Xinjiang, Qinghai, Gansu, Ningxia, and other provinces. Alxa Left Banner is a region within Inner Mongolia. As the main livestock in Alxa Left Banner, Bactrian camels are an important economic species for local herdsman. Camel milk has been part of the daily diet of nomadic people for centuries, and it has good nutritional and medicinal properties (Yagil, 1982; Shabo et al., 2005). Its biochemical composition is close to that of human milk, and it has a relatively low fat content and is rich in vitamins (Zibae et al., 2015). Moreover, camel milk contains all the essential nutrients needed for human, so it can serve as an alternative to bovine milk (Shamsia, 2007; El-Agamy et al., 2009; Bulca, 2017). One additional advantage is its low allergenicity (Teply, 1965; Zibae et al., 2015).

Aside from the physicochemical properties and nutritional composition of camel milk, a rich bacterial diversity exists in the milk, particularly in lactic acid bacteria (LAB; Khedid et al., 2009), which can contribute to the functional and desirable qualities of camel milk (Jans et al., 2012). Much effort has been devoted to research the yield of camel milk and its nutritional values (Bai et al., 2009; Zhao et al., 2015), as well as its medicinal values (Kula and Tegegne, 2016). However, the bacterial composition of camel milk has not been studied systematically. Lactic acid bacteria not only contribute to the sensorial and textural properties of dairy products but also help produce bioactive substances, such as bacteriocins and organic acids, that suppress the growth of potential pathogens (Leroy and De Vuyst, 2004). The presence of *Lactobacillus*, *Lactococcus*, *Streptococcus*, and *Enterococcus* in camel milk has been reported previously (Khedid et al., 2009).

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Other than gaining an understanding of the nutritional value of camel milk, it is important to profile its microbiota with respect to food safety and milk quality assessment (Benkerroum et al., 2003). Similar to other types of milk, camel milk contains a high level of microorganisms, reaching 10^5 cfu/mL (Dong et al., 2012). Therefore, studying the intrinsic microbiota composition is of high practical significance for the industrialization and promotion of camel milk. High-throughput sequencing has become a common technique to investigate microbial communities present in food products including milk (Ercolini, 2013; Li et al., 2017; Jin et al., 2018). It overcomes the limitation of isolating fastidious microbes using culture-dependent methods. For example, donkey milk bacterial communities were found to comprise 5 major phyla: *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Verrucomicrobia* (Soto Del Rio et al., 2017). Until now, most available reports studying camel milk microbiota have been based on culture-dependent methods (Benkerroum et al., 2003; Omer and Eltinay, 2008; Yamina et al., 2013; Ayyash et al., 2018). The complete profile of the camel milk microbiota has not yet been described by a high-throughput sequencing approach, although high-throughput sequencing has been used to identify possible sources of raw milk contamination (Heikkilä and Saris, 2003; Hou et al., 2015; Wang et al., 2018).

The application of PacBio single molecule real-time (SMRT; Pacific Biosciences, Menlo Park, CA) sequencing technology can achieve high taxonomic resolution, up to the species level, when combined with full-length 16S rRNA gene sequencing (Mosher et al., 2014). In the current study, we applied this technology to detect the bacterial microbiota of 10 camel milk samples collected in the Alxa Left Banner region. We focused on isolating and identifying LAB from 15 fresh camel milk samples by culture-dependent methods. Data generated from this work provide interesting information about the bacterial community of camel milk, which is valuable to the industry in search of novel functional LAB resources.

MATERIALS AND METHODS

Sample Collection

Fifteen fresh camel milk samples were collected on a farm in Alxa Left Banner of Inner Mongolia in March 2018. The teats of the cows were cleaned with 70% ethanol, and a few streams of foremilk were discarded before sample collection. All samples were stored in 50-mL sterile centrifuge tubes and stored at 4°C before being transported to the laboratory in an ice box within 24 h of collection. Upon arrival at the laboratory, half

of each sample was stored frozen at -80°C for DNA extraction and sequencing. The remaining half was used immediately for microbiological and physicochemical analyses.

Physicochemical Analyses of Fresh Camel Milk

Physicochemical analyses of 10 of the fresh camel milk samples were performed following the methods of the National Food Safety Standards (2010, 2016a,b,c,d) of China. The contents of fat, protein, nonfat milk solids, acidity, and density were measured by the methods described in the guidelines (GB5009.6-2016, GB5009.5-2016, GB5413.39-2010, GB5009.239-2016, and GB5009.2-2016, respectively).

Enumeration and Isolation of LAB

Camel milk LAB counts of all 15 samples were determined by plate count. One milliliter of sample was serially diluted with 9 mL of sterile physiological saline (0.85% NaCl, wt/vol) to a dilution of 10^{-3} . Then, a 1-mL aliquot of each diluted sample was used to determine counts (cfu) by pour plate method with de Man, Rogosa and Sharpe agar (MRS; Oxoid/Shanghai Harmony Biotechnology Co. Ltd., Shanghai, China). Agar plates were incubated at 37°C for 36 to 48 h anaerobically ($\text{CO}_2:\text{H}_2:\text{N}_2 = 10:10:80$). After incubation, only plates with 30 to 300 colonies were selected for counting. Data were expressed as colony-forming units per milliliter of milk. All experiments were performed in triplicate.

For isolation of LAB, 200 μL of diluted sample from each diluted gradient was spread on MRS or M17 agar (Oxoid/Shanghai Harmony Biotechnology Co. Ltd.). Plates were incubated at 37°C for 36 to 48 h anaerobically. Colonies with distinct morphology resembling that of LAB were selected and purified by streaking. All isolates were gram-positive and catalase-negative. Isolated strains were subcultured in M17 and MRS broths (both from Oxoid) to maintain purity and for subsequent experiments. Frozen stocks were made to preserve isolated strains.

Identification of Selected Isolates by 16S rRNA Sequencing

Genomic DNA of the purified isolates was extracted using a commercial kit (Tiangen Biotech Co. Ltd., Beijing, China), and the concentration and purity of the genomic DNA were checked with an ND-1000 type micro UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The 16S rRNA gene was amplified on a thermal cycler (PTC-200, MJ Research,

Waltham, MA) using genomic DNA as template with the universal bacterial 16S rRNA gene primers: 27F (5'-GCAGAGTTCTCGGAGTCACGAAGAGTTTGATCCTGGCTCAG-3') and 1495R (5'-AGCGGATCACTTCACACAGGACTACGGCTACCTTGT-TACGA-3') (Scarpellini et al., 2002). The 50- μ L PCR included 5 μ L of 10 $\times$ PCR buffer, 4 μ L of dNTP (2.5 mmol/L), 1.5 μ L of 27 F primer (10 mmol/L), 1.5 μ L of 1495R primer (10 mmol/L), 1.5 μ L of DNA template, and 0.5 μ L of *Taq* DNA polymerase (5 U/ μ L). The PCR amplification cycle parameters were as follows: predenaturation at 94°C for 5 min; denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min. Cycles were repeated 30 times, followed by an extension step at 72°C for 10 min. The PCR products were visualized using agarose gel electrophoresis and a gel imager. Sequencing was performed by Majorbio Bio-Pharm Technology Corp. Ltd. (Shanghai, China).

The 16S rRNA gene sequences were analyzed using the SeqMan software (DNA Star, Madison, WI), and the identities of the bacterial isolates were determined using the BLAST algorithm (<http://www.ncbi.nlm.nih.gov/blast>; Altschul et al., 1997). Sequences generated in this work were submitted to the National Center for Biotechnology Information database (<http://www.blast.ncbi.nlm.nih.gov>) under accession numbers MH891655 to MH891726 and IMAU11991 to IMAU12061. The 16S rRNA gene sequences of the type strains were retrieved from the List of Prokaryotic Names with Standing in Nomenclature (LPSN; <http://www.bacterio.net/>), and a phylogenetic tree was created with sequences of the isolated LAB using the MEGA software (version 7.0; <http://www.megasoftware.net>; Tamura et al., 2007).

DNA Extraction for SMRT Sequencing

The bacterial microbiota of 10 fresh camel milk samples were determined. Total DNA was extracted from 1.5 mL of each sample using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle et al., 2016) with the Soil DNA Extraction kit (Omega Bio-Tek, Norcross, GA). The quality of the extracted DNA was checked by agarose gel electrophoresis and spectrophotometry (Thermo Fisher Scientific).

PCR and SMRT Sequencing for Microbiota Profiling

The forward 27F (5'-GAGAGTTTGATCCTGGCTCAG-3') and reverse 1541R (5'-AAGGAGGTGATC-CAGCCGCA-3') primers were used to amplify the full-length 16S rRNA gene from genomic DNA extracted from the camel milk. The volume of reaction mixture was 25 μ L. The reaction conditions were as follows:

95°C for 3 min, 30 cycles of 98°C for 20 s, 58°C for 15 s, and 72°C for 1.5 min, with a final extension of 72°C for 1 min. The Agilent DNA 1000 Kit and an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) were used to check the quality of the PCR products. The purified amplification products were sequenced using the P6-C4 chemistry (Pacific Biosciences) on the PacBio RS II platform (Pacific Biosciences).

Bioinformatics Processing

Raw data were analyzed with RS-ReadsOfinsert.1 of SMRT Portal (version 2.7, Pacific Biosciences). The sequence filter parameter criteria, including the minimum full passes, minimum predicted accuracy, minimum read lengths, and maximum read lengths, were set to 5, 90, 1,400, and 1,800, respectively (Hou et al., 2015). The barcode and primer sequences were removed to create the data set. Then, PyNASt (Caporaso et al., 2010) was applied to align the extracted high-quality sequences, and UCLUST (Edgar, 2010) was used to classify operational taxonomic units (OTU) under a threshold of 97% (Lozupone et al., 2006). A de novo taxonomic tree was constructed as described in Price et al. (2010). Alpha diversity was evaluated with the Chao1 richness estimator, the Shannon-Wiener diversity index, and the Simpson diversity index.

Statistical Analysis

Graphic presentations were generated by the Origin software version 8.5 (OriginLab Corp., Hampton, MA). The co-occurrence and co-exclusion relationships were plotted with the Corrplot package under the R environment (<http://www.r-project.org>). Spearman correlation coefficients (*r*) were used to determine relationships between relative abundances of identified bacterial sequences.

RESULTS

Physicochemical Properties

The average contents of fat, total protein, nonfat milk solids, acidity, and density of the 10 milk samples were (mean $\pm$ SD) $4.40 \pm 0.82\%$ (range: 2.79–5.51%), $3.87 \pm 0.29\%$ (3.39–4.36%), $9.50 \pm 0.47\%$ (8.58–10.3%), 16.95 ± 1.65 °T (13.5–19.7 °T), and 1.02 ± 0.03 g/cm³ (0.98–1.06 g/cm³), respectively.

LAB Counts and Isolation

The average LAB viable count was 6.02×10^3 cfu/mL, ranging from 6.35×10^2 cfu/mL to 5.55×10^4

cfu/mL. A total of 72 LAB strains were isolated, 31 and 41 from MRS and M17 medium, respectively. They were all presumptive LAB based on gram-positive and catalase-negative characteristics. All were further identified to the species level by 16S rRNA gene sequencing. A phylogenetic tree was constructed to show evolutionary relationships among them and representative type strains (Supplemental Figure S1; <https://doi.org/10.3168/jds.2019-17023>). The 72 LAB strains belonged to 4 genera (*Lactobacillus*, *Lactococcus*, *Weissella*, and *Enterococcus*). They were identified as *Lactobacillus paracasei* (26 strains), *Enterococcus italicus* (23 strains), *Enterococcus durans* (12 strains), *Enterococcus faecium* (1 strain), *Lactococcus lactis* ssp. *lactis* (8 strains), and *Weissella confusa* (2 strains). Among them, *Lactobacillus paracasei* accounted for the highest proportion (36.1%), followed by *Enterococcus italicus* (31.9%). In addition to LAB strains, we found some potential pathogens in the fresh camel milk, including *Raoultella ornithinolytica*, *Streptococcus gallolyticus*, *Streptococcus suis*, *Staphylococcus aureus*, and *Streptococcus uberis*.

Richness, Diversity, and Composition of Camel Milk Bacterial Microbiota

Genomic DNA was extracted from 10 camel samples. A total of 58,806 high-quality sequences were obtained (average of 5,880.6 per sample), corresponding to 8,513 OTU (average of 851.3 OTU/sample). The α diversity indices of samples are shown in Table 1. The Shannon, Chao 1, and Simpson indices and the number of observed species ranged from 3.87 to 8.01, 527.49 to 2,243.24, 0.85 to 0.98, and 185.14 to 880.42, respectively.

Bacterial diversity and richness were also evaluated by rarefaction curves (Figure 1a) and Shannon diversity index curves (Figure 1b). As shown in Figure 1a, the rarefaction curves of all samples were not saturated, suggesting that new bacterial species might still be

detected if sequencing depth increased. The Shannon index curves (Figure 1b), however, reached a plateau, suggesting that the current sequencing depth was enough to cover the bacterial diversity. A large Shannon diversity index indicates high bacterial diversity. Based on the current data, samples XT10 and XT2 had the highest and lowest bacterial diversity, respectively.

Camel Milk Bacterial Microbiota

The 8,513 identified OTU were assigned to 32 phyla, 76 classes, 109 orders, 173 families, 377 genera, and 652 species (data not shown). All samples showed a similar microbiota structure at the phylum level. The dominant bacterial phyla (with mean relative abundance >1%) are shown in Table 2 and were *Proteobacteria* (50.34%), *Firmicutes* (27.95%), *Deinococcus-Thermus* (14.25%), *Bacteroidetes* (4.08%), and *Actinobacteria* (1.95%).

At the genus level (Table 2; Figure 2a), 10 dominant bacterial genera (average relative abundance >1%) could be identified, including *Pseudomonas* (29.92%), *Thermus* (14.10%), *Streptococcus* (10.04%), *Acinetobacter* (7.45%), *Lactobacillus* (3.67%), *Moraxella* (1.96%), *Lactococcus* (1.71%), *Naxibacter* (1.49%), *Leuconostoc* (1.43%), *Paenibacillus* (1.21%), *Propionibacterium* (1.05%), and *Neisseria* (1.04%). It is interesting to note that *Proteobacteria* and *Pseudomonas* were the predominant phylum and genus in sample XT2, comprising 94.44% and 93.70% (Figure 2a) of total sequences, respectively; these results were consistent with the observed low bacterial diversity of this sample.

At the species level, 12 dominant bacterial species (>1% of average relative abundance each) were identified: *Thermus scotoductus* (14.10%), *Streptococcus thermophilus* (8.57%), *Pseudomonas veronii* (7.44%), *Pseudomonas fragi* (5.37%), *Pseudomonas cedrina* (5.20%), *Acinetobacter lwoffii* (4.27%), *Acinetobacter johnsonii* (2.82%), *Lactobacillus helveticus* (2.33%), *Lactococcus lactis* (1.69%), *Naxibacter alkalitolerans* (1.45%), *Leu-*

Table 1. Sequence abundance and microbial diversity of samples

Sample	No. of reads	No. of OTU ¹	Shannon index	Simpson index	Chao1 index	No. of observed species
XT2	6,381	291	3.87	0.85	527.49	185.14
XT3	4,853	496	5.00	0.90	1,007.46	367.40
XT4	7,897	1,375	7.30	0.96	1,713.93	809.14
XT5	6,104	890	5.78	0.94	1,442.00	574.74
XT7	6,955	864	5.62	0.90	1,300.95	514.16
XT8	3,209	771	6.61	0.93	2,243.24	753.38
XT10	4,406	1,093	8.01	0.98	2,069.67	880.42
XT12	6,972	974	6.48	0.95	1,183.60	643.38
XT13	4,255	622	5.81	0.93	1,480.20	367.40
XT15	7,774	1,137	6.64	0.94	1,511.31	661.94
Mean $\pm$ SD	5,880.60 $\pm$ 1,609.25	851.30 $\pm$ 321.49	6.11 $\pm$ 1.17	0.92 $\pm$ 0.03	1,447.98 $\pm$ 497.50	575.71 $\pm$ 219.60

¹OTU = operational taxonomic units.

conostoc mesenteroides (1.43%), and *Propionibacterium acnes* (1.03%) (Table 2). Relatively large variation was found in the species-level composition between samples. For example, *Pseudomonas veronii* dominated samples XT2, XT5, XT12, contributing 26.69, 13.30, and 13.08% (Table 2) of the total sequence, respectively. The second dominant species, *Pseudomonas fragi*, represented 11.76 and 12.03% in samples XT5 and XT12, whereas *Pseudomonas cedrina* represented 18.98% of the total sequence in sample XT2 (Table 2). *Thermus scotoductus* was the predominant species in samples XT4, XT7, XT8, and XT15, representing

21.62, 38.12, 32.03, and 29.06% (Table 2) of the total sequence, respectively. Some samples had unique representative species. For example, the relative abundances of *Acinetobacter lwoffii* (23.41%) and *Lactococcus lactis* (14.84%) were highest in samples XT13 and XT10, respectively. Sample XT3 was enriched in *Streptococcus thermophilus* (46.57%) and *Lactobacillus helveticus* (15.91%) (Table 2).

Correlations Among Detected Camel Milk Taxa

Because the intrinsic microbes present in the milk interactively modulate the milk microbiota structure, correlation analysis was performed among detected camel milk taxa of relative abundance >0.3% (Figure 3). Interesting and significant co-occurrence relationships were detected among some LAB. For example, *Lactobacillus helveticus* showed strong correlations with *Streptococcus thermophilus* and *Lactobacillus fermentum* ($P < 0.001$; $r = 0.9084$ and 0.9211 , respectively). Moreover, the most dominant species of the camel milk microbiota, *Thermus scotoductus*, was positively and strongly correlated with *Brevundimonas terrae* ($P < 0.01$, $r = 0.7988$). A few taxa showed negative correlations. For instance, *Massilia timonae* co-excluded the presence of *Pseudomonas veronii* ($P < 0.05$, $r = -0.6849$) and the presence of *Pseudomonas cedrina* ($P < 0.05$, $r = -0.6712$; Figure 3).

DISCUSSION

Camels produce milk that provides valuable nutrients for nomadic families living in harsh environments (Alwan et al., 2014). In this study, we first assessed the physicochemical properties of camel milk. A previous work characterized the composition and physicochemical properties of Chinese Bactrian camel milk (Zhao et al., 2015), including the contents of total protein and fat, as well as milk acidity and density. Generally, the values for the current batch of camel milk were lower than those reported in Zhao et al. (2015). Many factors can affect the physicochemical parameters. For example, milk density and fat usually peak during the first stage of lactation (Hadeef et al., 2018). The lower acidity of the current batch of camel milk could be related to bacterial richness in the milk; specifically, LAB could decompose lactose to produce lactic acid and increase the milk acidity. The lower acidity could also result from the quantity and type of feeds available in the local area (Alwan et al., 2014). Protein content (casein and whey proteins) might vary due to camel breed and season of milk collection (Sawaya et al., 1984; Elamin and Wilcox, 1992; Al Haj and Al Kanhal, 2010). Nonfat milk solids refers to all substances except fat and water

Figure 1. Rarefaction curves (a) and Shannon diversity index (b) of all samples (XT). OTU = operational taxonomic units.

Table 2. Major taxa detected in the camel milk samples (XT; mean relative abundance >1%)

Phylum	Genus	Species	Relative abundance (%)											
			XT2	XT3	XT4	XT5	XT7	XT8	XT10	XT12	XT13	XT15	Mean	
Proteobacteria	Nazibacter	Nazibacter alkaliolerans	94.44	12.40	36.81	62.66	46.84	46.99	35.32	70.48	61.18	36.30	50.34	
			0	0.37	3.41	0.15	4.47	2.96	2.04	0.42	0.26	0.85	1.49	
	Pseudomonas	Pseudomonas veronii	0	0.37	3.37	0.15	4.39	2.71	2.02	0.40	0.26	0.84	1.45	
			93.70	4.16	14.99	55.47	25.42	14.02	2.93	54.72	25.38	8.41	29.92	
	Pseudomonas	Pseudomonas fragii	26.69	1.07	2.55	13.30	6.63	2.99	0.73	13.08	5.55	1.80	7.44	
		Pseudomonas cedrina	7.44	0.76	6.28	11.76	2.13	3.93	0.75	12.03	6.67	1.96	5.37	
Firmicutes	Acinetobacter	Acinetobacter lwoffii	18.98	0.56	1.56	9.27	5.25	1.87	0.59	9.14	3.69	1.13	5.20	
			0.08	3.26	6.36	0.20	8.66	14.96	1.29	0.77	28.67	10.21	7.45	
	Acinetobacter	Acinetobacter johnsonii	0.03	2.06	1.99	0.10	2.83	4.33	0.93	0.42	23.41	6.65	4.27	
			0.05	1.07	4.17	0	5.36	9.82	0.32	0.13	4.04	3.27	2.82	
	Lactobacillus	Lactobacillus helveticus	5.19	72.37	29.72	29.60	12.34	15.11	44.58	19.87	24.02	26.72	27.95	
			3.43	21.27	3.82	0.16	0.68	1.03	2.38	0.16	2.47	1.27	3.67	
Actinobacteria	Lactococcus	Lactococcus lactis	3.37	15.91	3.14	0.05	0.14	0.19	0.11	0.03	0	0.39	2.33	
			0.03	0.41	0.25	0.34	0.17	0.44	15.00	0.11	0.09	0.19	1.71	
	Streptococcus	Streptococcus thermophilus	0.03	0.41	0.25	0.34	0.17	0.41	14.84	0.11	0.09	0.18	1.69	
			0.13	47.15	5.98	0.90	4.80	3.49	10.89	3.90	9.40	13.74	10.04	
	Leuconostoc	Leuconostoc mesenteroides	0.09	46.57	4.25	0.33	4.20	1.99	5.72	1.70	8.81	12.04	8.57	
			1.16	0.02	0.37	9.14	0.10	0.56	0.34	2.19	0.31	0.09	1.43	
Deinococcus-Thermus	Propionibacterium	Propionibacterium acnes	1.16	0.02	0.37	9.13	0.10	0.56	0.34	2.19	0.31	0.09	1.43	
			0.03	2.02	2.14	2.15	0.56	1.34	6.56	1.41	1.65	1.70	1.95	
	Thermus	Thermus scotoductus	0.02	1.50	1.16	0.16	0.27	0.50	4.65	0.56	0.94	0.73	1.05	
			0.02	1.50	1.11	0.16	0.27	0.50	4.49	0.55	0.94	0.72	1.03	
	Thermus	Thermus scotoductus	0.03	11.50	21.70	0.20	38.15	32.16	3.00	1.36	5.03	29.41	14.25	
			0	11.40	21.62	0.20	38.12	32.03	2.29	1.33	4.96	29.06	14.10

Figure 2. Relative abundances and bacterial diversity at the genus (a) and species (b) levels. XT = sample.

Figure 3. Correlations among major bacterial species. Spearman rank correlation matrix of species with relative abundance >0.3% was included in the analysis. The color of the scale bar represents the nature and strength of correlation, with 1 indicating a perfectly positive correlation (dark blue) and -1 indicating a perfectly negative correlation (dark red) between 2 items. Only correlations with P -value < 0.05 are shown.

in milk, and it is often used as an indicator for measuring the quality of fresh milk (Ji et al., 2011). Compared with that reported in Zhang et al. (2016), a comparable level of nonfat milk solids was present in the current samples. In addition to the influencing factors stated above, factors such as geographic area, feeding conditions, physiological variations, and genetic or health status play important roles in shaping the composition of camel milks (Konuspayeva et al., 2009).

Fresh camel milk is a potential source of novel LAB of high probiotic potential (Abushelaibi et al., 2017; Ayyash et al., 2018). Our study isolated 72 LAB strains from fresh camel milk; the LAB plate count was 6.02

$\times 10^3$ cfu/mL. *Enterococcus* and *Lactobacillus* were the dominant genera and the predominant species were *Enterococcus italicus* and *Lactobacillus paracasei*. Benkerroum et al. (2003), however, reported a much higher LAB count (1×10^7 cfu/mL) in raw camel milk collected in Morocco. The bacteria in the study by Benkerroum et al. (2003) mainly belonged to the genus *Enterococcus*, particularly the species *Enterococcus faecalis*. A similar level of LAB count was reported in camel milk collected in south Morocco (Khedid et al., 2009). The dominant taxa were *Lactobacillus*, and *Lactococcus lactis* ssp. *lactis*. Yamina et al. (2013) found 30 strains of LAB from camel milk collected in Algerian,

and Ayyash et al. (2018) found 23 strains of LAB in camel milk collected from farms in Abu Dhabi, Saudi Arabia, and the United Arab Emirates. To sum up, the quantity and types of LAB can vary greatly among milk samples collected in different geographical regions.

Using SMRT technology, we profiled the bacterial microbiota of the camel milk samples. A high relative abundance of sequences representing the species *Streptococcus thermophilus*, *Lactobacillus helveticus*, *Lactococcus lactis*, and *Leuconostoc mesenteroides*. The rich LAB composition might function as natural antibacterial agents that enhance the shelf life of camel milk and its products, as well as serving as beneficial microorganisms (Benkerroum et al., 2003). The health-promoting effects of LAB have received much attention. Milk fermented by *Leuconostoc mesenteroides* shows high antioxidant activity; that is, 1,1-diphenyl-2-picrylhydrazyl (and 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activities, as well as high overall acceptance values compared with samples fermented by other strains (Soleymanzadeh et al., 2016). Lactic acid bacteria isolated from camel milk can also be used to produce other fermented dairy products because of their high acidifying and proteolytic activities (Fguiri et al., 2016).

Milk is generally produced in a traditional manufacture line, including the processes of milk collection, storage, handling, and transportation under strict sanitary conditions (David, 2012; Gautret et al., 2013). Some 16S rRNA sequences detected by SMRT technology represent potential contaminants and pathogens. The prevalence of certain microbes could undoubtedly reflect the hygienic conditions of milk production. For example, the *Pseudomonas* genus has been consistently reported to be dominant microbes of raw milk (Sørhaug and Stepaniak, 1997; Quigley et al., 2013; Soto Del Rio et al., 2017). Our current data showed a high relative abundance of *Pseudomonas* (>34%) in samples XT2, XT5, and XT12. The low storage temperature of milk (usually 1 to 4°C) makes it favorable for the survival of some common spoilage microbes such as *Pseudomonas* (De Jonghe et al., 2011). Our study also observed sequences representing the psychrotolerant species *Pseudomonas fragi*. In contrast, sequences of some extreme thermophilic bacteria, namely *Thermus*, were observed. These bacteria are not generally associated with milk. The current results, however, showed for the first time the connection between *Thermus scotoductus* and camel milk. This species can be isolated from hot tap water and other thermal sources, and they preferentially live at a temperature of 70 to 75°C (Brock and Freeze, 1969). Water can be a significant source of microbial contamination in milk because many local farms simply

wash milking equipment using waters from wells, lakes, and rivers. Thus, local waters and their associated microflora could become major milk contaminant sources. Dairy safety assessment based on *Propionibacterium* was described by Meile et al. (2008). Our work found sequences that corresponded to the species *Propionibacterium acnes*, which is generally associated with human skin. Most of the camels in the Alxa area are maintained by artificial rearing; thus, they might have frequent human contact, potentially leading to the transfer of this bacterium to camel milk. Some sequences represented members of other genera (namely *Acinetobacter*, *Moraxella*, *Nazibacter*, *Paenibacillus*, and *Neisseria*) that are considered environmental microorganisms from various sources, including soil, sludge, water, and dust (Spain et al., 1979; Berge et al., 2002; Carr et al., 2003; Xu et al., 2005). They could be acquired from the living environment of the camel, residing on the animal body and eventually contaminating camel milk during milk collection and production.

Strong positive correlations existed among the LAB species *Lactobacillus helveticus*, *Streptococcus thermophilus*, and *Lactobacillus fermentum*. These bacteria are commonly found in cheese whey starters, and together they contribute to the high viable counts of some related products (Rossetti et al., 2008). The distribution of these bacteria is mainly dairy plant-specific rather than correlated with geographical fluctuations (Rossetti et al., 2008).

Our study investigated the camel milk bacterial community using both culture-dependent methods and PacBio SMRT technology. Previous work showed that the results obtained from these 2 approaches are largely consistent (Mo et al., 2019). The data of Mo et al. (2019) from laboratory cultivation and PacBio SMRT technology consistently found that *Lactobacillus helveticus* was the most abundant species, although it has been hard to correlate the data set generated by the 2 approaches because of the principle of the methods (for culture-dependent methods, the separation of lactic acid bacteria is mainly dependent on the selected medium, and for PacBio SMRT technology, the method is mainly captured DNA sequences). However, we found no correlation between culture-dependent and culture-independent results. For example, in our study, *Lactobacillus paracasei* represented a high proportion of viable microorganisms but a low relative abundance of sequences detected by SMRT sequencing (0.08%; data not shown). Moreover, although a relatively high proportion of sequences represented *Streptococcus thermophilus*, this species was not recovered in culture. The discrepancies of results generated by culture-dependent and culture-independent approaches are likely explained

by technicalities such as specific growth requirements for certain bacteria and possible PCR amplification bias.

CONCLUSIONS

We analyzed the bacterial microbiota profile of 10 camel milk samples by PacBio SMRT sequencing technology; the bacterial microbiota profile was highly diverse among samples. We also isolated and identified 72 LAB strains (distributed in 6 different species) by culture-dependent techniques from 15 samples. Among the 6 species, *Lactobacillus paracasei* (36.1%) and *Enterococcus italicus* (31.9%) were most often encountered. This work provides interesting physicochemical and taxonomic data of camel milk, which has not been studied systematically before.

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