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Influence of Bactrian camel milk on the gut microbiota

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ABSTRACT

Bactrian camel milk has become popular in the market as an important source of nutrients with diverse functional effects. In this study, the influence of Bactrian camel milk on the gut microbiota of mice was studied using metagenomic-based sequencing of the V3 and V4 hypervariable regions of the 16S rRNA gene. Bioinformatics analysis showed that *Firmicutes* and *Bacteroidetes* were the predominant phyla, accounting for more than 80% of the bacteria present. At the genus level, *Allobaculum*, *Akkermansia*, *Romboutsia*, *Bifidobacterium*, and *Lactobacillus* were most abundant in the gut microbiota; of these, *Allobaculum* and *Akkermansia* were the predominant genera, representing 40.42 and 7.85% of all the bacteria present, respectively. Camel milk was found to reduce relative abundance of *Romboutsia*, *Lactobacillus*, *Turicibacter*, and *Desulfovibrio* (decreased by 50.88, 34.78, 26.67, and 54.55%, respectively) in the gut microbiota compared with the control. However, some genera such as *Allobaculum*, *Akkermansia*, and *Bifidobacterium* in the gastrointestinal flora increased in abundance in the presence of camel milk; these genera are correlated with beneficial effects for organisms. Our research suggests that the gut microbiota should be taken into account when conducting functional studies on camel milk, and this work provides a useful foundation for further study on functions of camel milk.

Key words: camel milk, gut microbiome, probiotic, high-throughput sequencing

INTRODUCTION

Camels are important nonbovine animals that produce milk rich in nutrients for human consumption (Lajnaf et al., 2017). Two species belonging to the *Camelidae* family include Bactrian camels with 2 humps (*Camelus bactrianus*) and Dromedary camels with a single hump (*Camelus dromedarius*; Cui et al., 2007). The studies estimated a total population of 22 million camels in the world, of which 89% were *C. dromedarius* located in North Africa and West Asia and the remaining 11% were *C. bactrianus* distributed mainly in central Asian countries, including China and Mongolia (Silbermayr et al., 2010; Mihic et al., 2016). China has only *C. bactrianus*, which is mainly distributed in the desert and grasslands of Xinjiang (55%) and Inner Mongolia (41%). There are 3 breeds within the species, namely the Xinjiang camel, the Alxa camel, and the Sonid camel, named according to the geographic area in which they are found (Sa et al., 2015). Commercial Bactrian camel milk can be found in local markets and has become popular in China in recent years.

Although the numbers of *C. bactrianus* are relatively low compared with *C. dromedarius*, the nutrients in the milk of Bactrian camels are higher in protein, DM, and fat and lower in lactose than milk from Dromedary camels (Konuspayeva et al., 2009). Studies on the functions of camel milk have shown that it has good properties for human health, including prevention of diabetes, cancer, immune disorders, allergic symptoms, Crohn's disease, hypertension, oxidative stress, lipid peroxidation, and autism (Yadav et al., 2015; Kaskous, 2016). It has high levels of MUFA and PUFA, vitamin C, lactoferrin, immunoglobulins, serum albumin, lysozyme, insulin, iron, and manganese and low levels of α-CN and β-LG (Brezovečki et al., 2015; Kaskous, 2016).

Interplay among food, disease, and the gut microbiota has been studied in recent years (Dolan and Chang, 2017; Espín, 2017). Several studies have shown that certain foods can modulate the species composition and community structure of the gut microbiota due to changes in the ecological environment in the gut (e.g., bile acids and pH) and that different nutrients in foods can be selectively used by different microbes (McKenzie et al., 2017). The gut microbiota can be changed, even within a day, when the diet is changed (Koropatkin et al., 2012). Meanwhile, species composition of the gut microbiota can be different in individuals with various

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diseases compared with healthy individuals (Cani et al., 2016). Reports have indicated correlations between gut microbiota and obesity, diabetes, inflammatory bowel disease, and cancer; in particular, changes in the quantity of some microbial genera could either induce certain diseases or provide health benefits (Cani et al., 2016; Erdman, 2016; Knip and Siljander, 2016; Miyoshi and Chang, 2017). Comparative studies led us to conclude that although there are abundant nutrients in foods that have beneficial functional effects on human health, we cannot neglect the fact that these functional studies should not be independent of the gut microbiota. Therefore, when we studied the function of camel milk, its influence on microbiota should be investigated to comprehensively understand its function. In this research, the V3 and V4 hypervariable regions of 16S rRNA gene amplicon sequencing was used to investigate the effects of camel milk on the gut microbiota to provide a fundamental basis for functional studies on camel milk.

MATERIALS AND METHODS

Collection of Gut Microbiota Samples

Twelve-week-old C57BL/6J male mice were housed with 12-h light–dark cycles at a temperature of 22 ± 2°C and a humidity of 45 ± 5% and fed sterilized standard food and distilled water ad libitum. The animals received humane care, and all procedures involving them were performed in accordance with institutional guidelines.

After acclimation for 1 wk, the mice were allocated randomly to 2 groups (n = 6 mice/group): mice that received 10 mL of sterile distilled water/kg of BW intragastrically (**DW**) and mice that received 10 mL of camel milk/kg of BW intragastrically (**CM**; Arab et al., 2017). Each group was caged individually (1 mouse/cage) to avoid any direct contact between animals. Commercial UHT Bactrian camel milk, which had a 6-mo shelf life, was purchased from the market and stored at 4°C; the same batch of UHT camel milk was used for the entire duration of the experiment. All groups of mice were treated once a day for 4 consecutive weeks. Fecal samples were collected on d 29 and placed in liquid nitrogen and stored at −80°C before metagenomic DNA extraction.

Metagenomic DNA Extraction

Metagenomic DNA from the microbiome present in fecal samples was extracted and analyzed. For extraction, we used the commercial kit (QIAamp DNA Stool Mini Kit; Qiagen, Valencia, CA) according to the manufacturer's instructions. The concentration and purity of the metagenomic DNA were evaluated using a spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific, Waltham, MA); the quality of the metagenomic DNA was assessed by 1% agarose gel electrophoresis at a voltage of 100 V for 40 min. High-quality DNA was diluted to 1 ng/μL in sterile water as the template for PCR.

High-Throughput Sequencing of V3–V4 Regions of 16S rRNA Gene

Amplification of the V3–V4 regions of the 16S rRNA gene was achieved using specific primers with a set of 12-nucleotide barcodes (Table 1). We used the universal forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and the reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′) for PCR, which was done with Phusion High-Fidelity PCR Master Mix (Thermo Fisher Scientific Inc.; Zhang et al., 2017). Amplified PCR products were detected by electrophoresis in 2% agarose gels at a voltage of 80 V for 40 min. The PCR products were purified using the Qiagen Gel Extraction Kit (Qiagen Inc., Germantown, MD). A TruSeq DNA PCR-Free Sample Preparation Kit (Illumina Inc., San Diego, CA) was used to construct the DNA library. The library was quantified with a Qubit fluorometer (Thermo Fisher Scientific) and an Agilent 2100 Bioanalyzer system (Agilent Technologies, Santa Clara, CA). The sequencing was done using an Illumina HiSeq 2500 system (Illumina Inc.), and 250-bp paired-end reads were generated.

Bioinformatics Analysis of the Sequence Data

Paired-end reads from different samples were separated based on barcode sequences. Flash (v. 1.2.7;

Table 1. Amplicon sequencing information of the gut microbiota from the camel milk (CM) group and the distilled water (DW) group of mice

Sample	Barcode sequence	Effective tags (no.)	Q20[1]
CM1	GATCAG, ACTGAT	55,457	97.25
CM2	TAGCTT, ACTGAT	51,562	97.33
CM3	GGCTAC, ACTGAT	51,241	97.35
CM4	CTTGTA, ACTGAT	56,028	97.33
CM5	ATCACG, ATGAGC	56,107	97.26
CM6	CGATGT, ATGAGC	54,278	97.25
DW1	ATCACG, ACTGAT	52,918	97.32
DW2	CGATGT, ACTGAT	50,338	97.36
DW3	TGACCA, ACTGAT	53,306	97.25
DW4	ACAGTG, ACTGAT	52,146	97.39
DW5	GCCAAT, ACTGAT	54,978	97.24
DW6	ACTTGA, ACTGAT	56,317	97.50

[1]Q20: The bases with minimum base call accuracy of 99% in effective tags.