Compositional and functional comparisons of the microbiota in the colostrum and mature milk of dairy goats

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Abstract

Background

Goat milk is essential for the initial development of kids by providing a great source of commensal bacteria. Here we analyzed the microbiota of goat colostrum which was collected daily for five days post delivery and mature milk collected at the 7th, 10th, 20nd, 30th, 40th, 50th and 60th days, respectively, from three farms of Shaanxi province.

Results

The result showed that microbial alpha diversity was higher in the mature milk compared with that in the colostrum. According to taxonomy results, *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* were the predominant bacteria phyla in both colostrum and mature milk. In addition, lactation stage noticeably influenced the composition of milk microbiota. Specifically, *Novosphingobium*, *Brachybacterium*, *Psychrobacter*, *Lactobacillus*, *Yersinia*, *Roseatelesand*, *Rothia*, *Sanguibacter*, *Cloacibacterium*, *Variovorax*, *Sphingobacterium*, *Coxiella* were enriched in the colostrum while *Georgenia*, *Peptostreptococcus*, *Bacteroidales*, *Yaniella*, *Planomicrobium*, *Cloacibacterium*, *Azospirillum*, *Turicibacter*, *Cupriavidus*, *Herbaspirillum*, *Rhodobacteraceae*, *Aeromonadales* were the dominant genera in the mature milk. The enriched metabolic functions of the goat milk microbiota were predicted by PICRUST and classified by KEGG pathway. Moreover, the abundances of the environmental information processing, cellular processes pathway, genetic information processing pathway, organismal systems pathway and metabolism pathway were significantly different between microbiota of colostrum and mature milk.

Conclusions

Altogether, our study disclosed the significant difference between the microbial communities of colostrum and mature milk and provided grounds for further research in dairy microbiology.

Background

Goat milk production accounts for about 2.1% of global milk production. About 95% of the world's goat population is located in Asia, Africa, and Latin America. Among them, Asia accounts for approximately 60% of the total goat population [1]. Goat milk production in China rose from 54, 000 tons in 1969 to 223, 134 tons by 2018 at a remarkable yearly average rate of 3.31% (<https://knoema.com/data/china+agriculture-indicators-production+goats+milk>). In China, Shaanxi province possesses the largest storing facility for goat milk.

Goat milk contains relatively lower lactose and fat but rich in calcium, antimicrobial factors, antioxidants and other functional components which is essential for the health of human being [2]. Similar to human breast milk, goat milk contains a high level of antimicrobial enzyme such as lysozyme that boosts the immunity of infants against numerous infections [3]. In addition, goat milk shares several key features

with human breast milk and has been regarded as the most important replacement of human breast milk [4, 5].

Colostrum is a nutrient-rich milk particularly contains a high amount of immunoglobulin, lactoferrin and a variety of other growth factors that are produced for few days post parturition. It provides necessary protection to newborns through passive immunity against pathogens. The colostrum ingestion by newborn ruminants is the only source of immunoglobulins (Ig) during the first month of life and is further maintained in their whole lifespan [6]. Moreover, not only metabolic and immunological alterations but also hormonal and nutritional changes occurred over the transition period which influences incidence in the infectional and metabolic diseases. Lactation stages are mostly gradual but sometimes sudden changes may occur in composition and properties [7]. For comparing components of mature milk to colostrum, contents of proteins and minerals were lower in mature milk. In contrast, carbohydrates, and lipids contents were higher in colostrum. [8]. By developing intestinal immune balance the infants adopt to the extra-uterine environment. In order to complete the development of the intestinal immune system, an optimum amount of essential bacteria is very crucial in defining the fate of infant health system [9].

The main goal of this research is to examine the microbial diversity and community on goat colostrum and mature milk which was sampled at their different lactation stages from goat milk collected from different areas of Shaanxi province. The findings of this study indicated that the lactation stage influences the milk microbiota of goats, and microbial composition differed significantly between the colostrum and mature milk.

Results

Differences of the Microbial Diversity and Compositions in the colostrum and mature milk from different farms

We obtained 1,010,969 bacterial sequences from the 30 different colostrum and mature milk samples. Among them, the median number of reads in colostrum was 498,864 and that in the mature milk was 512,105, respectively. All these reads were classified into 15,430 OTUs which used for downstream analyses. The indices of bacterial alpha diversity in the colostrum and mature milk of dairy goat were listed in Fig. 1. There was no consequential difference in the Shannon index (Fig. 1A, B and C) and Simpson index (Additional file 1) between the colostrum and mature milk from different farms. The Shannon index showed that microbiota alpha diversity was greater in the mature milk than that in the colostrum in farm A (Wilcoxon; $p = 0.84$), farm B (Wilcoxon; $p = 0.84$) and farm C (Wilcoxon; $p = 0.56$), indicating the richness and diversity of the milk microbiota (Fig. 1A, B and C). However, the Simpson index of bacterial diversity in farm C indicated that there was no considerable difference between the two lactation stages (Additional file 1C). The bacterial diversity was markedly higher in the mature milk than that in the colostrum from farm A and farm B (Additional file 1A and B). However, the milk microbiota alpha diversity neither in colostrum nor in mature milk samples was not significant statistically ($P > 0.05$).

Comparing the community of bacterial composition in the colostrum and mature milk, the beta diversity analysis was represented by Principal coordinate analysis (PCoA). The samples were moderately clustered in accordance with the colostrum and mature milk lactation stages in the PCoA plot in the three different farms (Fig. 2A, B, and C). According to the association of OTUs, similar OTUs were classified in the table to estimate the Bray-Curtis distance. Based on the t-test and Wilcoxon signed-rank test, we observed clear differences of microbial beta diversity in the colostrum compared with the mature milk of a goat.

Total bacterial numbers in dairy goat colostrum and mature milk from the different farms

The results of the most prevalent OTUs were used to create Venn diagrams showing the numbers of microbes and variances of each colostrum and mature milk samples (Additional file 2) from the different farms (Fig. 3A, B and C). The numbers of mutual OTUs that were shared by colostrum and mature milk samples from farm A was 1411, representing 48.1% of all OTUs; from farm B was 1750, representing 60.5% of all OTUs and from farm C was 1570, representing 57.1% of all OTUs, respectively.

Taxonomic compositions and differences of microbiota in colostrum and mature milk of dairy goat

In this study, we focused on taxonomic compositions and taxonomic differences of the relative abundant bacteria obtained from the 16S rRNA gene sequences from colostrum and mature milk samples. Figure 4 displayed the average prevalence of the most predominant bacterial genera in the two lactation stages of dairy goat milk from the three different farms.

Furthermore, the abundant microbial taxa were assigned by LEfSe (Linear discriminant analysis Effect Size) with a log LDA score > 2.0 , Kruskal-Wallis test and the Wilcoxon test ($P < 0.05$) that were statistically different between the colostrum and mature milk. LEfSe analysis indicated the taxonomic differences between the colostrum and mature milk in farm A (13; 17 phylotypes) (Fig. 5A) and in farm B (13; 32 phylotypes) (Fig. 5C). The LDA scores revealed 25 phylotypes with enriched functions of the colostrum microbiota on the negative scale and 9 phylotypes of discriminative functions of the mature milk microbiota on the positive scale in farm C (Fig. 5E), respectively. The relative abundances of *Firmicutes* and *Bacteroidetes* were relatively greater in the mature milk (Fig. 5B). We illustrated that *Proteobacteria* were significantly enriched in the colostrum from Farm A (Fig. 5B), and in the mature milk from farm C (Fig. 5F) which was a potential biomarker for farm C's goat milk microbiota. The relative abundance of *Bacteroidetes* was higher in the mature milk, while the relative abundances of *Burkholderiales* and *Lactobacillales* were higher in the colostrum in the Cladogram of farm B (Fig. 5C and D). The phylum *Proteobacteria* including the *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* significantly influenced bacterial abundance in the colostrum from Farm A (Fig. 5B).

A total of twenty-four genera of bacteria dominated in the milk samples from the three different farms (Fig. 5B, C, and D). For instance, the bacterial predominant genera in the colostrum of Farm A were *Novosphingobium*, *Brachy bacterium* and *Psychrobacter*, while the colostrum of farm B were *Lactobacillus*, *Yersinia* and *Roseateles* and the colostrum of farm C were *Rothia*, *Sanguibacter*, *Cloacibacterium*, *Variovorax*, *Sphingobacterium*, and *Coxiella*, respectively.

Moreover, predominated bacterial genera detected from mature milk of farm A were *Georgenia*, *Peptostreptococcus*, and *Bacteroidales*. In comparison, those for farm B's mature milk were *Yaniella*, *Planomicrobium*, *Cloacibacterium*, *Azospirillum*, *Turicibacter*, and *Cupriavidus*, and farm C were *Herbaspirillum*, *Rhodobacteraceae*, *Aeromonadales*, respectively (Fig. 5A, C and E). In addition, relative abundances in individual milk samples of colostrum and mature milk from dairy goat identified by LEfSe were significantly different ($P < 0.05$).

Functional characterization of goat milk microbiome between colostrum and mature milk

In order to determine the essential role of microbiota in goat colostrum and mature milk, the Phylogenetic Investigation of Communities by Reconstruction of Unobserved State (PICRUSt) [10] program was applied to predict microbiota metabolic functions hinged on bacterial 16S rRNA via high-throughput sequencing data. The metabolic pathways were classified into six categories by the analysis of the Kyoto Encyclopedia of Genes and Genomes pathway (KEGG, <http://www.genome.jp/kegg/pathway.html>) database: "Metabolism", "Genetic information processing", "Environmental information processing", "Cellular processes", "Organismal systems and human diseases" and "Metabolic pathway".

Significantly enriched pathways of milk from farm A includes membrane transport ($p = 0.02$) and signal transduction ($p = 0.03$) that are associated with environmental information processing, transport and catabolism pathways ($p = 0.05$), replication and repair pathways ($p = 0.05$), transcription ($p = 0.02$) and translation ($p = 0.05$) pathways, enzyme families ($p = 0.04$), metabolism of terpenoids and polyketides ($p = 0.03$), nucleotide metabolism ($p = 0.05$), xenobiotics biodegradation and metabolism ($p = 0.04$) and amino acid metabolism ($p = 0.03$) pathways, and excretory system and endocrine system ($p = 0.04$) pathways (Fig. 6A). Whereas environmental information processing related pathways including membrane transport ($p = 0.02$); transport and catabolism ($p = 0.04$) associated with cellular processes; cellular processes and signaling ($p = 0.05$) and amino acid metabolism ($p = 0.04$) from metabolic pathways; carbohydrate metabolism ($p = 0.0007$) from organismal systems were significantly higher in milk from farm B (Fig. 6B). On the other hand, genes involved in carbohydrate metabolism (9.8%), membrane transport (11.3%) and amino acid metabolism (10.4%) are predominated in milk from farm C (Fig. 6C), and there were no statistically significant differences between the two lactation stages of milk samples from farm C.

Ultimately, we found a high prevalence of microbial community and diversity in both populations of colostrum and mature milk from the three different farms (Fig. 6).

Discussion

This study performed 16S rRNA sequencing techniques on V3 and V4 regions of bacterial communities in the colostrum and mature goat milk via the high-throughput sequencing. Whereas, various bacterial species in milk are hypothetically essential for maternal and infant's health [11, 12]. In the last decade, maternal milk has been known for harboring a complex bacterial community that helps the establishment of the intestinal microbiota in both newborns and infants [13]. To the best of our research, few studies have reported microbial composition in goat milk during different lactation stages. Results here indicate that the lactation stage has a significant influence on goat milk microbial and functional composition and bacterial composition was different in colostrum and mature milk. Microbial community which were found in the colostrum and mature milk could potentially establish goat kid's gut microbiota and influence the development by its microbial components and that have the potential in goat health promotion.

In this study, it was shown that bacterial and functional compositions were highly different in all the colostrum and mature milk samples from farms A, B and C, indicating that lactation stages play crucial roles in the composition of the goat milk microbiota. A previous finding showing the predominant phyla (*Proteobacteria*, *Firmicutes*, *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes*) from goat milk was similar to the results of the present study (Fig. 4A-C). Besides, Moossavi et al. (2019) found that *Proteobacteria* and *Firmicutes* were the predominant phyla in human breastmilk at 3–4 months postpartum [14]. Our findings elucidated that different lactation stages significantly affect the predominant phylum and genus of milk microbiota. Interestingly, the *Proteobacteria* is the largest phylum in both the colostrum and mature milk, while *Actinobacteria* is the second in colostrum and *Firmicutes* in mature milk, respectively. *Firmicutes*, *Proteobacteria*, and *Actinobacteria* are also important phylum in cow milk microbiota, but the specific role of bacteria in the milk microbial community is yet to be determined [15]. Moreover, the phylum *Bacteroidetes* increases corresponding to lactation stages at both the colostrum and mature milk. Therefore, these phyla might play more critical roles in the microbial ecosystem of the goat colostrum and mature milk than the other phyla. On the other hand, Zhao et al. (2020) identified that *Proteobacteria*, *Firmicutes*, *Deinococcus-Thermus*, *Bacteroidetes*, and *Actinobacteria* were the predominant phyla of camel milk [16]. These results suggested that fresh milk contains high bacterial diversity and is a valuable natural source of various kinds of bacteria.

Sonnenburg and Bäckhed (2016) reported that gut commensal microbiota contributes to the establishment and the maintenance of the innate immune function [17]. In line with that study, we found the colostrum bacteria contain several key components, including *Novosphingobium*, *Brachy bacterium*, *Psychrobacter*, *Lactobacillus*, *Yersinia*, *Roseateles*, *Rothia*, *Sanguibacter*, *Cloacibacterium*, *Variovorax*, *Sphingobacterium*. Moreover, we also identified bacterial genera from mature milk such as *Georgenia*, *Peptostreptococcus*, *Bacteroidales*, *Yaniella*, *Planomicrobium*, *Cloacibacterium*, *Azospirillum*, *Turicibacter*, *Cupriavidus*, *Herbaspirillum*, *Rhodobacteraceae*, and *Aeromonadales* from three different areas' goat milk samples. Other studies also focused on the affection of lactation stages on the immune and physical characteristics of goat colostrum until 5 days postpartum [18].

Several factors such as the lactation stage, genetic specificity, feedings, geographic locations, milking equipment as well as milk transportation and storage could impact on the overall milk microbial community [19]. Doyle and Conor studied the cow milk lactation stage effects on microbial composition and they found that *Bacteroides*, *Faecalibacterium*, *Campylobacter*, and *Rhodanobacter* as predominant genera in the mid-lactation, which was unlike to our study with dairy goat milk. [20]. However, the predominant phylum in these studies was *Actinobacteria* in late-lactation milk, which was in resemblance with our investigated mature milk samples from farm A. The lactation stages are being described as an influencing factor of the milk microbiota community [21], as a higher microbial diversity has been mainly reported in the mature milk than in the colostrum samples.

Tormo and Delacroix-Buchet (2011) described that the *Staphylococcus*, *Arthrobacter*, and *Serratia* were the predominant genera in the goat milk from the goat of Languedoc Roussillon and Midi-Pyrenees regions in France [22]. Besides, Zhang et al. (2017) found *Enterococcus* and *Lactobacillus* as predominant lactic acid bacteria genera in Saanen and Guanzhong dairy goats [23]. On the other hand, the report of Oliszewski, Van Nieuwenhove, González and Pérez Chaia (2006) described that Argentinean goat milk and cheeses contain 60% of *Lactobacillus* showing a dominance similarity as well as that in farm B goat's colostrum of this study [24]. Additionally, the *Lactobacillus* isolated from breast milk shown inhibition of adhesion and growth of gastrointestinal pathogens such as *Escherichia coli*, *Shigella spp*, *Pseudomonas spp*, and *Salmonella spp* strains [25, 26].

Moreover, we analyzed the abundances of the functional metabolism of the milk microbiota of goats from the three farms such as an environmental information processing ($p < 0.03$), cellular processes pathway ($p < 0.05$), genetic information processing pathway ($p < 0.05$), organismal systems pathway ($p < 0.05$), metabolism pathway ($p < 0.05$), and the microbiota functional metabolism abundances were significantly distinctive between in the colostrum and mature milk. Among these metabolism pathways, the result of the carbohydrate metabolism ($p = 0.0007$) was presented the highest significance in the Chuantoucun farm's goat milk microbiota in this research likewise the research of Zhang et al. (2017) who demonstrated the functional genes involved in amino acids metabolism and carbohydrate metabolism enriched in the goat milk microbiota representing 11.93% and 11.23%, respectively [23].

Further investigation is needed to effect of goat colostrum and mature milk to establish a bacterial community in the goat kid's gut on the gastrointestinal colonization and the stimulation of the kid's immune system. Studies are also needed to identify additional factors that influence the bacterial community; characterize other non-bacterial fractions of the goat milk microbiota, including fungi and viruses; and determine the relation between mother gut's health and milk microbiota composition during different lactation stage. Finally, the effect of milk microbiota during different lactation stage on goat kid's gut microbial ecosystem could increase our understanding of the role of the gut microbiota in health and development.

Conclusions

In conclusion, this research studied the overall compositions of the bacterial communities and diversities of the goat colostrum and mature milk collected from different farms (farms A, B and C) of dairy goats in China based on 16S rRNA gene sequencing on V3 and V4 regions by using high-through sequencing. Additionally, *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* were the most predominant bacterial phyla in the goat milk from the three different farms of dairy goats. Moreover, the dominance of the microbial community and their specific compositions in the colostrum and mature milk were diversified along with changes of the lactation stage period and dominant bacterial genera of the colostrum (*Novosphingobium*, *Brachybacterium*, *Psychrobacter*, *Lactobacillus*, *Yersinia*, *Roseatelesand*, *Rothia*, *Sanguibacter*, *Cloacibacterium*, *Variovorax*, *Sphingobacterium*, *Coxiella*) and mature milk (*Georgenia*, *Peptostreptococcus*, *Bacteroidales*, *Yaniella*, *Planomicrobium*, *Cloacibacterium*, *Azospirillum*, *Turicibacter*, *Cupriavidus*, *Herbaspirillum*, *Rhodobacteraceae*, *Aeromonadales*) were identified, respectively. Therefore, our findings revealed that the goat lactation stages significantly effect the milk microbial community and diversity. Furthermore, the abundance of the microbiota metabolic functions such as the environmental information processing ($p < 0.03$), cellular processes pathway ($p < 0.05$), genetic information processing pathway ($p < 0.05$), organismal systems pathway ($p < 0.05$), metabolism pathway ($p < 0.05$) of the goat milk were discriminated by PICRUST and classified by KEGG PATWAY, where the abundance of the metabolism pathways of the goat milk microbiota were significantly different between the colostrum and mature milk. Altogether our study provided a good comprehension of the bacterial ecosystem development and differences in the goat milk at their colostrum and mature milk lactation stages.

Methods

Sample collection

In this study, a total of 30 goat milk samples were randomly selected from three local farms (farm A, B and C, respectively) in Shaanxi province of China (Table 1) during the spring of 2019. The samples including colostrum and mature milk were collected before their milking. The colostrum samples ($n = 15$) were collected in the first five days after the delivery and mature milk samples ($n = 15$) were collected at 7th, 10th, 20nd, 30th, 40th, 50th, 60th days, respectively.

Before conducting the manual milk sample collection, the breast surface of goat was washed and sterilized with 70% of ethyl alcohol and then the sample was collected into a sterile plastic tube after discarding the first drop of milk. Each sample was numbered and refrigerated immediately at 4°C and that was transported to the laboratory inside an icebox.

Table 1
Information of selected colostrum and mature milk samples

Area	Samples		Given names
	Colostrum	Mature milk	
Qian Yang	5	5	farm A
Chuan Tou-Cun	5	5	farm B
Yang Ling	5	5	farm C
Total	15	15	30

DNA Extraction

Microbial community in 1 mL of each milk sample was accumulated at 12000 rpm for 5 min and the pellets were suspended 3 times in 200 μ L 0.8% NaCl solution and bacterial genomic DNA was isolated by CTAB (cetyl trimethylammonium bromide) method that was frozen at -80°C till the next experiment. The DNA bands were identified in 0.8% (w/v) agarose gel and each DNA concentration were detected by a Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Amplification of V3 and V4 regions of 16S rRNA gene

The V3 and V4 regions in 16S rRNA genes were determined with the specific primers 338F: 5'-ACTCCTACGGGAGGCAGCA – 3'; 806R: 5'-GGACTACHVGGGTWTCTAAT – 3' with a nucleotide barcode, respectively. Each PCR reaction amplified about 10 ng of template DNA along with 0.2 μ L of each primer in 15 μ L of Phusion High - Fidelity PCR Master Mix (New England Biolabs, Ipswich, Massachusetts, United States). PCR thermal cycling condition was reacted at 98°C as an initial denaturation for 1 min and following 30 times of cycles in which 98°C of 10-sec denaturation, 50°C for annealing in 30 s and 72°C as elongation for 30 sec in each cycle and amplification was terminated by 5 min final extension at 72°C . The amplified PCR products were joined with Synergy Brands (SYBR) green dye and specific products (between 200 bp and 450 bp) were determined in 2% agarose gel and purified from the gel by Qiagen Gel Extraction Kit (Qiagen, Hilden, Nordrhein-Westfalen, Germany).

Library preparation and sequencing

Sequencing libraries were prepared by using TruSeq DNA PCR – Free Sample Preparation Kit (Illumina, San Diego, USA) with unique indices followed by instructions of the manufacture. The 5' ends of the amplicons were removed by using the Fast DNA Mix 2 End Repair Kit (Thermo Scientific, Waltham, MA, USA), and overhang adenine residues were added at the 3' blunt end and then phosphorylated at the 3' end which prevents the DNA fragment from self-joining. The termination sequence was contained library-specific tags (i.e., Index sequence) at the 5' end so that DNA molecules can be modified on the Flow Cell. PCR was performed on the modified DNA template constructed with the termination sequence was to amplify the sequencing library template, and then AMPure XP Beads (BECKMAN Coulter, Indianapolis, United States) were used to purify the enrichment products of the library. The final fragment

of the library selection and purification was conducted by gel electrophoresis in 2% gel. The quality of the library assess was performed on the Qubit 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 (Agilent Technologies, USA) system according to the instructions of manufacture. In the end, the sequencing of the library was completed and 480 bp paired-end sequences were generated on an Illumina MiSeq-PE250 platform.

Bioinformatical and statistical analysis

In this study, Illumina MiSeq-PE250 platform was used to conduct Paired-end sequencing on the DNA fragments and original sequencing data were converted into FASTQ format [27]. The raw tags quality filtering was performed with a particular mode of filtering to attain the high-quality tags for the process of quality control according to the Quantitative Insights Into Microbial Ecology (QIIME) [28, 29]. In order to detect and remove chimera sequences, the tags were compared to the Silva database by UCHIME algorithm [30] and removed [31] and abundance tags were gained.. The sequenced were analyzed by Uparse software [32]. For instance, $\geq 97\%$ of similarity sequences were designated to the same operational taxonomic units (OTUs) [33]. Representative sequence for each OTU was evaluated for further definition and for each representative sequence, the method of the Quast (2012) was applied for taxonomic annotation based on Mothur algorithm [34]. The MUSCLE (multiple sequence comparison by log-expectation) software was operated with the variations of dominant species in particular samples and Multiple Sequence Alignment (MSA), concerning the phylogenetic correlation of distinctive OTUs [35].

With the corresponding standard sequence, the community abundance information of OTUs was assimilated in which subsequent analysis were confirmed for alpha and beta diversity. Diversity of species and its complexity were analyzed through alpha diversity with Shannon and Simpson indices. Beta diversity estimation was performed by Bray-Curtis distance matrices and visualized by principal coordinate analysis (PCoA) [36, 37]. The indices mentioned above were computed by QIIME (Version 1.7.0) and represented by R software (Version 3.6.1).

Variations of abundant taxa and functions between the groups were characterized by the Linear discriminant analysis Effect Size (LEfSe) in accordance with taxonomic and functional profiles of genes (<http://huttenhower.sph.harvard.edu/galaxy/>) [38].

In addition, the Phyloseq package [39] was used to accomplish bioinformatics analysis in R. Metagenome function was predicted by PICRUST [40]. In order to compare microbiota significant difference between in colostrum and mature milk of goat, the description of Wilcoxon rank sum test with Phyloseq was run in R software.

Abbreviations

Ig: Immunoglobulin; DNA:Deoxyribonucleic acid; CTAB:Cetyl trimethylammonium bromide; rRNA:Ribosomal ribonucleic acid; PCR:Polymerase chain reaction; FLASH:Fast length adjustment of short; FASTQ:Format is a text-based format for storing both a biological sequence and quality scores;

QIIME:Quantitative insights into microbial ecology; UCHIME:Undetected chimeras; OTU:Operational taxonomic unit; MUSCLE:Multiple sequence comparison by log-expectation; MSA:multiple sequence alignment; PCoA:principal coordinate analysis; LEfSe:Linear discriminant analysis Effect Size; PICRUST:Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; LDA:Linear discriminant analysis; KEGG:Kyoto Encyclopedia of Genes and Genomes.

Declarations

Availability of data and materials

All dataset generated and/or analyzed during this current study are included in this published article, and also available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The animal protocol in this study was approved by the Research Ethics Committee of Northwest Agriculture and Forestry University according to the Guidelines of Ministry of Health, China. The sample collection was done after getting permission from respective farm owners.

Consent for publication

Not applicable

Competing interests

The authors have no conflicts of interest to declare.

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Authors' contributions

ZN, WTM, and DKC designed and conducted the study. XTY, MJL, NB, JJZ, JJC, YW, and LL helped in collecting the data and performing the experiment. All authors read and approved the final manuscript.

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Figures

Figure 1

Alpha diversity measured (within sample diversity) by Shannon index, Statistical (p) value generated by Wilcoxon test, respectively. The alpha diversity of the bacterial community richness in the mature milk were higher than colostrum, but these differences were not statistically significant in farm A ($p = 0,84$), B ($p = 0,84$) and in C ($p = 0,56$) farms. The horizontal bars within boxes represent median values, the tops and bottoms of boxes represent 75th and 25th quartile values, respectively.

Figure 2

The beta diversity (between microbial diversity) of the colostrum and milk microbiota from three farms based on PCoA plot (principal coordinate analysis) using the Bray-Curtis distances. Each point in a PCoA plots represents the microbial community from a single sample. Samples with the most similar microbial communities cluster together. The first coordinate (Axis 1) explained 54.8 % (A), 29,2 % (B), 76.1 % (C) and the second coordinate (Axis 2) explained 26.2 % (A) , 22,2 % (B), 12.2 % (C) of the variation between

colostrum and mature milk samples. Colostrum cluster very closely with no discernible difference in farm A and farm B. But mature milk clusters were long distance from each other (the colostrum and mature milk did not statistically differ).

Figure 3

Bacterial OTUs shared between the colostrum and mature milk of goat in three farms. The number of mutual OTU in colostrum and mature milk samples from farm A were 1411, representing 48.1 % of all OTU; from farm B were 1750, representing 60.5 % of all OTU and from farm C were 1570, representing 57.1 % of all OTU.

Figure 4

The relative abundance of the top genera between in the colostrum and mature milk from three farms. The genus with relative values is represented in red [0-2] and low values in blue [0-(-2)]. Goat milk microbiota composition at genus level was significantly different between the colostrum and mature milk in three farms (A, B, C), respectively.

Figure 5

Linear discriminant analysis Effect Size (LEfSe) analysis of dairy goat colostrum and mature milk microbiota taxonomic differences from three different farms. Differently abundant taxa were identified using linear discriminant analysis (LDA) combined with effect size (LEfSe) algorithm. Histograms of linear discriminant analysis scores of 16S gene sequences in A, C and E figures were shown, with a cut off value of LDA score > 2 (log10). Figures A, C, and E colostrum-enriched taxa were indicated with a negative LDA score (red), and taxa enriched in the mature milk were characterized by a positive score (green). B, E, and F were clipped from LEfSe analysis of differential colostrum and mature milk microbial taxa. The root of the bacteria tree was displayed as the central point and enlarged into rings as different taxonomic levels from phylum to genus. Small diameter of circles on the rings were represented the relative abundance (non-significance in yellow and biomarkers in red and green) of each classification. Proteobacteria were significantly enriched in the farm A's goat colostrum (B), and in the mature milk microbiota of farm C (F) goat. The relative abundance of Bacteroidetes was higher in the mature milk in Cladogram of farm C (C and D). The Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria were significantly high classes in the colostrum of the A farm's dairy goat.

Figure 6

The main categories of the functional analysis based on the KEGG pathways. Relative number of genes in microbiota from goat milk (colostrum and mature milk) from three farms. Signal transduction ($p = 0.03$), membrane transport ($p = 0.02$), transcription ($p = 0.02$) and amino acid metabolism ($p = 0.03$) pathways were most significantly different in A farm. Carbohydrate metabolism ($p = 0.0007$) were most significantly higher in B farm goat milk microbiota.

Supplementary Files

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