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Diversity and specificity of the bacterial community in Chinese horse milk cheese

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Abstract

The nutrition and flavor of cheese are generated by the microbial community. Thus, horse milk cheese with unique nutrition and flavor, an increasingly popular local cheese of the Xinjiang Uygur Autonomous Region of China, is considered to have diverse and specific bacterial community. To verify this hypothesis, horse, cow, and goat milk cheese samples produced under the same environmental conditions and manufacturing process were collected, and the 16S rRNA gene was targeted to determine the bacterial population size and community composition by real-time quantitative PCR and high-throughput sequencing. The bacterial community of horse milk cheese had a significantly larger bacterial population size, greater species richness, and a more diverse composition than those of cow and goat milk cheeses. Unlike the absolute dominance of *Lactococcus* and *Streptococcus* in cow and goat milk cheeses, *Lactobacillus* and *Streptococcus* dominated the bacterial community as the starter lactic acid bacteria in horse milk cheese. Additionally, horse milk cheese also contains a higher abundance of unclassified secondary bacteria and specific secondary bacteria (e.g., *Psychrobacter*, *Sulfurisoma*, *Halomonas*, and *Brevibacterium*) than cow and goat milk cheeses. These abundant, diverse, and specific starter lactic acid bacteria and secondary bacteria may generate unique nutrition and flavor of horse milk cheese.

KEYWORDS

diverse secondary bacteria, horse milk cheese, *Lactobacillus*, MiSeq sequencing, real-time quantitative PCR

1 | INTRODUCTION

Chinese artisanal cheese is one of the most common and popular fermented foods consumed in the Xinjiang Uygur Autonomous Region of China (Zheng, Liu, Li, et al., 2018). In addition to cow milk cheese and goat milk cheese, horse milk cheese is a recent popular local cheese of the Xinjiang Uygur Autonomous Region because of its specific nutrition and flavor. It has been suggested that the nutrition and flavor of cheese are generated by the different metabolites of diverse microbial communities (Zheng, Liu, Shi, et al., 2018). The microorganisms that ferment and mature horse milk cheese mainly originate from raw horse milk because it is made by nomadic people via a natural fermentation process without a starter culture (Azat et al., 2016; Cogan, Guinee, McSweeney, & Fox, 2000). Raw horse

milk has lower protein and fat contents but higher lactose and vitamin C contents than raw cow and goat milk (Claeys et al., 2014) and possesses an optimum whey protein/casein ratio (Uniacke-Lowe, Huppertz, & Fox, 2010). All of these distinctive features may act as specific environmental factors for the microorganisms in horse milk cheese and may drive the microbial community in horse milk cheese to differ from that in cow and goat milk cheeses. Thus, we assume that the microbial community of horse milk cheese is different from that of cow and goat milk cheeses, which may play a potential role in the formation of its unique nutrition and flavor.

The bacterial community of cheese may be divided into starter lactic acid bacteria and secondary bacteria (Cogan et al., 2000). Starter lactic acid bacteria, common species from the genera *Lactococcus*, *Lactobacillus*, and *Streptococcus*, are the dominant groups in cheese microbial communities; these bacteria are involved in acid production by degrading lactose during the manufacturing process and contribute to the ripening process by producing proteases (Fox, 1999; Settanni & Moschetti, 2010). Secondary bacteria, including non-starter lactic acid bacteria (NSLAB) and non-lactic acid bacteria, have a low abundance in cheese and begin to increase during the ripening process. They can affect cheese flavor by hydrolyzing casein and peptides into free amino acids (FAAs), which are precursors for other catabolic reactions, giving rise to volatile aroma compounds (Settanni & Moschetti, 2010). Thus, clarifying the diversity of the starter lactic acid bacteria and secondary bacteria present in horse milk cheese is conducive to understanding the formation of the specific nutrition and flavor of horse milk cheese.

In this study, we focus on the diversity and specificity of the bacterial community of Chinese artisanal cheese made from horse milk. The cheese samples, cow milk cheese, goat milk cheese, and horse milk cheese, were collected from the same farmhouse in Yili, Xinjiang Uygur Autonomous Region, and the partial sequence of the bacterial 16S rRNA gene was targeted and quantified by real-time quantitative PCR (qPCR) and identified by high-throughput sequencing (HTS). The bacterial population size and community diversity in horse milk cheese were determined and compared with those in cow and goat milk cheeses, and the starter lactic acid bacteria and secondary bacteria were annotated. This comprehensive knowledge can provide insights into the diverse and specific microorganisms in horse milk cheese and discuss their potential role in the formation of the unique nutrition and flavor of horse milk cheese.

2 | MATERIALS AND METHODS

2.1 | Sample collection and preparation

The cheese samples were collected from the same local ethnic minority farmhouses in the Yili area, Xinjiang Uygur Autonomous Region, China. The milk sources for the cheese samples were horse milk, cow milk, and goat milk, and the milk-producing animals had the same living environment and food (Figure A1). Based on the local traditional preparation method, natural horse milk cheese (HC), cow milk cheese (CC), and goat milk cheese (GC) were prepared from the above three fresh raw milk products on 12 July 2017. In brief, the freshly collected milk was filtered and placed in a shaded place (about 20°C) for natural fermentation without any starter cultures for about 36 hr, and then, the fermented curd was stirred to remove the whey under the 75°C and finally poured into the mold to dry without a press for 7 days. Microbial genomic DNA from three random replicates of each cheese type was extracted using the PowerFood Microbial DNA Isolation Kit (MoBio Laboratories Inc.; Quigley et al., 2012). One gram of cheese was combined with 9 ml 2% trisodium citrate and homogenized before DNA extraction.

2.2 | qPCR and PCR amplification of the microbial community 16S rRNA genes

Absolute quantification methods (Klein, 2002) were used to quantify the 16S rRNA gene copy of each cheese sample. *Escherichia coli* DH5α, grown in LB medium at 37°C, was used as the host strain for all transformation procedures. *Pseudomonas stutzeri* JCM-5965 was used as the standard bacterial strain, the V4 region gene of its 16S rRNA genes was cloned into the *E. coli* plasmid pUCm-T. Recombinant *E. coli* transformed using pUCm-T was cultured in LB medium at 37°C with the addition of 100 μg/ml of ampicillin. A 10-fold serial dilution series of the pUCm-T, ranging from 1.9×10^5 to 1.9×10^9 copies/μl, was used to construct the standard curves for 16S rRNA genes. Using the gradient concentration plasmid and the extracted genomic DNA of the cheese sample as a template, quantitative PCR was carried out in the StepOne™ Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific Inc.), targeting the V4 region of the bacterial 16S rRNA genes. The qPCR amplification was carried out with the Toyobo KOD SYBR qPCR Mix Kit. The reaction system was as follows: 10 μl KOD mix, 0.4 μl ROX, 0.4 μl Primer *F* (515F, 5′-GTGCCAGCMGCCGCGGTAA-3′), 0.4 μl Primer R (806R, 5′-GGACTACHVGGGTWTCTAAT-3′), 2 μl BSA (5 mg/ml), 1 μl DNA template, and nuclease-free H_2O to a final reaction volume of 20 μl. The reaction conditions were as follows: the preheating stage: 98°C for 2 min; the cycle stage: 40 cycles of denaturation at 98°C for 10 s, annealing at 55°C for 10 s, and extension at 68°C for 30 s; and the amplification stage: 95°C for 15 s and 60°C for 1 min. StepOne software was used to monitor the amplification process in real time and analyze the results. The amplification efficiency of the plasmid standard sample was 85.031%.

DNA samples were amplified by PCR using the universal prokaryotic primers 515F and 806R for the V4 region of the 16S rRNA gene. PCRs were carried out in 25 μl reactions with 2.5 μl of a buffer, 2 μl of Mg^{2+} (25 mmol/L), 2 μl of dNTPs, 2.5 μl of BSA (5 mg/ml), 0.1 μl of Taq enzyme, 0.5 μl of each primer (200 nmol/L), 1 μl of template DNA, and nuclease-free H_2O to a final reaction volume of 25 μl. PCR amplification was performed using a G-Storm thermal cycler (Thermo Fisher Scientific Inc.). The amplification program

consisted of an initial denaturation step at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 40 s. A final elongation step at 72°C for 10 min was also included. Amplicons were cleaned using GeneJET (Thermo Fisher Scientific Inc.). The quantity of extracted DNA was assessed using a NanoDrop 3300 Fluorospectrometer (Thermo Fisher Scientific Inc.).

2.3 | HTS and bioinformatics analysis

The 16S rRNA V4 amplicons were sequenced on an Illumina MiSeq platform according to paired-end protocols, and the average read length is 252 bp. The quality control process of tags refers to Qiime (v1.7.0; http://qiime.org/scripts/split_libraries_fastq.html) (Caporaso et al., 2010), and raw tags are truncated from the first low-quality base site with three consecutive low-quality value (≤19) bases; the tags dataset obtained after the truncation of tags is further filtered to remove tags whose continuous high-quality base length is <75% of the tag length. The tag sequence obtained after the above processing is compared with the database Gold database (Gold database, http://drive5.com/uchime/uchime_download.html) using UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) to detect the chimera, and the effective tags is obtained after removing the chimera sequence (Haas et al., 2011). Statistical analysis to measure the sequencing diversity, including Chao1 richness, ACE index, Shannon index and Simpson index, and to monitor the results for sequencing abundance using rarefaction was performed with the MOTHUR package (Schloss et al., 2009). Principal coordinate analysis (PCoA), measuring dissimilarities at phylogenetic distances based on weighted UniFrac analysis, was performed using the QIIME suite (Caporaso et al., 2010). An unweighted pair-group method with arithmetic mean, measuring the similarity between different samples based on the weighted UniFrac analysis and dissimilarities at phylogenetic distances, was performed using the QIIME suite (Caporaso et al., 2010). Uparse (Uparse v7.0.1001; http://drive5.com/uparse/) (Edgar, 2013) was used to cluster all the effective tags of all samples, the sequences are clustered into OTUs (Operational Taxonomic Units) with 97% identity, and the sequences with the highest frequency of OTUs are selected as the representative sequences of OTUs for further taxonomic annotation. The Mothur method and the SSUrRNA database (Wang, Garrity, Tiedje, & Cole, 2007) of SILVA (http://www.arb-silva.de/) (Quast et al., 2012) were used to annotate the taxonomic information. A species abundance heatmap was drawn based on the relative abundances of secondary bacteria, which were normalized by *Z* values because of the low relative abundance of secondary bacteria. The *Z* value is the number of standard deviations between a certain eigenvalue and the mean, which is a commonly used statistic of the statistical process control (SPC). Statistical significance was determined by using the nonparametric Kruskal–Wallis test (Kruskal & Wallis, 1953) in the Minitab statistical package.

3 | RESULTS

3.1 | The abundance and diversity of the bacterial 16S rRNA gene from the cheese samples

The partial sequence of the bacterial 16S rRNA gene of the three cheese samples was quantified by qPCR. The abundance of the bacterial 16S rRNA gene in horse milk cheese was significantly higher than that in cow and goat milk cheeses ($p < .05$) (Figure 1a). This result indicated that the bacterial population size in horse milk cheese was significantly higher than that in cow and goat milk cheeses.

Then, these partial sequences of the bacterial 16S rRNA gene were identified by an HTS approach. After the quality control steps were performed, a total of 651,728 high-quality 16S rRNA gene sequences with an average length of 250 bp were recovered from the three cheese samples, which were clustered into 1,153 operational taxonomic units (OTUs) at a 97% identity level (Table 1). All sequence coverages were >99.0% (Table 1), indicating that the microbial communities in the samples were adequately represented. Rarefaction curve analysis showed that the OTU number in horse milk cheese was consistently higher than that in cow and goat milk cheeses when the same number of sequences was abstracted (Figure 1b). The average OTU number in horse milk cheese was 396, which was significantly higher than that in cow and goat milk cheeses ($p < .05$). Based on the OTUs from all cheese samples, the Shannon index and Simpson index of the bacterial 16S rRNA genes in all cheese samples were determined to evaluate the diversity and evenness of bacterial community composition. Both Shannon index and Simpson index of the bacterial community in horse milk cheese were significantly lower than those in cow milk cheese ($p < .05$), but significantly higher than those in goat milk cheese ($p < .05$). Then, Chao1 index and phylogenetic diversity index were determined to evaluate the species richness and phylogenetic diversity of the bacterial community. The horse milk cheese had the highest Chao1 value (464.71) and phylogenetic diversity index (34.3), indicating that the bacterial community in horse milk cheese has greater bacterial species richness and evolutionary diversity than that in cow and goat milk cheeses (Table 1).

A Venn diagram was used to evaluate the distribution of OTUs among the different samples, as shown in Figure 1c. In addition to the 415 OTUs shared by the three cheese samples, the horse milk cheese also had 275 specific OTUs, which was the highest specific OTU number among the three samples. PCoAs were performed to identify the clustering patterns of microbial communities in all cheese samples (Figure 1d). The contribution of PC1 to the sample difference was 80.13%, and the first principal component coordinate of horse milk cheese was 0.129, which was far from the cow and goat milk cheese samples (cow milk cheese −0.062, goat milk cheese −0.066). However, the first principal component coordinates of the cow and goat milk cheese samples exhibited almost no significant difference, which suggests that the microbial community composition of cheeses made from different milk sources

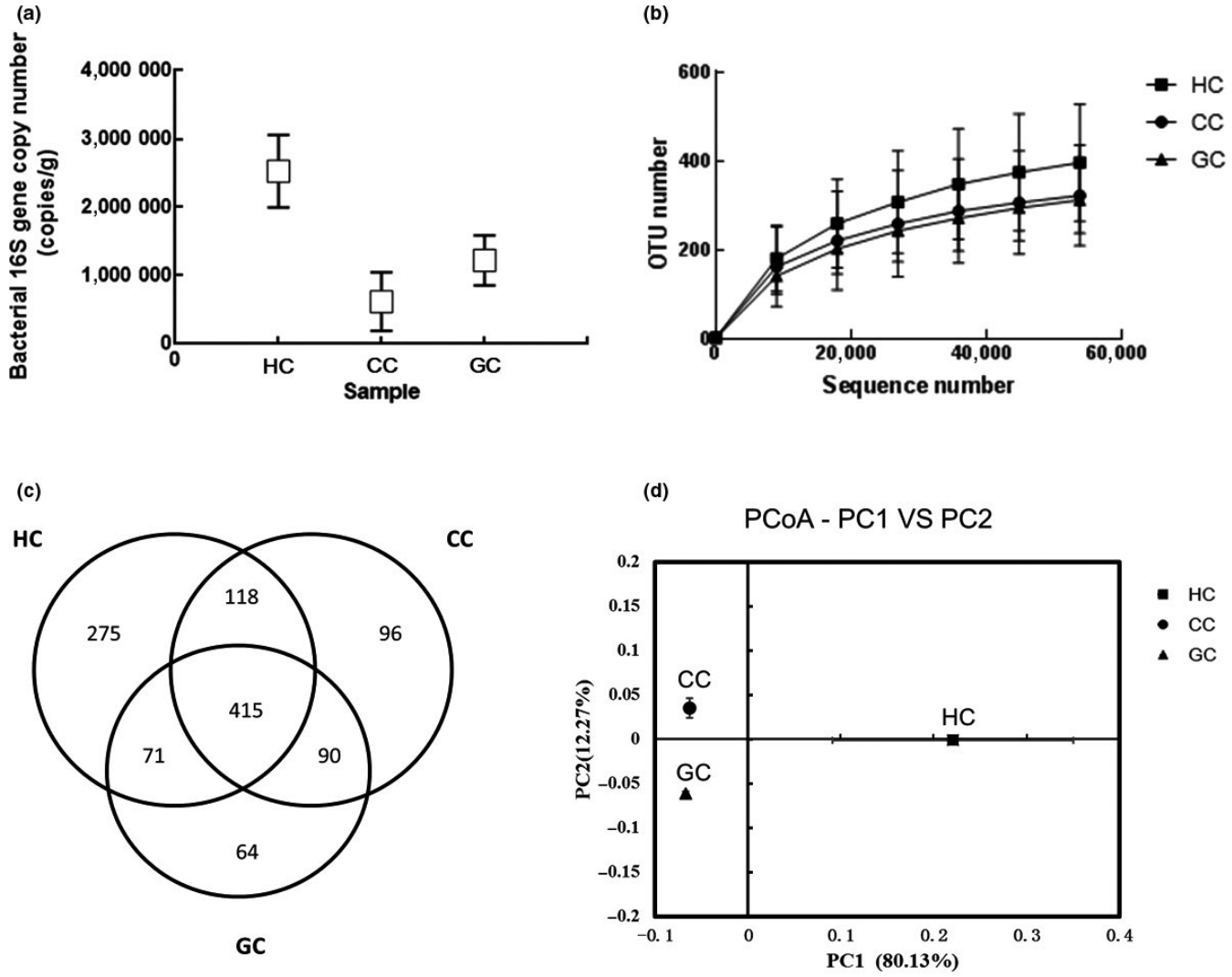

FIGURE 1 Abundance and diversity of bacterial communities in the three cheese samples based on qPCR and high-throughput sequencing of the bacterial 16S rRNA gene. (a) Bacterial 16S rRNA gene copy number. (b) Richness rarefaction curves based on the OTUs. (c) Venn diagrams based on the OTUs. (d) PCoA based on the OTUs. CC, cow milk cheese, GC, goat milk cheese; HC, horse milk cheese

differs. The unweighted pair-group method with arithmetic mean analysis (Figure 2) based on species abundance and evolutionary relationships was also used to evaluate bacterial community structure differences between the three samples. Both of these analyses indicated that the bacterial community composition of horse milk cheese was distinguished from that of cow and goat milk cheeses.

3.2 | Characteristics of the community of the starter lactic acid bacteria

Based on the annotation of the OTUs, the microbial communities of the three kinds of cheese were dominated by their starter lactic acid bacteria (Figure 2). Cow milk cheese was dominated by the starter *Streptococcus* (56.9%) and *Lactococcus* (27.6%), and goat milk cheese was dominated by the starter *Lactococcus* (91.9%) (Figure 2). Notably, horse milk cheese was dominated by the starter *Lactobacillus* (51.5%), *Lactococcus* (9.8%), and *Streptococcus* (34.8%) (Figure 2).

3.3 | Characteristics of the community of secondary bacteria

The secondary bacteria in the horse, cow, and goat milk cheese samples accounted for lower relative abundances of 9.8%, 5.0%, and 9.0% than those of starter lactic acid bacteria, respectively (Figure 2). All of these secondary bacteria corresponded to five different phyla, with Firmicutes being the most abundant, followed by Proteobacteria, Actinobacteria, Bacteroidetes, and Deinococcus–Thermus. Although horse milk cheese contained the highest relative abundance of unclassified OTUs on the genus level (5.8%),

the genus number of secondary bacteria in horse milk cheese (280) was still significantly larger than that in cow and goat milk cheeses ($p < .05$). In the species abundance heatmap at the genus level based on *Z* values (Figure 2), *Psychrobacter*, *Janthinobacterium*, *Alteromonas*, *Thiobacillus*, *Acinetobacter*, *Sulfurisoma*, *Halomonas*, and *Brevibacterium* in horse milk cheese had a positive *Z* value, which indicated that these genera were the specific dominant genera in horse milk cheese.

4 | DISCUSSION

The qPCR and HTS results suggest that Chinese horse milk cheese has a larger bacterial population size and species richness and a more diverse bacterial community composition than Chinese traditional cow and goat milk cheeses. It has been reported that the distribution of cheese microbial communities is influenced by various factors, such as dairy processing (Bokulich & Mills, 2013; Stellato, De Filippis, La Storia, & Ercolini, 2015), fermentation environment (Bokulich, Ohta, Lee, & Mills, 2014), and milk sources (Alessandria et al., 2016; Zheng, Liu, Li, et al., 2018). In this study, because the dairy processing and fermentation environment were the same for all of the samples, the milk source may have been the main factor influencing the abundance and composition of the cheese microbial community. Moreover, animal species are a significant influential factor of the nutritional composition of cheese (Lucas, Rock, Agabriel, Chilliard, & Coulon, 2008). Thus, the specific nutrition of horse milk may act as the specific environmental factor for the microorganisms and may cause the microbial community of horse milk to differ from that of cow and goat milk, and the metabolites of this microbial community may consequently generate the unique nutrition and flavor of horse milk cheese.

The broad variety of flavors produced from carbohydrates, proteins, and fats by the different starter lactic acid bacteria were quite different (Pastink et al., 2009). *Lactobacillus*, the most abundant genus in horse milk cheese, was correlated with the production of ethanol, alanine, and short-chain fatty acids (e.g., butanoic acid and acetic acid) in the cheese through the catabolic pathway of carbohydrates, proteins, and fats. Moreover, it has been verified that *Lb. rhamnosus* and *Lb. casei* are capable of producing flavor compounds such as ketones, aldehydes, alcohols, and acids, which may contribute to the organoleptic features of cheese (Santarelli et al., 2013). *Lactococcus*, the most abundant genus in goat milk cheese, mainly produces carboxylic acids, which are important compounds for cheese aroma (Kieronczyk, Skeie, Langsrud, & Yvon, 2003). *Streptococcus*, the most abundant genus in cow milk cheese, contributed to the increase in ethyl lactate, ethyl-2-methylbutanoate, and acetoin (Zheng, Liu, Li, et al., 2018). Thus, the type of bacterial starter may influence the flavor of cheese through the different catabolic pathway of the nutrients (e.g., carbohydrates, proteins and fats).

In this study, cow and goat milk cheese were always dominated by *Streptococcus* and *Lactococcus* as previously reported and had very few *Lactobacillus* (Aldrete-Tapia, Escobar-Ramírez, Tamplin, & Hernández-Iturriaga, 2014; Dalmasso et al., 2016; Fuka et al., 2013; Psoni, Tzanetakis, & Litopoulou-Tzanetaki, 2003; Quigley et al., 2012). In contrast, horse milk cheese was dominated by *Lactobacillus*. Thus, horse milk cheese may have more specific nutrition and flavor than cow and goat milk cheeses because of its specific starter lactic acid bacteria.

Some specific dominant genera of secondary bacteria in horse milk cheese, including *Psychrobacter*, *Janthinobacterium*, *Alteromonas*, *Thiobacillus*, *Acinetobacter*, *Sulfurisoma*, *Halomonas*, and *Brevibacterium*, were determined, and they also showed very low relative abundance in the tested cow and goat milk cheeses. Therefore, we believe that these bacterial genera should more likely come from the cheese production and ripening environments, and they might be more suitable for the distinctive features of horse milk as previously described. Based on previous reports, only minor flavor differences have been observed in cheese trials conducted with starter lactic acid bacteria with altered peptidase activities, indicating that the conversion of amino acids to flavor compounds by secondary bacteria is the rate-limiting step in flavor formation from proteins (Savijoki, Ingmer, & Varmanen, 2006; Steele, Broadbent, & Kok, 2013). Thus, secondary bacteria may play an important role in the formation of the unique flavor of horse milk cheese. Among the previously mentioned specific dominant genera in horse milk cheese, *Psychrobacter* can produce branched-chain aldehydes, alcohols, and esters, which are the compounds of cheese flavor (Deetae, Bonnarme, Spinnler, & Helinck, 2007). *Sulfurisoma* is a sulfur-oxidizing bacterium, presumably related to the production of sulfur-containing flavor substances in cheese. *Halomonas*, which has previously been identified on the surface of Irish artisanal cheeses (Mounier et al., 2005), is a salt-tolerant bacterium that grows at an absolute salinity (NaCl) of 0%–32%; additionally, this bacterium is leucine aminopeptidase-positive and can

TABLE 1 Summary of sequencing library information of bacterial 16S rRNA gene in the three cheese samples

Sample ID	Total sequences	OTUs observed	Shannon index	Simpson index	Phylogenetic diversity	Chao1 index	Goods coverage
HC	225,879	396 ± 108^{b}	1.61 ± 0.32^{b}	0.43 ± 0.04^{b}	34.3 ± 9.4^{b}	464.7 ± 97.6^{b}	99.8%
CC	216,092	322 ± 92^{a}	2.20 ± 0.39^{c}	0.60 ± 0.06^{c}	27.8 + 5.5^{a}	398.5 ± 67.1^{a}	99.8%
GC	209,757	312 ± 62^{a}	0.95 ± 0.19^{a}	0.19 ± 0.03^{a}	28.8 + 4.1^{a}	382.7 ± 53.5^{a}	99.8%

Note: Different lowercase letters indicate significant difference at $p < .05$ in each cheese sample. Each sample has three parallel treatments.
Abbreviations: CC, cow milk cheese; GC, goat milk cheese; HC, horse milk cheese.

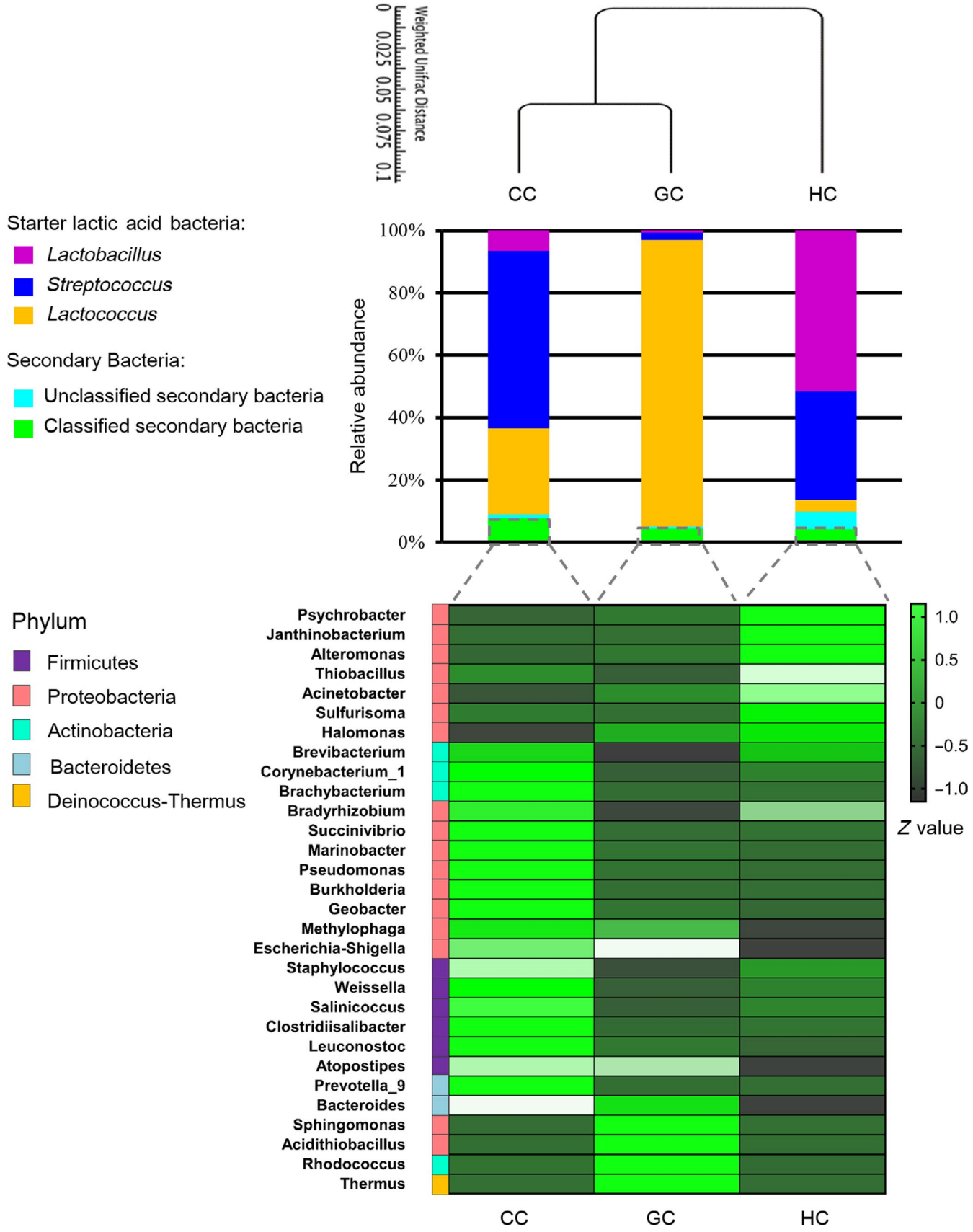

FIGURE 2 The community characteristics of starter lactic acid bacteria and secondary bacteria in the three cheese samples based on the multianalysis. The weighted UniFrac distance evaluated the similarity of bacterial community structure; the genera relative abundance of starter lactic acid bacteria was shown; the heatmap analysis of Top 35 secondary bacteria was performed based on *Z* values. CC, cow milk cheese, GC, goat milk cheese; HC, horse milk cheese

degrade DL-lactic acid to cheese flavor precursors. *Brevibacterium*, especially *Brevibacterium linens*, is a major surface microorganism of surface-ripened cheeses, which can play an important role on the proteolytic, peptidolytic, esterolytic, and lipolytic activities in the ripening process (Rattray & Fox, 1999). Simultaneously, volatile sulfur compounds (VSCs) produced by *B. linens* are the main source of cheese aroma (Forquin et al., 2011). These results indicated that the specific annotated secondary bacteria may play a crucial role in the formation of the unique nutrition and flavor of horse milk cheese. Furthermore, the unclassified secondary bacteria as the specific bacterial groups also have the potential to influence the formation of the nutrition and flavor of horse milk cheese.

5 | CONCLUSION

Under the same dairy environment, preparation environment, and cheese processing conditions, traditional Chinese horse milk cheese had a significantly larger bacterial population size, species richness, and phylogenetic diversity than Chinese cow and goat milk cheeses. Unlike the lactic acid bacteria *Lactococcus* and *Streptococcus*, which dominated the bacterial community of cow and goat milk cheeses, the bacterial community of horse milk cheese was dominated by the lactic acid bacteria *Lactobacillus* and *Streptococcus*. Furthermore, the bacterial community of horse milk cheese also had a higher abundance of secondary bacteria, including a large proportion of unclassified secondary bacteria and several specific secondary bacteria, than that of cow and goat milk cheeses. All of these starter lactic acid bacteria and secondary bacteria in horse milk cheese may be associated with the differences in the nutritional metabolism and flavor formation between horse milk cheese and cow and goat milk cheeses. Thus, the diverse and specific bacterial community of horse milk cheese may play a crucial role in the formation of its unique nutrition and flavor. In future studies, to isolate the previously annotated starter lactic acid bacteria and secondary bacteria of horse milk cheese will contribute to unveil and grasp the formation of the horse milk cheese's unique nutrition and flavor.

ETHICS STATEMENT

None required.

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CONFLICT OF INTERESTS

None declared.

AUTHORS CONTRIBUTION

Lin Zhu: Conceptualization (lead); funding acquisition (lead); supervision (equal); writing – original draft (lead); writing – review & editing (lead). **Chunlin Zeng:** Data curation (lead); investigation (lead); methodology (lead); project administration (lead); validation (lead); writing – original draft (equal); writing – review & editing (equal). **Sai Yang:** Data curation (equal); investigation (equal); methodology (equal); resources (equal); validation (equal); writing – original draft (equal); writing – review & editing (equal). **Zhaozhi Hou:** Data curation (equal); investigation (equal); resources (equal); validation (equal); writing – original draft (equal); writing – review & editing (equal). **Yuan Wang:** Investigation (equal); resources (equal); validation (equal); writing – original draft (equal); writing – review & editing (equal). **Xinyu Hu:** Investigation (equal); resources (equal); validation (equal); writing – original draft (equal); writing – review & editing (equal). **Keishi Senoo:** Conceptualization (equal); formal analysis (equal); writing – original draft (equal); writing – review & editing (equal). **Wei Wei:** Conceptualization (lead); data curation (lead); funding acquisition (lead); project administration (lead); supervision (equal); writing – original draft (lead); writing – review & editing (lead).

DATA AVAILABILITY STATEMENT

The raw sequence data were deposited in the GenBank Data Libraries with accession number PRJNA626479: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA626479.

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APPENDIX

FIGURE A1 The appearance of the tested cow, goat, and horse milk cheeses