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Using PacBio sequencing to investigate the bacterial microbiota of traditional Buryatian cottage cheese and comparison with Italian and Kazakhstan artisanal cheeses

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ABSTRACT

Traditional fermented dairy foods including cottage cheese have been major components of the Buryatia diet for centuries. Buryatian cheeses have maintained not only their unique taste and flavor but also their rich natural lactic acid bacteria (LAB) content. However, relatively few studies have described their microbial communities or explored their potential to serve as LAB resources. In this study, the bacterial microbiota community of 7 traditional artisan cheeses produced by local Buryatian families was investigated using single-molecule, real-time sequencing. In addition, we compared the bacterial microbiota of the Buryatian cheese samples with data sets of cheeses from Kazakhstan and Italy. Furthermore, we isolated and preserved several LAB samples from Buryatian cheese. A total of 62 LAB strains (belonging to 6 genera and 14 species or subspecies) were isolated from 7 samples of Buryatian cheese. Full-length 16S rRNA sequencing of the microbiota revealed 145 species of 82 bacterial genera, belonging to 7 phyla. The most dominant species was *Lactococcus lactis* (43.89%). Data sets of cheeses from Italy and Kazakhstan were retrieved from public databases. Principal component analysis and multivariate ANOVA showed marked differences in the structure of the microbiota communities in the cheese data sets from the 3 regions. Linear discriminant analyses of the effect size identified 48 discriminant bacterial clades among the 3 groups, which might have contributed to the observed structural differences. Our results indicate that the bacterial communities of traditional artisan cheeses vary depending on geographic origin. In addition, we isolated novel and valuable LAB resources for the improvement of cottage cheese production.

Key words: bacterial diversity, Russia, single molecule, real time, traditional artisan cheese

INTRODUCTION

Buryatia, a federal subject of Russia belonging to the Siberian Federal District, is located in the south of Eastern Siberia along Lake Baikal. Traditional fermented dairy products have become an indispensable part of the Russian diet and life. Since ancient times, traditional Russian dairy products have mainly included cottage cheeses (soft cheese, milk tofu), butter, yogurt, sour cream, fermented dairy drinks, and kefir (Tsydenova, 2008). In fact, each nation has its own unique culture and customs, as well as its own types and methods of making dairy products (Kamber, 2007; Litopoulou-Tzanetaki and Tzanetakis, 2014). In general, cottage cheeses are made by first storing raw milk in porcelain or casks for natural fermentation for approximately 3 d. Then the fermented milk is transferred to a pot and cooked slowly on the stove with continuous stirring until it becomes thick. To improve the sensory quality of the final product, cream or butter is usually added to the mixture before allowing it to ripen. Finally, the mixture is shaped into a mold. The ripening process involves many natural microorganisms. The complex microbial communities are mainly from the raw material and the direct environment in which the cheeses are made, such as the utensils and containers for cheese production. As a part of Russia, Buryatia has preserved its regional and traditional cottage cheeses.

The physical properties and sensory quality of cheeses are consequences of fermentation by a complex natural microbial community (Escobar-Zepeda et al., 2016). The consumption of raw milk cheese has positive effects on enteric microbiota and immunity (Koning et al., 2008; Montel et al., 2014). High-throughput sequencing has been applied to investigate the microbial communities present in ecological environments, including food products (Ercolini, 2013). In particular, this method

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allows rapid and accurate identification of microorganisms, including those that are difficult to culture and those present in low abundance (Walsh et al., 2017). This approach has been used to study the bacterial diversity of various cheeses. Quigley et al. (2012) analyzed the microbiological compositions of 62 Irish soft, semi-hard, and hard cheeses using high-throughput sequencing techniques based on cheese type, milk, and production techniques. The results showed that the microbial structure varied by cheese type, animal milk source, additives used, and whether the milk was pasteurized. Other traditional cheeses have also been studied, such as Latin-style cheese, Kazakhstan cheese, Italian cheese, and Mexican cheese (Lusk et al., 2012; González-Córdova et al., 2016; Li et al., 2017; Silvetti et al., 2017). However, the microbial communities present in Buryatian cheeses have yet to be fully explored.

The third-generation DNA sequencer, PacBio RS II (Pacific Biosciences, Menlo Park, CA), is an advanced DNA sequencing platform that produces sequences of long read lengths and a low degree of bias. This technique is useful for generating microbiota profiles based on full-length 16S rRNA gene sequences at a high taxonomic resolution at the species level (Nakano et al., 2017). Earlier sequencing technologies, such as 454-pyrosequencing and the Illumina platform, provided sequence information based only on the partial 16S rRNA gene, which limited the taxonomic resolution. Singer et al. (2016) compared the results of microbiota profiling of samples taken from meromictic Sakinaw Lake using the PacBio SMRT sequencing and Illumina platform. PacBio SMRT sequencing resulted in less ambiguous classification while allowing wider species diversity identification. Thus, the PacBio SMRT platform could be used to describe the microbial communities more accurately with higher phylogenetic resolution (Singer et al., 2016). This method has successfully been applied to evaluate the safety of infant formula, based on the bacterial population of traditional fermented koumiss and milk products enhanced with probiotics (Gesudu et al., 2016; Zheng et al., 2016; Xu et al., 2017). However, few studies have applied the PacBio SMRT sequencing technology to study the microbiota composition of traditional cheeses, with the exception of a previous study that depicted the bacterial profiles in artisan cheese from Kazakhstan (Li et al., 2017).

With the modernization of society and the industrialization of cheese making, conventional homemade cheese production has declined. As the Buryatia region still maintains its traditional methods and starter strains in cheese production, we anticipated a high microbial richness and diversity in cheese samples collected from local households. Common to other fermented foods, lactic acid bacteria (**LAB**) are the most important bacteria responsible for the sensory quality and physical properties of traditional fermented dairy products (Yu et al., 2012). These bacteria can serve as valuable resources for future improvements in the production of cheeses and other fermented products. Thus, we characterized the microbiota communities of cheese samples collected in Buryatia based on full-length 16S rRNA profiling using PacBio technology. We isolated LAB from a variety of samples with the aim of preserving these valuable microbial resources for subsequent research and industrial application. Moreover, we compared the bacterial microbiota of Buryatia cheese with those from Kazakhstan and Italy using the data sets of 18 Kazakhstan and Italian cheeses retrieved from public databases.

MATERIALS AND METHODS

Samples

A total of 7 traditional fermented cheeses were obtained from 7 different farms in Buryatia, represented as BL1 to BL7. All farms produced traditional cottage cheeses using similar methods. The samples were collected using a sterile spoon, stored in sealed bags, and sent to our laboratory packed on ice. Upon delivery to the laboratory, samples were stored at −80°C and analyzed within a few days of collection. Data sets from 18 cheese samples from Italy and Kazakhstan were retrieved for comparative analyses (Table 1; De Filippis et al., 2014).

Enumeration and Isolation of LAB

One gram of sample was serially diluted with 9 mL of saline (0.85% NaCl) for each gradient. The pour plate method was used to determine the bacterial load in the cheese samples. A total of 200 μL of diluted sample was plated onto de Man, Rogosa, and Sharpe (MRS, Difco Laboratories, Detroit, MI) and M17 (Oxoid Ltd., Waltham, MA) agar plates. The inoculated plates were cultured in an anaerobic incubator at 37°C for 48 to 72 h. Colonies were counted and grouped based on colony morphology, including color, shape, and size. Representative single colonies were streaked and propagated on the corresponding culture medium. All isolates were tested for catalase activity and Gram staining. Gram-positive and catalase-negative isolates were recultivated for further identification. Meanwhile, the isolates were kept at −40°C for long-term storage.

Identification of LAB

Genomic DNA of the selected isolates was extracted based on previously reported method (Yu et al., 2009).