A survey of the bacterial composition of kurut from Tibet using a culture-independent approach

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ABSTRACT

Kurut (fermented yak milk) made by natural fermentation is a very important dairy food for the local people in Tibet (China). It is important to fully understand the bacterial composition of kurut for quality improvement and industrial production. Because more than 99% of prokaryotes cannot be cultured and identified by methods currently used in taxonomy, we applied a culture-independent approach to explore the microbial biodiversity of this traditional food. In this study, a bacterial 16S rRNA gene clone library, including 460 clones, was constructed using total DNA extracted from 30 samples of kurut. After screening by restriction fragment length polymorphism (RFLP) analysis, 56 operational taxonomic units (OTU) with unique RFLP patterns were obtained. Then, 1 representative sequence of every OTU was sequenced and phylogenetically analyzed. The representative phylotypes were affiliated with 5 groups, including *Lactococcus lactis* ssp. *lactis*, *Lactobacillus helveticus*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, and *Acetobacter*. In addition, nearly one-third of the representative clones (132 clones) had low similarity to species in GenBank (<97%), and these phylotypes were regarded as unknown bacteria. The characteristics of kurut are determined not only by lactic acid bacteria well known by the culture-dependent approach but also by bacteria that have not yet been identified.

Key words: kurut, 16S rRNA gene clone library, restriction fragment length polymorphism, bacterial biodiversity

INTRODUCTION

Kurut (fermented yak milk) is a naturally fermented dairy product and a main source of livelihood for Tibetan households in the Qing-Tibetan highlands. It has long played important economic and dietary roles to the people of Qinghai and Tibet (Cao et al., 2004). Kurut is a pure white, viscous, fermented dairy food with unique organoleptic sensations of acidity and alcohol; fermentation usually takes 3 to 6 d at an ambient temperature around 10 to 20°C to achieve the desired properties. Kurut contains 5.37% milk fat and approximately 5.44% total protein, on average, which is about twice as rich in fat and protein as in yogurt. The contents of calcium (140 mg/100 g), phosphorus (146 mg/100 g), magnesium (154 mg/100 g), zinc (5.74 mg/100 g), and B vitamin in kurut are also higher than in yogurt (Zhang et al., 2008). Because of the desirable nutritious and organoleptic properties of kurut, it is important to fully understand its microbiological composition for the design of starter cultures and industrial production.

Microorganisms such as lactic acid bacteria (**LAB**) and yeast play key roles during the making of kurut, and *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* are the predominant populations in kurut (Zhang et al., 2008; Sun et al., 2010). However, knowledge concerning the microflora of dairy products often comes from traditional culture-dependent methods. These methods can give significant insights into specific isolates and microbial populations in different environments, but the culture media used may not be sufficiently selective for monitoring population dynamics and may fail to recover unculturable bacteria, resulting in an underestimation of microbial diversity. Generally, more than 99% of prokaryotes cannot be cultured using traditional methods (Amann et al., 1990; Schloss and Handelsman, 2005). In addition, the traditional classification is time-consuming and lacks wide coverage needed to analyze temporal variations in complex bacterial communities in natural food ecosystems (Yanagida et al., 2007; Scheirlinck et al., 2008).

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Novel molecular approaches, especially those based on the utilization of rRNA genes, have provided us with the opportunity to analyze complex communities based on sequence diversity (Amann et al., 1995). In particular, metagenomics was coined as a new and rapidly developing field in the analysis of microbial community about 10 yr ago (Handelsman et al., 1998), and this culture-independent method has opened up fresh perspectives in microbiology (Hugenholtz and Tyson, 2008).

In recent studies, both culture-dependent and culture-independent methods were applied to the analysis of microbial biodiversity and ecology in different environments (Pidiyar et al., 2004; Obodai and Dodd, 2006; Chen et al., 2008). In the food research field, Aquilanti et al. (2007) showed that the microbial diversity of fermented meat products could be successfully investigated by culture-dependent and culture-independent approaches. This reliable and rapid approach was also used to study the composition of LAB in probiotic products (Temmerman et al., 2003) and in cheese (Randazzo et al., 2002). However, we know of no research on the analysis of the bacterial composition of kurut in Tibet using culture-independent methods.

Therefore, we intended to investigate the bacterial composition of kurut using a culture-independent approach, which will be useful in future studies to improve the quality and optimize the manufacturing process of kurut. In this study, a bacterial 16S rRNA gene clone library containing 460 clones was constructed and the bacterial diversity in kurut was systematically studied.

MATERIALS AND METHODS

Sample Collection

Thirty kurut samples were collected from 16 villages located in 3 regions of Tibet during the period from July 25 to August 10, 2007. Samples were aseptically collected and kept in ice boxes during transportation. Once the samples arrived at the laboratory, they were immediately analyzed for microbial composition.

Extraction of Bacterial DNA

Bacterial DNA extraction was carried out with some modifications to the protocol of Lick et al. (1996). In brief, 1 mL of sample was frozen in liquid nitrogen for 5 min and thawed in a water bath at 60°C. Then, 600 μ L of freshly prepared 400 mmol/L NaOH and 300 μ L of 40% (wt/vol) trisodium citrate dihydrate (wash buffer A) were added to the thawed sample. After mixing, the sample was incubated for 15 min at room temperature and then centrifuged for 4 min at 10,000 $\times g$. The su-

pernatant fluid containing the fat layer was discarded. This step (addition of wash buffer A) was repeated twice. After that, the sediment was dissolved in sterile ultrapure water. Then, 50 μ L of 10% SDS and 10 μ L of proteinase K solution (10 mg/mL) were added, and the mixture was incubated at 55°C for 1 h. At the end of the incubation, 10 μ L of NaCl (5 M) and 100 μ L of cetyltriethylammonium bromide/NaCl (10%, wt/wt) were added, followed by incubation for 10 min at 65°C. Deproteinization was conducted by extraction with 1 volume of phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isoamyl alcohol (24:1) twice. Finally, DNA was precipitated by adding 0.1 volume of 3 M sodium acetate to the water phase followed by 1 volume of ice-cold isopropyl alcohol. The DNA was collected, washed, and dissolved in 50 μ L of sterile ultrapure water.

16S rRNA Amplification by PCR

Amplification of the bacterial 16S rRNA gene was carried out on an automatic thermal cycler (MJ Research PTC-200, Bio-Rad, Hercules, CA) using the universal primers of 16S rRNA gene (Lane, 1991; Delbes et al., 2007); the primer sequences were (27f) 5'-AGAGTTT-GATCCTGGCTCAG-3' and (1495r) 5'-CTACGGC-TACCTTGTTACGA-3'. The PCR reaction mixture was prepared according to the protocol of Wang et al. (2008). Each sample contained 1 $\times$ Taq buffer (Takara Bio-Company, Shiga, Japan), 1.5 mM MgCl₂, 0.2 mmol/L of each dNTP, 0.2 μ M of each primer, 50 ng of template bacterial DNA, 1.0 U of Ex Taq polymerase (Takara Bio-Company), and 50 μ L of deionized water. The PCR amplification procedure was as follows: 94°C for 5 min, 31 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 2 min, and then 72°C for 8 min, and finally 4°C for 30 min. The approximately 1,450-bp PCR products were obtained from each sample of total DNA, which was tested by 1% agarose gel electrophoresis.

Construction of 16S rRNA Clone Library

The PCR products were purified by DNA purification kit (Takara Bio-Company), and these PCR products were inserted into PMD18-T vector (Takara Bio-Company) between *Bam*HI and *Hind*III sites. The recombinant plasmid was transformed into *Escherichia coli* DH5 α and induced with 1 mmol/L isopropyl- β -D-thiogalactoside. Recombinant plasmid was confirmed by restriction enzyme digestion and PCR amplification. Randomly selected positive colonies were used to construct 16S rRNA gene clone libraries. The purified product of PCR was linked to PMD18-T vector following the instructions in the vector kit. Briefly, the total 10- μ L reaction system consisting of 4 μ L of products

of PCR, 1 μ L of PMD18-T, and 5 μ L of solution I was incubated for 10 h at 16°C. Then, the recombinant plasmid was transformed into *E. coli* strain DH5 α by heat-shock transformation (Sambrook et al., 2002). The plates were incubated overnight (for 12 to 16 h) at 37°C. β -Galactosidase-positive white colonies were analyzed by restriction enzyme digestion. Subsequently, 460 transformants with the proper inserts confirmed by PCR were used to construct the metagenomic library.

Scanning of 16S rRNA Library by RFLP

Screening of this library was carried out according to the methods described by Sato et al. (2000) and Weidner et al. (2007) with some modifications. The DNA of recombinant plasmid was extracted by plasmid mini preparation kit (Takara). The 16S rRNA gene fragment of each clone was amplified using the universal primers 27f and 1495r. Five microliters of each PCR amplified product was digested with the restriction endonucleases *AluI*, *HaeIII* and *HinfI* (Boehringer Mannheim, Mannheim, Germany) as recommended by the manufacturer. The RFLP profiles were analyzed, and all the clones were divided into different groups (operational taxonomic units, OTU) according to RFLP profiles, which represented the different bacterial phylotypes in kurut.

Sequencing of Library Clones

Partial 16S rRNA gene (approximately 1,400 bp for each representative OTU) was sequenced on ABI Prism 3730XL DNA Analyzer (Sunny Biotechnology Co. Ltd., Shanghai, China). All the sequences were assembled using DNASTar software package (1989, DNASTar Inc., Madison, WI).

The determined 16S rRNA gene sequences were aligned and the phylogenetic tree was constructed by the neighbor-joining method using MEGA software version 4.0 (Kumar et al., 2004). The representative sequences of every species were selected from isolates for phylogenetic tree construction. The species identification of phylotypes was confirmed by comparing the sequences of OTU with the type strains using the phylogenetic tree. The sequences were analyzed and determined through BLAST search on the National Center for Biotechnology Information website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The sequences obtained in this study for all phylotypes were deposited in GenBank.

Analysis of Data

The diversity coverage of the clone library was estimated by rarefaction analysis with Analytical Rarefac-

tion software version 1.3 (S. M. Holland, University of Georgia, Athens; <http://www.uga.edu/strata/software.html>). The diversity and species richness were analyzed by the Species Prediction and Diversity Estimation software (SPADE; Chao and Shen, 2003).

RESULTS AND DISCUSSION

Within the complex bacterial community of traditional fermented dairy products, LAB are considered to be the dominant microflora, and the variety of LAB in fermented dairy products depends on the starter culture used and the raw milk. Traditional research on the microflora of dairy products relies heavily on pure culture isolation, suitable substrates, and culture media. However, cultivable microorganisms are not always representative of the microflora in a complex ecosystem. It has been reported that only a small fraction of microorganisms are obtained by conventional methods (Ward et al., 1990; Engelen et al., 1998). In contrast, culture-independent methods can provide a more realistic view of the microbial diversity in the ecosystem, as demonstrated in Mozzarella cheese (Coppola et al., 2001) and typical Italian salami (Aquilanti et al., 2007).

To clarify the microbial diversity in the traditional kurut in Tibet, we constructed a bacterial 16S rRNA gene clone library. The microbial diversity and species richness were studied by RFLP and sequences analysis.

Analysis of 16S rRNA Gene Clone Library

A bacterial 16S rRNA gene clone library containing 460 clones was successfully constructed with total DNA extracted from the kurut samples, and the rarefaction analysis revealed that the clone library was sufficient to cover the bacterial diversity (Figure 1 and Table 1). The coverage rate of the library was 92.1%, Shannon index was 5.4, and Simpson index was 0.1. According to the report by Kemp and Aller (2004), the size of this 16S rRNA gene clone library was sufficient for the analysis of microbial diversity.

The phylotype diversity analysis was conducted by scanning the clone library using RFLP and sequences analysis, and 56 phylotypes were identified based on the RFLP profiles. Then, each phylotype was confirmed by 16S rRNA sequence analysis. Partial sequences of the 16S rRNA gene (1.4 kb) of all the representative clones with unique RFLP patterns were sequenced and phylogenetically analyzed. All of the representative sequences in the present study were deposited in GenBank with accession numbers GQ267858 to GQ267992.

Based on the BLAST search results, the sequences were classified into 2 parts. Part 1 contained 5 OTU

Table 1. Diversity estimate of 16S rRNA clone library by SPADE software (Chao and Shen, 2003)¹

No. of OTU	C	Species richness		Shannon index		Simpson index	
		FSR	95% CI	Chao and Shen (2003)	95% CI	MLE	95% CI
55	92.1	34.5	(81.8, 229.2)	5.4	(61.3, 84.1)	0.1	(56.0, 56.7)

¹OTU = operational taxonomic unit; C = estimated sample coverage; FSR = estimation of species richness; Chao and Shen (2003) = based on Horvitz-Thompson estimator and sample coverage method; MLE = maximum likelihood estimator.

(OTU 1 to 5) that exhibited high similarity (>97%) to the type strains or identified species in GenBank. Part 1 was the major component of the 16S rRNA gene clone library, occupying about 70.5% of the total clones. Part 2 was composed of 51 OTU, including 132 clones in the library with low phylogenetic similarity (<97%) to the identified species (Table 2). To identify these species, phylogenetic analysis was conducted. Some sequences of type strains and representative sequence of each clone were used to construct a phylogenetic tree.

Identification of Phylotypes with High Phylogenetic Similarity

In the 16S rRNA gene clone library, part 1 included major sequences that could be identified by BLAST search, and the sequences in this part were resolved to 5 OTU. Among them, OTU 1 was the largest group, containing 155 clones, and was identified as *Lactobacillus helveticus* with 97 to 100% similarity to the type strains or identified species in GenBank by BLAST search. The second group, comprising 15.6% of the total clones, was identified as *Lb. delbrueckii* ssp. *bulgaricus*, with 97 to 100% similarity. The third group, which contained 58 clones (12.4% of total clones), was

assigned to *Streptococcus thermophilus* with similarity of 100% to the identified sequences in GenBank. The remaining 29 clones were regarded as *Lactococcus lactis* ssp. *lactis*, with 99% similarity.

Twenty sequences with high similarity (>97%) to the identified species in GenBank or type strains were used to construct the phylogenetic tree (Figure 2). These phylotypes were classified into 6 clusters. Cluster I contained 7 sequences identified as *Lb. delbrueckii* ssp. *bulgaricus* with 97 to 100% similarity. Cluster II (including 4 sequences) was identified as the *Lb. helveticus* group with 97 to 100% similarity to the type strain of *Lb. helveticus* in GenBank. Cluster III was *Lc. lactis* ssp. *cremoris*, including 2 sequences with 97 to 99.8% similarity to the type strain in GenBank, and cluster IV was *Strep. thermophilus* with 100% similarity to the 2 sequences listed in the GenBank. The remaining phylotypes with high similarity to the species in GenBank were the *Pseudomonas* group (cluster V) containing 2 sequences and the *Acetobacter* group (cluster VI) including 3 sequences.

Part 1 was the major segment in the library, including 328 clones representing 71.2% of the total clones in the bacterial 16S rRNA gene clone library. Our results indicated that *Lb. delbrueckii* ssp. *bulgaricus*, *Lb. helveticus*, *Lactococcus lactis*, and *Strep. thermophilus* were the major microbial flora in kurut from Tibet. This result was in agreement with the previous study, which found that *Lb. delbrueckii* ssp. *bulgaricus* and *Strep. thermophilus* were the predominant species in kurut from Qinghai (Sun et al., 2010). However, different results were reported in a previous study described by Yu et al. (2009), which found the presence of *Lactobacillus fermentum*, *Lactobacillus casei*, and *Enterococcus* in kurut. We also found *Lb. helveticus*, *Lb. delbrueckii* ssp. *bulgaricus*, and *Strep. thermophilus* in kurut from Tibet. The major reason for this difference could be ascribed to the different seasons of sample collection, the composition of yak milk, and environmental factors (Zhang et al., 2008; Sun et al., 2010).

As the major dairy product consumed daily, kurut could make a beneficial contribution to the health of local people as a large number of LAB are ingested

Figure 1. Rarefaction curve for bacterial composition of kurut samples. The x-axis represents the numbers of the total samples of 16S rRNA gene clones, and the y-axis represents operational taxonomic units (OTU) clustered by 16S rRNA gene clones. We showed that OTU were close to their saturation curve and that the clone library was sufficient to cover the bacterial diversity.

Table 2. Restriction fragment length polymorphism types of 16S rRNA gene sequences from the clone library, listed with phylogenetic affiliation, accession number of representative clone, similarity (NCBI), and percentage similarity

Part ¹	RFLP profiles	No. of clones	Top match species (BLAST)	GenBank accession no. of representative clone	Similarity (%)	Distribution (%)
1	OTU 1	155	<i>Lactobacillus helveticus</i>	GQ267860	97–100	33.7
	OTU 2	73	<i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i>	GQ267859	97–100	15.9
	OTU 3	58	<i>Streptococcus thermophilus</i>	GQ267961	97–100	9.0
	OTU 4	29	<i>Lactococcus lactis</i> ssp. <i>lactis</i>	GQ267990	97–100	4.5
	OTU 5	13	<i>Acetobacter</i>	GQ267927	97–100	2.0
2	OTU 6	12	Unknown bacteria	GQ267864	<97	2.0
	OTU 7	9	Unknown bacteria	GQ267928	<97	1.4
	OTU 8	9	Unknown bacteria	GQ267861	<97	1.4
	OTU 9	8	Unknown bacteria	GQ267878	<97	1.25
	OTU 10	7	Unknown bacteria	GQ267959	<97	1.1
	OTU 11	6	Unknown bacteria	GQ267963	<97	0.9
	OTU 12	6	Unknown bacteria	GQ267920	<97	0.9
	OTU 13	6	Unknown bacteria	GQ267954	<97	0.9
	OTU 14	4	Unknown bacteria	GQ267979	<97	0.6
	OTU 15	4	Unknown bacteria	GQ267931	<97	0.6
	OTU 16	4	Unknown bacteria	GQ267873	<97	0.6
	OTU 17	3	Unknown bacteria	GQ267976	<97	0.6
	OTU 18	3	Unknown bacteria	GQ267893	<97	0.5
	OTU 19	3	Unknown bacteria	GQ267985	<97	0.5
	OTU 20	3	Unknown bacteria	GQ267969	<97	0.5
	OTU 21	3	Unknown bacteria	GQ267867	<97	0.5
	OTU 22	3	Unknown bacteria	GQ267987	<97	0.5
	OTU 23	2	Unknown bacteria	GQ267866	<97	0.3
	OTU 25	2	Unknown bacteria	GQ267967	<97	0.3
	OTU 26	2	Unknown bacteria	GQ267973	<97	0.3
	OTU 27	2	Unknown bacteria	GQ267986	<97	0.3
	OTU 28	2	Unknown bacteria	GQ267917	<97	0.3
	OTU 29	2	Unknown bacteria	GQ267908	<97	0.3
	OTU 30	2	Unknown bacteria	GQ267980	<97	0.3
	OTU 31-56	1	Unknown bacteria	GQ267858-GQ267992	<97	0.15
Total		460				

¹Part 1 includes operational taxonomic units (OTU) with high similarity to sequences in GenBank (OTU 1 to OTU 5); part 2 includes those with low similarity (OTU 6 to OTU 56).

simultaneously. Besides the health benefits, LAB play major roles in the quality of fermented dairy products, such as the key flavor, texture, and preservative qualities (Atrih et al., 2001; Messens and Vuyst, 2002; Ahmad and Irene, 2007). *Streptococcus thermophilus* is an industrially important species used in the production of fermented dairy products, and its main industrial roles are the production of lactic acid and the formation of texture and flavor during cheese and yogurt production (Tamime and Deeth, 1980; Grappin et al., 1985). An increase in yogurt acidity during storage is often caused by strains of *Lb. delbrueckii* ssp. *bulgaricus*, because of its acidifying activity at high acidity and low temperature (Lourens-Hattingh and Viljoen, 2001). *Lactobacillus delbrueckii* ssp. *bulgaricus* serves as the proteolytic organism in yogurt rather than *Strep. thermophilus* because the former has a very extensive set of proteolytic enzymes (Sieuwerds et al., 2008). In the study of contribution of LAB in flavor formation, Imhof et al. (1995) indicated a synergistic effect between *Lb. delbrueckii* ssp. *bulgaricus* and *Strep. thermophilus* and underlined the important contribution of the flavor

compound acetaldehyde to the resulting aroma. *Lactobacillus helveticus* is a lactic-acid-producing strain, which could provide the acid for yogurt formation. In addition, *Lb. helveticus* could provide essential amino acids via hydrolysis of casein with its proteolytic enzymes; these proteolytic enzymes are involved in the development of flavor in fermented dairy products (Fernández et al., 1994). Therefore, it is possible that *Lb. helveticus*, *Lb. delbrueckii* ssp. *bulgaricus*, and *Strep. thermophilus* play major roles in the quality and flavor formation of kurut.

Identification of Sequences with Low Phylogenetic Similarity

The second part included phylotypes with low similarity (<97%) to the identified species by BLAST. It is well known that high similarity (>97%) is regarded as a threshold for species identification by 16S rRNA gene (Stackebrandt and Goebel, 1994). Thus, these groups including 28.8% of the clones in the library were assigned as unknown bacteria. These sequences

Figure 2. Phylogenetic positions of 16S rRNA gene obtained in this library with high similarity to species in GenBank. IMAU clone designations refer to nomenclature from Inner Mongolia Agricultural University.

and some type strains were used to construct another phylogenetic tree (Figure 3), which was divided into 3 large clusters and 8 small ones. The 3 large clusters were cluster I, cluster II, and cluster III. Cluster I was composed of LAB and *Acetobacter*, whereas cluster II was composed of *Pseudomonas*. Cluster III contained only one clone, namely clone 421 (GQ267967), which was located in an independent branch of the phylogenetic tree and could not be identified to any species. Thus, clone 421 was regarded as an unknown species. The largest cluster was composed of 8 small groups, including *Strep. thermophilus*-related group (cluster i), *Lactococcus cremoris*-related group (cluster ii), *Lactobacillus plantarum* and *Lactobacillus casei* group (cluster iii), *Lactobacillus acidophilus*-related group (cluster iv), *Leuconostoc mesenteroides*-related group (cluster v), and *Acetobacter*-related group (vi). The similarity of all of these sequences to species in NCBI was <97% by BLAST analysis. They were regarded as different groups related to the type strains in GenBank. These clones probably represented some new isolates in kurut,

which could not be recovered by traditional isolation method.

Microbial composition, especially LAB and yeasts, in traditional fermented dairy products is regarded as an important topic because it plays a critical role in kurut quality formation and potential health or nutritional benefits (Zhang et al., 2008; Sun et al., 2010). However, based on previous studies, many microbes were not recovered by pure culture isolation, and consequently microbial ecology in different environmental niches was underestimated (Amann et al., 1995; Streit and Schmitz, 2004; Xu, 2006; Kennedy et al., 2008).

The occurrence of an *Acetobacter*-related group and a *Pseudomonas*-related group in kurut was noteworthy. In fact, strains of *Acetobacter* could not be isolated by the traditional agar plate technique, and the culture-independent method is a powerful tool for microbial composition analysis (Ongol and Asano, 2009). *Acetobacter* spp. can produce high amounts of acetic acid from alcohol produced by yeast present in kurut. *Acetobacter* spp. have a high ability to oxidize

Figure 3. Phylogenetic positions of 16S rRNA gene obtained in this library with low similarity to species in GenBank. IMAU clone designations refer to nomenclature from Inner Mongolia Agricultural University.

ethanol to acetic acid, which is dependent on the enzyme alcohol dehydrogenase (Adachi et al., 1978; Yoo et al., 1994). *Acetobacter* is important for the quality of certain fermented dairy products with regard to flavor formation (Park et al., 2006). The pseudomonads are highly proteolytic and lipolytic, activities that accelerate the deterioration of milk products (Lengkey and Adriani, 2009). However, these strains are destroyed by pasteurization during processing of milk products. Because of the low level of bacteria in finished products, the count of pseudomonads could be underestimated by traditional plate count methods (Bedeltavana et al., 2010). In this study, the phylotype and proportion of *Pseudomonas* were comprehensively obtained. Interestingly, one clone (clone 421) located in the independent branch of the phylogenetic tree could not be identified to any species; therefore, we temporarily designated it as an unknown new bacterium in kurut, which should be further studied in single pure culture for its physiological and biochemical characteristics and function.

CONCLUSIONS

As a geographically specific dairy food, kurut is of considerable economic and dietary importance to the local people. It shows a great diversity in fat and dry matter levels because of the different ways of processing it in different localities. Production of kurut relies heavily on its microbial composition, with different systems of proteolytic and lipolytic enzymes. In this study, most of the clones (>70.5%) were regarded as LAB, including *Lactobacillus helveticus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus*, and *Lactococcus lactis* ssp. *lactis*. The results generally agreed with previous literature. Interestingly, we also detected sequences of the genera *Acetobacter* and *Pseudomonas* in kurut. Furthermore, some sequences of unknown bacteria in the kurut found in this study were novel and not mentioned in previous reports. Our results may be valuable for quality improvement and industrial production of kurut in the future. To provide a better understanding of microbial ecology in kurut, the application of traditional culture method combined with molecular biology technology would be very useful, and future studies on microbial diversity in traditional fermented dairy products should employ both culture-dependent and culture-independent methods.

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