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Full Length Research Paper

Isolation and identification of cultivable lactic acid bacteria from traditional goat milk cake in Yunnan province of China

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Thirteen goat milk cake samples which were collected from different households in Yunnan province in China were used for isolation and identification of lactic acid bacteria (LAB). The results showed that LAB and yeasts were the dominant microflora in the samples with the LAB counts in all samples ranged from 8.32 log_{10} CFU/g to 10.16 log_{10} CFU/g. The number of yeasts in most samples ranged from 1.60 log_{10} CFU/g to 6.15 log_{10} CFU/g respectively. Seventy-six strains of LAB were isolated from these samples and identified by conventional and 16S rRNA gene sequence analysis methods. The LAB strains were found to belong to five general and nine different species, among which the ratio of cocci bacteria (96.1%) was higher than that of rod (3.9%), and was considered to be the major population. The results also showed that *Lactococcus lactis, subsp. lactis* (72.4%) and *Lactococcus garvieae* (10.5%) could be considered as the dominant groups population in these samples. The obtained LAB pure cultures may serve as valuable sources for further selection of starter cultures in dairy industry.

Key words: Goat milk cake, Lactic acid bacteria, isolation, identification and 16S rRNA gene sequencing analysis.

INTRODUCTION

Lactic acid bacteria (LAB) belong to a large family of fermentative gram-positive bacteria, which can ferment glucose with lactic acid as the major metabolic end product. LAB and yeast play important roles in the production of certain traditional fermented dairy products, such as cheese, dairy fan, koumiss and kurut (Florez et al., 2006; Liu et al., 2009; Meng et al., 2009; Sun et al., 2010a). These traditional dairy products could be a good resource for obtaining novel LAB with desirable properties. In recent decades, studies on isolation, identification and screening of probiotic LAB and their application in dairy products have expanded (Beukes et al., 2001; Stanton et al., 2001; Saavedra et al., 2003; Meng et al., 2009; Sun et al., 2010a; 2010b). Further research on LAB isolated from the traditional dairy products will be valuable to the development of industrial starter cultures.

Goat milk cake is a traditional fermented milk product, which has been consumed and produced for over six hundreds years by people of minority ethnic region in Yunnan province of China. Nutritional value of goat milk cake has high contents of fat acid, crude protein, vitamins and diversified trace elements, which are essential for the human body (Chen et al., 2009). Goat milk cake of high quality is fresh cheese-like goat milk product which is yellow or white in colour, without sour taste. Goat milk cake could be prepared and eaten in many ways, for example, raw, fried, boiled, grilled or steamed with vegetables. Also milk cake can be used in sandwiches (Zhang et al., 2009). As a natural milk product, the production of goat milk cake is very unique. Generally, it is always made by traditional method in summer time. Firstly, fresh goat milk was boiled and acidified by using an acidulant. Then acidified milk was mixed well and held for 30 min for whey separation. The whey was removed

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by filtration using cheese cloth and the curd obtained was transferred to cake molds and pressed to obtain goat milk cake. Sometimes, during this procedure, the whey from the production of goat milk cakes was set into a container for spontaneous fermentation. The acid whey was preserved and used as the source of acidulant for the next milk cake production. Therefore, abundant microflora such as LAB was deposited in the acid whey and goat milk cake after several generations.

Till now, the production of milk cake in Yunnan province is still in the stage of traditional family workshops. However, with the development of modern economy and advance in industry, home-made goat milk cake of ethnic minority will be reduced in this region and rich microbial resources deposited in these traditional dairy products would be lost or even go into extinction in future. Therefore, it is necessary to study and preserve these LAB resources in goat milk cake. Since, the documents about microbial diversity of LAB in traditional goat milk cake in China were scant and scattered, this research aimed to systematically study on the enumeration, isolation, identification and characterizion of the dominant LAB in the thirteen samples of traditional goat milk cake in Yunnan province, China, using combination of the conventional methods and 16S rRNA sequence analysis. This study is valuable for designing and selecting starter culture for dairy cake production.

MATERIALS AND METHODS

Collection of goat milk cake

Thirteen goat milk cake samples were collected from different households in Jianchuan region of Yunnan province, China in the period of 5th to 10th June, 2008. The temperature of milk cake samples at sampling ranged from 24.3 to 42.4℃ with an average of 28.8℃. All these samples were aseptically collected and kept cool in an ice box and then transported to laboratory for analysis immediately.

Reference strains

Reference strains were as follows: *Lactobacillus acidophilus* 1.1878T, *delbrueckii* subsp. *bulgaricus* JCM 1002T, *helveticus* 1.1877T, *coryniformis* subsp. *coryniformis* 1.1879T and *Lactococcus lactis subsp. lactis* 1.1936T were obtained from China Generally Microorganism Culture Collection Center (CGMCC). *Streptococcus thermophilus ATCC19258NT, Leuconostoc mesenteroides* subsp. *dextranicum* ATCC19255T and *Enterococcus facealis* ATCC19433 were obtained from American Type Culture Collection (ATCC).

Enumeration of LAB and yeasts

Samples were divided into four equal portions. From each quadrant, 5 g of milk cake were used to determine the acid content by titration. Meanwhile, the samples were aseptically diluted in a sterile plastic tub containing 45 ml of 0.9% sterile NaCl. Further decimal dilutions were made by adding 1 ml of the blended samples to 9 ml sterile 0.9% NaCl solution. Serial 10-fold dilutions of 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} were performed. Appropriate diluted samples were plated on Brom Cresol Purple (BCP) agar, gotten from Nissui Pharmacy, Tokyo, Japan and 30℃ for 48 h to enumerate the total LAB under anaerobic conditions (Florez et al., 2006; Ishii, 2003). The appropriate dilutions were surface plated on acidified PDA agar plates (Potato Dextrose Agar, Nissui Pharmaceutics Co, LTD, Japan, pH3), and incubated aerobically at 28℃ for 72 h to obtain yeast count (Ishii, 2003). The total concentration of individual species was calculated using total number of isolates which were typed and identified. Enumeration of each sample was averaged among the four quadrants.

Isolation of LAB

For LAB isolation, samples were plated on MRS agar (DifcoTM) and M17 agar (Oxoid CM0785) with 0.5% (w/v) lactose (Sigma, St. Louis, MO, USA) and with 0.5% (w/v) glucose. The plates were incubated anaerobically for 2 to 3 days at 30℃ using anaerobic jars. Representative single colonies were randomly selected from MRS and M17 plates on the basis of colonies morphological differences, such as colour, shape and examined microscopically. All isolates were initially examined for gram reaction, and then tested for catalase reaction and cell morphology. Only Gram-positive, rod or sphere shaped, catalase negative, non-spore forming bacterial isolates were purified and freeze-dried in protective reagent (10% skim milk broth containing 0.1% sodium glutamate), and then stored at -80℃ for further studies.

Identification of LAB by conventional method

All purified isolates were examined as follows: colonial morphology, cell appearance, motility, gas production from glucose, NH_3 production from arginine, salt tolerance, growth temperature and pH values and optical rotation of lactic acid (D – or L +) from sugar fermentation. The experimental tests were carried out using the method described in previous studies with some modifications (Airidengcaicike et al., 2010; McDonough et al., 1963; Wang et al., 2008). The lactic acid isomers produced by glucose growth were detected spectrophotometrically (OD340) in the supernatant fluids of 24 h cultures using a commercial kit (Hoffman La Roche Diagnostic Mannheim, Germany) which used D- and L-lactate dehydrogenases. However, the enzyme reaction system was reduced to 1/20 using NanoDrop micro-ultraviolet spectrophotometer (Gutmann and Wahlefeld 1974). All the above methods were used to identify the isolates to genus level.

After that, twenty-two kinds of sugar fermentation tests were performed according to the methods as described by Airidengcaicike et al. (2010) and Ling (1999). Each test was done in triplicate. Results were confirmed by using Bergey's Manual of Systematic Bacteriology (Sneath et al., 1986).

Molecular identification of LAB by 16S rRNA gene sequence analysis

Seventy-six isolated strains grown in MRS broth at 30/37℃ (Lactobacilli at 37℃, cocci at 30℃) were used for DNA extraction and purification as described by Wang et al. (2008). A specific 16S rDNA PCR assay was performed with PTC-200 Peltier Thermal Cycler (MJ Research Corporation) using PCR buffer containing 1.5 mM $MgCl_2$, 200 mM of each dNTP, 25 μM of each primer, 1U Taq DNA polymerase (TaKaRa Biomedical Technology (Dalian) Co, Ltd), and 50 to 100 ng of template DNA in a total volume of 25 μL. The universal 16S rRNA gene primer sequences were as follows: forward primer 27F, 5′-AGAGTTTGATCCTGGCTCAG-3′ (Mora et al., 1998) and reverse primer 1495R, 5′-CTACGGCTAC CTTGTTACGA-3′ (Jensen et al., 1993). The amplification program consisted of 1 cycle at 94℃ for 4 min; 30 cycles at 94℃ for 1 min,

Table 1. Counts average of the LAB and yeasts in goat milk cake samples.

No. samples	LAB average counts (log_{10} CFU/g)	Yeasts average counts (log_{10} CFU/g)	No. samples	LAB average counts (log_{10} CFU/g)	Yeasts average counts (log_{10} CFU/g)
A	8.78 ± 0.2	3.81 ± 0.3	H	9.66 ± 0.1	5.07±0.2
B	10.16 ± 0.3	5.50 ± 0.1	I	8.70 ± 0.2	1.60 ± 0.1 [b]
C	8.45 ± 0.1	5.69 ± 0.1	J	10.10 ± 0.2	2.00 ± 0.1
D	8.63 ± 0.2	6.15 ± 0.3 [a]	K	8.32 ± 0.3[b]	2.70 ± 0.2
E	9.60 ± 0.2	4.18 ± 0.4	M	10.34 ± 0.3 [a]	4.27 ± 0.3
F	10.03 ± 0.2	4.72 ± 0.1	N	9.96 ± 0.3	5.85 ± 0.3
G	9.56 ± 0.3	4.56 ± 0.2			

a. The highest count. b. The minimum count.

58℃ for 1 min and 72℃ for 2 min and finally 1 cyc le at 72℃ for 7 min. PCR products were checked by electrophoresis in 1.0% agarose gels. Approximately 1,500 bp fractions between two primers corresponding to the nucleotide numbers were amplified. Positive products were purified using a commercial DNA fragment purification kit (TaKaRa Biomedical Technology (Dalian) Co, Ltd), and the purified fragments was connected with pMD 18-T Vector (Takara) and cloned. Then the ligation reaction products were verified with PCR and Restriction enzymes (*Hin*d III and *Bam*H I, TaKaRa Biomedical Technology (Dalian) Co, Ltd) methods. The recombined plasmids were sequenced on ABI Prism 3730XL DNA Analyzer by Shanghai Sangni Biosciences Corporation of China.

Sequences were aligned using DNASTAR Lasergene software package (DNASTAR, Inc., Madison, WI). The obtained sequence similarity searches were examined by comparing the obtained sequence with those in the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/).

Phylogenetic trees were constructed by using the neighbor-joining method, available in the MEGA version 4.0 software package (Center for Evolutionary Functional Genomics, The Biodesign Institute, Arizona State University, Phoenix, AZ, USA) (Tamura et al., 2007).

RESULTS

Enumeration of LAB and yeasts

LAB and yeasts strains in each sample were counted by standard plate count method (Table 1). The counts of LAB in 13 samples ranged between 8.32 log_{10} CFU/g and 10.34 log_{10} CFU/g, whereas the yeasts counts in 13 samples ranged between 1.60 log_{10} CFU/g and 6.15 log_{10} CFU/g. Overall, the count of LAB in most sample was significantly higher than those of yeast with at least three logarithms. Therefore, LAB was the predominated microflora in the samples.

Isolation and identification of LAB by conventional method

From 13 samples of goat milk cake collected in Yunnan, many isolates were obtained by pure culture techniques. Most of the isolates were shown to be LAB by their positive gram reactions, absence of catalase, lack of motility and cell morphology as cocci or rods, after which a few key conventional phenotypic tests were used to identify them tentatively. Finally, seventy-six strains of LAB were obtained consisting of 73 strains of cocci (96.1% in total) and 3 strains of Lactobacilli (3.9% in total). These strains belonged to 5 groups: *Lactococcus, Enterococcus, Streptococcus, Leuconostoc* and *Lactobacillus* (Table 2).

Lactic acid fermentation of the 73 cocci strains was all considered as homofermentation because of absence of gas production from glucose. These cocci were divided into four groups by conventional identification method (Table 2). Group 1 was identified as *Lactococcus lactis*. They produced L-Lactic acid from glucose. All isolates in this group can grow at 10℃, but could not grow at 45℃. Strains in group 2 were identified as *Enterococcus*. In this group, all the strains could grow at 45℃, as well as in the presence of 6.5% NaCl and on MRS agar adjusted to pH 9.0. They also produced L-lactic acid. Growth at 45℃ suggested that they were thermophilic. They fermented most carbohydrates, grew at high temperature and in a wide pH range.

Group 3 was considered as *Leuconostoc* due to the production of gas from glucose, and the production of D- lactic acid. The strains included in group 4 were identified as *streptococcus*. Strains in this group could not grow at 10℃, but grew well at 45℃ suggesting its thermophilic nature.

There were 3 strains *Lactobacillus* including IMAU50151 (YNK-2-1), IMAU50154 (YNK-4-1-1) and IMAU50127 (YNF-5). YNK-2-1 and YNK-4-1-1 grew well at 45℃, produced L-lactic acid, and

Table 2. Physiological and biochemical characteristics of lactic acid bacterial isolates.

	Cocci (73)							Lactobacilli (3)	
Groups [a]		1			2	3	4	5	
Sub-group [a]	1.1	1.2	1.3	2.1	2.2			5.1	5.2
No. isolates	55	8	2	3	3	1	1	2	1
R/C [b]	C	C	C	C	C	C	C	R	R
Gram-staining	55/55[c]	8/8	2/2	3/3	3/3	1/1	1/1	2/2	1/1
Lactic acid isomer [d]	L	L	L	L	L	D	L	DL	D
Catalase reaction	0/55	0/8	0/2	0/3	0/3	0/1	0/1	0/2	0/1
Gas from glucose	0/55	0/8	0/2	0/3	0/3	1/1	0/1	0/2	0/1
Growth at 10℃	55/55	8/8	2/2	3/3	3/3	1/1	0/1	NT [e]	NT
15℃	NT	NT	NT	NT	NT	NT	NT	0/2	0/1
40℃	30/55	1/8	0/2	3/3	3/3	1/1	NT	0/2	1/1
45℃	0/55	0/8	0/2	3/3	3/3	0/1	1/1	2/2	1/1
4% NaCl	NT	8/8	0/2	NT	NT	0/1	NT	NT	0/1
6.5 % NaCl	55/55	NT	0/2	3/3	3/3	1/1?	0/1	NT	NT
pH 3.5	0/55	NT	NT	NT	0/3	0/1	0/1	2/2	0/1
pH4.5	55/55	NT	2/2	3/3	3/3	0/1	0/1	2/2/	1/1
pH9.0	55/55	8/8	2/2	3/3	3/3	1/1	0/1	0/2	1/1
NH_3	55/55	0/8	0/2	0/3	0/3	0/2	0/1	0/2	0/1
Arabinose	0/55	0/8	0/2	0/3	0/3	0/1	0/1	0/2	0/1
Xylose	3/55	0/8	1/2	0/3	0/3	0/1	0/1	0/2	0/1
Ribose	55/55	8/8	0/2	0/3	0/3	0/1	0/1	0/2	0/1
Mannose	55/55	8/8	2/2	3/3	3/3	0/1	1/1	2/2	0/1
Fructose	55/55	8/8	2/2	3/3	3/3	1/1	1/1	2/2	1/1
Galactose	55/55	8/8	2/2	3/3	2/2	1/1	1/1	2/2	0/1
Sucrose	30/55	2/8	1/2	0/3	2/2	1/1	1/1	0/2	0/1
Maltose	55/55	1/8	1/2	3/3	2/2	1/1	1/1	0/2	0/1
Cellobiose	55/55	8/8	2/2	3/3	0/2	0/1	0/1	0/2	0/1
Lactose	55/55	8/8	2/2	3/3	2/2	1/1	1/1	2/2	1/1
Trehalose	55/55	8/8	1/2	3/3	0/2	0/1	0/1	0/2	0/1
Melibiose	0/55	0/8	0/2	3/3	0/2	0/1	0/1	0/2	0/1
Raffinose	0/55	0/8	0/2	0/3	0/2	0/1	0/1	0/2	0/1
Melezitose	0/55	0/8	0/2	0/3	0/2	0/1	0/1	0/2	0/1
Dextrin	55/55	8/8	2/2	0/3	0/2	0/1	0/1	NT	0/1
Inulin	0/55	0/8	0/2	0/3	0/2	0/1	0/1	NT	0/1
Mannitol	35/55	1/8	0/2	0/3	0/2	0/1	0/1	0/2	0/1
Sorbitol	0/55	0/8	0/2	0/3	0/2	0/1	0/1	0/2	0/1

Table 2. Contd

Inositol	0/55	0/8	0/2	0/3	0/2	0/1	0/1	0/2	0/1
Aesculin	55/55	2/8	2/2	0/3	1/2	0/1	1/1	0/2	0/1
Salicin	55/55	8/8	2/2	3/3	2/2	0/1	0/1	0/2	0/1
Amygdalin	55/55	8/8	2/2	3/3	0/2	1/1	0/1	0/2	0/1

a. Group 1: *Lactococcus lactis* group (1.1 *Lactococcus. lactis* subsp. *lactis,* 1.2 *Lactococcus garvieae*, 1.3 *Lactococcus. lactis* subsp*. cremoris*). Group 2: *Enterococcus* group (2.1 *Enterococcus. durans*, 2.2 *Enterococcus saccharominimus*). Group 3: *Leuconostoc* group; Group 4: *Streptococcus* group; Group 5: *Lactobacillus* group (5.1 *Lactobacillus. helveticus*, 5.2: *Lactobacillus. delbrueckii* subsp. *bulgaricus*). b. R, rod; C. cocci. c. number of positive strains or number of strains. d. L: L-lactic acid, DL: DL-lactic acid, D: D- lactic acid. e. NT: not tested.

utilized glucose, galactose, lactose, mannose, maltose and fructose. These two strains had the same morphological, staining and biochemical characteristics as those found with type strain *Lactobacillus helveticus* 1.1877^T. Therefore, these two isolates were identified as *Lactobacillus helveticus*. Strain YNF-5 grew well at 45℃, but it expressed very slight growth at 10℃ and belonged to *Lactobacillus*. YNF-5 fermented glucose, lactose, fructose, but could not ferment cellobiose, raffinose, ribose, melezitose and sucrose. Its characteristics were consistent with the reference strains *Lactobacillus delbrueckii* subsp. *bulgaricus* JCM 1002. According to Bergey's Manual (Sneath et al., 1986), this strain was identified as *Lactobacillus dellbrueckii* subsp. *bulgaricus*. Furthermore, acid-producing ability of YNF-5 (0.61 %) was lower than *Lactobacillus delbrueckii* subsp. *bulgaricus* JCM 1002 (1.35%) in skimmed milk.

The strains isolated from milk cake samples had high acid-producing ability ranging between 0.61 and 1.23%. All these strains coagulated the milk when incubated in skim milk at 37 ℃ for 24 h. Most of these strains produced L-lactic acid.

Identification of LAB by molecular method

The nucleotide sequences of the 16S rRNA gene of all the isolates were analyzed, and each strain identity was determined by BLAST program on NCBI (http://www.ncbi.nlm.nih.gov/) and phylogenetic tree analysis. Sequences of 16S rRNA gene obtained in this study were deposited in the NCBI database with the accession numbers from FJ749490 to FJ749564 and FJ915635.

Phylogenetic tree based on 16S rRNA gene sequence of LAB is depicted in Figure 1. Seventy-six strains of LAB were divided into 4 large clusters: Cluster I was the *Lactococcus* group, which was composed of Lactococcus lactis subsp. lactis, *Lactococcus lactis subsp. cremoris, Lactococcus garvieae and Streptococcus thermophilus*; Cluster II comprised *Enterococcus durans* and *Enterococcus saccharominimus*; Cluster III comprised *Leuconostoc lactis* was classified as cluster III and Cluster IV was the *Lactobacillus* group which was composed of *Lactobacillus helveticus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. However, the genetic relationship between *Lactococcus and Streptococcus* was closer than that of *Enterococcus, Leuconostoc and Lactobacillus*.

Species and numbers of LAB identified from milk cake by molecular method were showed in Table 3. Seventy-three cocci strains were identified as follows: 55 strains of *Lactococcus lactis subsp. lactis* (72.4% of total isolates), 8 strains of *Lactococcus garvieae* (10.5% of total isolates), 2 strains of *Lactococcus lactis subsp. cremoris*, 3 strains of *Enterococcus durans*, 3 strains of *Enterococcus saccharominimus*, and 1 strain each of *Streptococcus thermophilus* and *L. lactis*. Three strains of *Lactobacillus* included 2 strains of *Lactobacillus helveticus* and 1 strain of *Lactobacillus dellbrueckii* subsp. bulgaricus. The overall results showed that *L. lactis subsp. lactis* could be considered as the dominant strain and *Lactococcus garvieae* was the second most numerous.

DISCUSSION

Foods in each region or area have their own particular characteristics because of the uniqueness of local ingredients and production techniques, which are deeply rooted in tradition and linked to the territory (Duan et al., 2008; Duthoit et al., 2003; Harun-ur-Rashid et al., 2007). Goat milk cake is one of the popular traditional milk products in Yunnan province of China. Commonly, it contains two distinct microbes, namely bacteria and fungi. Unique quality was partly due to the interactions that occurred between yeasts and LAB in the milk ecosystem (El Khalifa and El Tinay, 1994). In the present study, LAB was found as the predominated microflora in the samples collected. Similar results were also obtained with other fermented goat milk samples (Zhang et al., 2009).

Further research was focused on identification

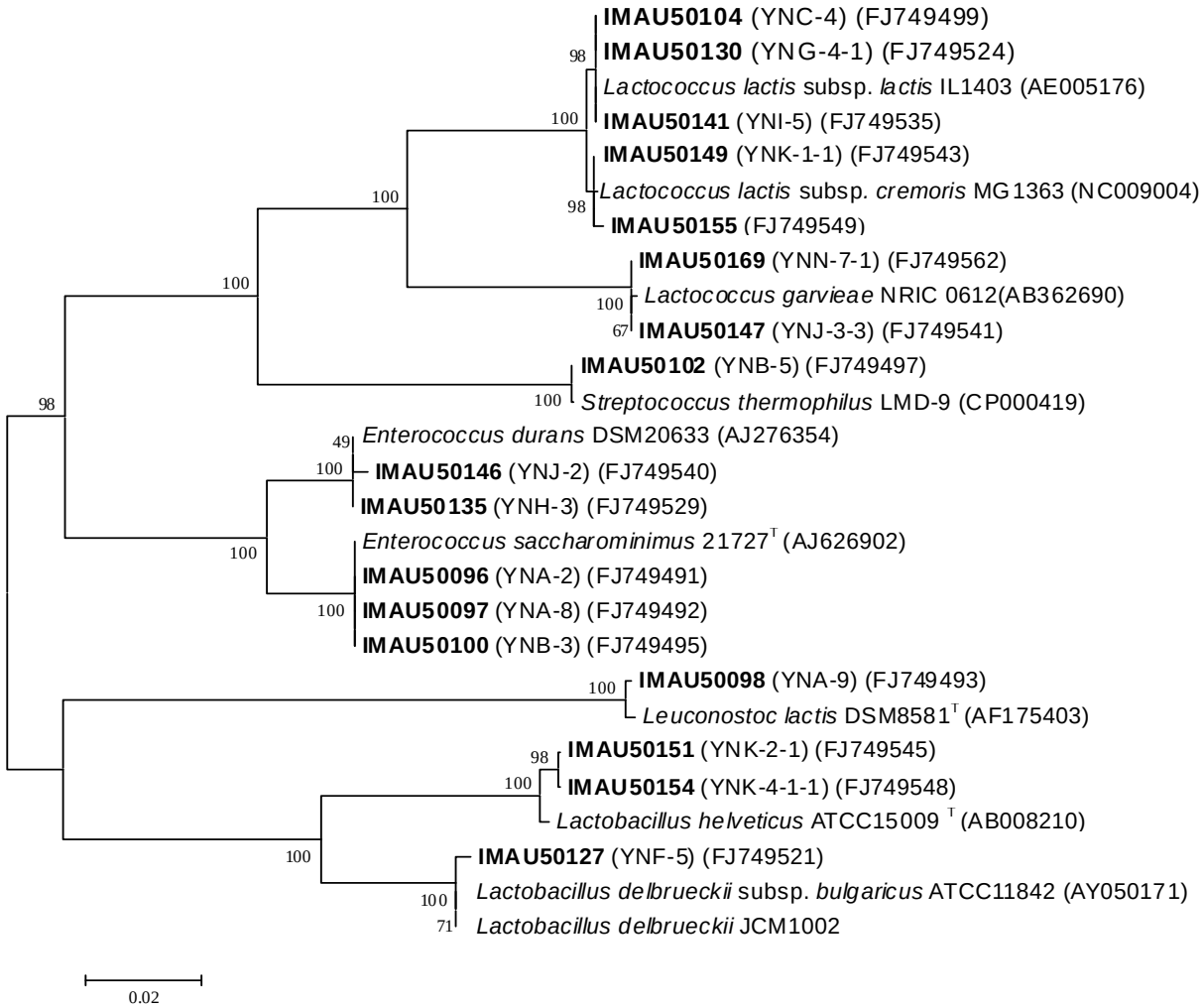

Figure 1. Phylogenetic tree of LAB isolated from traditional goat milk cake in Yunnan province of China. 'IMAU' represent the strain isolated in this study. Numbers in bracket is the accession numbers for the strains.

Table 3. Distribution of LAB species in goat milk cake in Yunnan province.

Genus	Strains species	No. of isolates	Percent (%)	
Lactococcus	*Lactococcus lactis* subsp. *lactis*	55	72.4	85.5
	Lactococcus garvieae	8	10.5	
	Lactococcus lactis subsp. *cremoris*	2	2.6	
Enterococcus	*Enterococcus durans*	3	3.9	7.8
	Enterococcus saccharominimus	3	3.9	
Leuconostoc	*L. lactis*	1	1.3	1.3
Streptococcus	*Streptococcus thermophilus*	1	1.3	1.3
Lactobacillus	*Lactobacillus helveticus*	2	2.6	3.9
	Lactobacillus delbrueckii subsp. *bulgaricus*	1	1.3	
	Total	**76**		

and characterization of these LAB isolates. Given that the conventional method based on biochemical tests is limited in identifying the species level of strains (Ercolini et al., 2001; Holzapfel et al., 2001). So, in this study, a combination of phenotypic and molecular approach was applied for identification of LAB isolated from thirteen goat milk cake samples in Jianchuan region in Yunnan province of China. Finally, all seventy-six LAB belonged to five genera and nine different species. There were more obvious differences in the species of LAB isolated from these milk cake samples compared with isolates from other traditional milk products such as koumiss, kurut and yak milk in different regions of China or Mongolia (Meng et al., 2009; Sun et al., 2010a; Zhang et al., 2008a). It was well established that traditional dairy products made in different locales appeared to have regional uniqueness in LAB biodiversity. Commonly, environmental factors are considered as important agents that influence the characteristics of microbiota component in local traditional milk products. Microbial composition in local traditional milk products is affected not only by environmental factors, but also by milk source, processing temperature, coagulation time and coagulation factors.

To take species abundance into account, *Lactococcus lactis subsp. lactis* was the dominant LAB in Yunnan milk cake samples. *Lactococcus lactis* is one of the most important micro-organisms involved in the dairy industry, especially in the production of buttermilk and many cheeses including Brie, Camembert, Cheddar, Colby, Gruyère, Parmesan and Roquefort (Coffey and Ross 2002). It is obvious that we isolated so *many Lactococcus lactis subsp. lactis* from goat milk cake when compared with the previous studies on the predominant microflora in other traditional dairy products. For example, Zhang et al. (2008b) found that the species and numbers of *Lactobacillus* isolated from Qinghai were higher than that of *Lactococcus*; moreover, *Lactobacillus delbrueckii* was the predominant microflora in naturally fermented goat milk in Qinghai province, China. Similar results were also described by Mathara et al. (2004), Sun et al. (2010b) and Watanabe et al. (2008). Overall, the Lactococci isolated in this study represent a unique genetic pool. The sources from which these organisms were isolated are hitherto unexplored by indigenous dairy products made in the unorganized sector in Yunnan Province in China. At present, the industrially important dairy *Lactococci* strains comprise a limited genetic pool. Several investigators have emphasized the need to isolate new *Lactococcus* strains (with unique flavor producing abilities and displaying resistance to prevailing phages) and other desirable genetic characteristics to extend the number of useful *Lactococcus* strains for use in dairy starters. The *Lactococci* isolated in this study might fulfill this need. Additionally, two of the isolates typed out to be *Lactococcus lactis* subsp. *cremoris* (Table 3). The isolation of *cremoris* subspecies from nature (including from raw milk) is rare. Prevenient investigators have reported that the *cremoris* subspecies is preferable for use in Cheddar cheese (Salama et al., 1991). Florez also reported that in cheese *Lactococcus* dominated at early stage of manufacture and reached approximately 9.6 $\log^{10}$ CFU/g within 3 days' fermentation and maintained this level even throughout the ripening period. However, Lactobacilli remained at a lower level of 8.5 $\log^{10}$ CFU/g in 3 day-old Cabrales, a traditional Spanish cheese manufactured using cow, goat and sheep's milk mixture (Florez et al., 2006).

In our study, 8 strains of *Lactococcus garvieae* were isolated from milk cake samples. This species could be considered as the second dominant groups. From Figure 1, we can see that 16S rDNA sequencing analysis could distinguish *Lactococcus garvieae* and *L. lactis*, which are phenotypically similar. *Lactococcus garvieae* (junior synonym *Enterococcus seriolicida*) originally described from a bovine with mastitis (Collins et al., 1984). Soon afterwards, different biotypes of *Lactococcus garvieae* were isolated from non-dairy sources, such as water buffalos, fish, human (Zlotkin et al., 1998), as well as some dairy sources, like Italian artisanal cheeses (Fortina et al., 2007) and Spanish traditional raw milk cheese (Fernandez et al., 2010). Although the role of *Lactococcus garvieae* as an infectious agent for fish remains unclear, however, the strains belonging to the *lactococcus* species are commonly used as starter cultures for a variety of fermented and cultured dairy products and are generally recognized as safe (GRAS) microorganisms (Villani et al., 2001). The use of *Lactococcus garvieae* strains as starter or adjunct cultures might be recommended for certain cheese types (Fernandez et al., 2010).

In conclusion, this paper systematically analyzed the composition of LAB in goat milk cake samples in Yunnan province of China by conventional and molecular genetic methods. The results showed that goat milk cakes in Yunnan province have abundant and important LAB resources, and the dominant LAB was L. lactis subsp. lactis. It provides basic data for LAB composition in goat milk cakes, which may become valuable source for further selection of starter cultures. On the other hand, it showed that a combined use of biochemical tests and molecular technique is needed for obtaining exact identifications.

The current study constitutes the first step in the designing process of LAB starter cultures in order to protect the typical organoleptic characteristics of traditional goat milk cake. For future study, characterization and selection of the most desirable strains for potential as starter cultures for commercial use is needed.

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