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To cite this version:

Bao, Yu, Liu, Qing, Chen, et al.. Predominant lactic acid bacteria in traditional fermented yak milk products in the Sichuan Province of China. Dairy Science & Technology, 2012, 92 (3), pp.309-319. 10.1007/s13594-012-0061-x . hal-00930623

HAL Id: hal-00930623

https://hal.science/hal-00930623v1

Submitted on 11 May 2020

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Dairy Sci. & Technol. (2012) 92:309–319
DOI 10.1007/s13594-012-0061-x

NOTE

Predominant lactic acid bacteria in traditional fermented yak milk products in the Sichuan Province of China

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Received: 30 September 2011 / Revised: 11 February 2012 / Accepted: 13 February 2012 /
Published online: 13 March 2012
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Abstract Yak milk products have been consumed for hundreds of years in the Sichuan province of China where complex microbial populations contribute to their unique flavor and functional properties. Sixty-four samples of traditional yak milk products (kurut, qula cheese, raw milk, whey, and butter) were collected from widely distributed households in Sichuan province, China. In total, 213 strains of lactic acid bacteria (LAB) were obtained by traditional pure culture method and all strains were identified to species level by phenotypic characterization, 16S rRNA gene sequence analysis, species-specific PCR, and tuf-PCR-RFLP technologies. The result showed that a complex LAB composition was present in these products, and all the isolates belonged to six genera and 17 different species and subspecies. The distribution of the isolates by genus was as follows: *Leuconostoc* (40.8%), *Lactobacillus* (39.0%), *Streptococcus* (13.2%), *Lactococcus* (5.6%), *Enterococcus* (0.94%), and *Weissella* (0.46%). Among these isolates, *Leuconostoc mesenteroides* subsp. *mesenteroides* (61 strains, about 28.6%) and *Lactobacillus helveticus* (41 strains, about 19.2%) were the predominant populations in yak milk samples. This paper systematically studied the LAB composition in various yak milk products in Sichuan province of China, which provides raw data and LAB strain resource for further studies involving probiotic strain selection and starter culture design pertaining to the industrial production of traditional fermented milk.

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在传统的发酵牦牛奶产品在中国四川省的主要乳酸菌

摘要：中国四川地区的牦牛乳资源丰富，当地牧民生产和销售传统发酵牦牛乳制品已经有几百年的历史，其中蕴藏着的复杂微生物区系赋予牦牛乳制品独特的组织形态、风味和功能等品质特征。本研究通过纯培养的方式对从四川省不同牧民家庭采集的64份牦牛乳制品（酸牦牛乳，曲拉，鲜牦牛乳，乳清和奶油）中的乳酸菌进行分离，并通过形态观察、16S rDNA序列分析、种特异性PCR及*tuf*-PCR-RFLP等技术对其进行准确地鉴定，最终获得乳酸菌213株。结果表明这些传统乳制品中蕴含着丰富的乳酸菌资源，所获得的乳酸菌属于6个属17个不同种和亚种。6个属的分布如下：明串珠菌属占总分离株数的40.8%，乳杆菌属占39.0%，链球菌属占13.2%，乳球菌属占5.6%，肠球菌属占0.94%和魏斯特菌属占0.46%。其中，肠膜明串珠菌肠膜亚种（61株，约占总分离株的28.6%）和瑞士乳杆菌(41株，约占总分离株的19.2%)是牦牛乳制品中所有乳酸菌分离株的优势菌群。本文系统地研究了中国四川地区各种牦牛乳制品中乳酸菌的组成，为益生菌和发酵剂的筛选提供了宝贵的资源，同时为工业化生产传统发酵乳制品的发酵剂的设计提供了基础数据。

Keywords Yak milk products · Lactic acid bacteria · Isolation · Identification · 16S rDNA · Species-specific PCR · PCR-RFLP

关键词 牦牛乳制品 · 乳酸菌 · 分离 · 鉴定 · 16S rDNA序列分析 · 种特异性PCR · PCR-RFLP 技术

1 Introduction

Yaks are members of the subfamily Bovinae (Ding et al. 2008) and live at high altitude in the Qinghai–Tibetan plateau (China) and the Himalayan regions (Nepalese Himalayas, Indian Kashmir, and Mongolia). The total population of yaks all over the world is estimated to be around 14.2 million, with more than 93.7% located in parts of China, in regions as diverse as the Sichuan, Qinghai, Tibet, and Gansu provinces (Ding et al. 2008; Liu et al. 2011).The Sichuan province is located in the southwest of China, bordered by the Tibetan Plateau in the west and by the Three Gorges and the Yangtze River in the east with an average altitude of 4,200 m. Owing to the high altitude and the related harsh environment, crop cultivation is not practical in most areas of the plateau, yet the grazing of live stock of hardy animals which can tolerant the cold such as yak and Tibetan sheep is more feasible.

In general, the lactation period of yaks is 150–180 days, and the average milk production is about 270 kg per year. Therefore, the Sichuan province possesses abundant yak milk resources. Yak milk is a highly nutritious product rich in fat, protein, essential minerals, and healthy polyunsaturated fatty acids such as conjugated linoleic acid and omega three fatty acids (Ding et al. 2008; Kandeepan and Sangma 2010). Thus yak milk plays an essential role in vital functions and in providing nutrients for human health for the native people (Liu et al. 2011). To extend the shelf-life of yaks' milk, local herdsmen in the Sichuan province use raw yak milk in the production of various home-made fermented yak milk products including kurut, qula, butter, and whey, etc. Kurut is a naturally fermented yak milk which is viscous, and ivory and yellow in color (Airidengcaicike et al. 2010). Qula is a grainy, hard, yellow or white cheese made in the Tibetan plateau from yak's milk (Duan et al. 2008). Kurut, qula, and butter are three kinds of common naturally fermented yak milk products and popular with Tibet herdsmen. These products are

fermented by the interaction of distinct types of microorganisms. Lactic acid bacteria (LAB) is one of the major components of these products (Coeuret et al. 2003; Wouters et al. 2002) and they play an essential role in the aroma, texture, and acidity of the product as well as exerting beneficial effects for human health (Ahmad and Irene 2007; Zhang et al. 2008). Over hundreds of years, a rich resource of LAB derived from traditional yak products have been handed down from one generation to the next.

Over the past few years, the microbiological characteristics of traditional fermented milk products have been studied in Algeria (Guessas and Kihal 2004), Bangladesh (Rashid et al. 2007), Iran (Azadnia and Khan Nazer 2009), Moroccan (Ouadghiri et al. 2005; Ouadghiri et al. 2009), Mongolia (Uchida et al. 2007; Yu et al. 2011), and Inner Mongolia as well as China (Airidengcaicike et al. 2010). According to the previous studies, LAB are widely distributed in nature and occur naturally as indigenous microflora in raw milk, yogurt, etc. Furthermore, the composition of LAB in these products is more varied and inconstant. Therefore, these traditional fermented yak milk and raw milk foods could be considered as an abundant resource for obtaining safe LAB.

To the best of our knowledge, published literature regarding the biodiversity and composition of LAB in yak milk products in the Sichuan province in China are scarce. In addition, with the development of a modern economy and the technological advances in industry, the nomadic life of ethnic minorities in China has declined more and more. As a result, the rich microbial resources of traditional dairy products could be lost forever in the near future. Therefore, the objective of this study was to isolate and identify the LAB strains from various yak milk products collected from the Sichuan province in order to constitute an original collection of LAB strains from this area. This study will provide useful information on the flora diversity and predominant LAB species in yak milk products in the Sichuan province.

2 Materials and methods

2.1 Collection of samples

Sixty-four yak milk products (including kurut, qula cheese, raw milk, whey, butter) were collected from 12 widely distributed sites located in two counties of the Ngawa prefecture in the Sichuan province of China over the period of 4th to 12th, September, 2009 (Table 1). Kurut is a naturally fermented yak milk product and qula cheeses were made by local Tibetans using traditional methods described in Duan et al. (2008). Before collection, samples of liquid milk were mixed thoroughly in the fermentation vat, and then transferred aseptically in sterile tubes, and kept in an ice-box until transported to the laboratory. The solid samples, such as qula, were collected using aseptic plastic packaging. These samples were analyzed immediately upon arrival with the exception of the raw milk samples which were incubated at 30 °C for 24 h in the laboratory before analysis. The remainder of the samples were frozen by liquid nitrogen and kept at −80 °C for further analysis.

Table 1 The sampling location and enumeration of viable LAB on BCP agar in the fermented yak milk products

Type	No. of samples	Sampling location in Sichuan		LAB counts ($\log_{10}$ CFU·mL^{-1} or $\log_{10}$ CFU·g^{-1})	
		County	No. of villages	Average	Range
Kurut	6	Norgay	3	7.93±0.90	6.01–8.79
	14	Hongyuan	7	8.11±0.69	7.51–9.15
Whey	10	Hongyuan	3	8.47±0.25	8.21–9.02
Qula	17	Hongyuan	5	7.18±1.49	4.00–8.62
	1	Norgay	1	8.26	
Butter	1	Hongyuan	1	8.14	

2.2 LAB enumeration and isolation

One milliliter (or 1 g) of sample was homogenized with 9 mL of 0.85% (*w*/*v*) sterile physiological saline to make an initial dilution (10^{-1}). Serial dilutions of the suspended samples were performed and 0.1 mL aliquots of the appropriate dilution (10^{-5}, 10^{-6}, and 10^{-6}) spread plated in triplicate on three universal media which were used for microbial enumerations and isolation. Firstly, plate count agar containing bromocresol purple (BCP agar) was incubated at 30 °C for 48 h under anaerobic conditions for the enumeration of total LAB (Rashid et al. 2007; Zhang et al. 2008). To inhibit the growth of yeast, 0.01% (*v*/*v*) cycloheximide was added into BCP agar plate. After incubation, colonies were enumerated, and recorded as colony-forming units (CFU) per milliliter or per gram of milk products.

For LAB isolation, samples were plated on M17 agar (Oxoid CM0785) and MRS agar (Difco™). For M17 agar, 0.5% (*w*/*v*) lactose (Sigma, St. Louis, MO, USA) and 0.5% (*w*/*v*) glucose were added. Plates were incubated anaerobically for 3 day at 30 °C using anaerobic jars. MRS agar was mainly used for the isolation of lactobacilli (De Man et al. 1960) and M17 agar was mainly used for the isolation of cocci such as enterococci and *Streptococcus thermophilus* (Terzaghi and Sandine 1975). Colonies with distinct morphological characteristics such as color, shape, and size were selected randomly from each plate containing 30–300 colonies and purified by streaking on the same medium for further identification.

Gram-positive, catalase-negative bacterial isolates were purified. Frozen stocks of the purified isolates were made in 10% (*w*/*v*) skim milk and stored at −80 °C until use. Lyophilization of isolates was also performed for long-term storage of strains.

2.3 Isolation and identification of LAB

DNA extraction and 16S rDNA sequencing Four milliliters of each late exponential phase culture grown in TPY broth at 30 °C was collected by centrifugation at 4 °C at 8,000×*g* for 3 min. DNA extraction was performed as previous described (Yu et al.

2009). Purified DNA was diluted to a final concentration of 100 ng·μL^{-1} for further use.

A fragment of approximately 1,500 bp of the 16S rDNA was amplified using the forward primer FA-27F (GCAGAGTTCTCGGAGTCACGAAGAGTTTGATCCTGGCTCAG) and the reverse primer RA-1495R (AGCGGATCACTTCACACAGGACTACGGCTACCTTGTTACGA) as described by Sun et al. (2010). Nucleotides 1 to 21 (underlined) of both primers (FA-27F and RA-1495R) are specific sequencing primers. All PCR amplifications of the 16S rRNA gene were performed on a PTC-200 Peltier Thermal Cycler (MJ Research Corporation, USA), using 50 μL PCR buffer containing 1.5 mM $MgCl_2$, 200 mM of each dNTP, 25 μM of each primer, 0.4 U Taq DNA polymerase (TaKaRa Corporation, Dalian, China), and 50–100 ng of template DNA. The amplification program consisted of 1 cycle at 94 °C for 4 min; 30 cycles at 94 °C for 1 min, 58 °C for 1 min and 72 °C for 2 min; and finally 1 cycle at 72 °C for 7 min.

The sequencing of purified products was performed in the Shanghai Sangni Biosciences Corporation of China. The resulting 16S rDNA sequences were initially determined using the BLAST program in NCBI GenBank (www.ncbi.nlm.nih.gov/blast; Altschul et al. 1997). Then sequences of representative isolates and their related type strains were imported into MEGA version 4.0 software to create the sequence alignment and phylogenetic trees based on the neighbor-joining method (Tamura et al. 2007). The percentage of bootstrap confidence levels for internal branches, as defined by the MEGA program, was calculated from 1,000 random reassembling. The 16S rDNA sequences of all isolates were submitted to NCBI (http://blast.ncbi.nlm.nih.gov).

Discrimination of closely related species For further identification of the *Lactobacillus plantarum* group and the *Lactobacillus casei* group strains, species-specific multiplex PCR assay of *rec*A gene and *tuf*-PCR-RFLP techniques were also used. All type strains used in this study were bought from Am5erican Tissue Culture Collection (ATCC). Those strains were preserved in milk containing 0.1% sodium glutamate and stored at −80 °C.

Five strains of the *Lb. plantarum* group (*Lb. plantarum*, *Lb. paraplantarum*, and *Lb. pentosus*) were distinguished by multiplex PCR using species-specific *rec*A gene-based primers as described by Torriani et al. (2001). The PCR amplifications of the *rec*A gene in LAB were performed on a PTC-200 Peltier Thermal Cycler, with an initial denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C (30 s), annealing at 56 °C (10 s), elongation at 72 °C (30 s), and final extension at 72 °C for 5 min. The PCR products were visualized on 1% agarose gel.

PCR-RFLP analysis of the *tuf* gene was used for discrimination of the *Lb. casei* group. A fragment of approximately 850 bp of the *tuf* gene was amplified by the primer *tuf*-1 (GATGCTGCTCCAGAAGA) and *tuf*-2 (ACCTTCTGGCAATTCAATC), and then 3 μL PCR amplified product was digested with restriction endonuclease *Hae* III using the manufacturer's recommendations (37 °C, 3 h). Restriction digests were analyzed on a 5.0%polyacrylamide gel run at 150 V for 1.5 h in 0.1× TBE (40 mM Tris–acetate and 1 mM EDTA pH 8.0).

3 Results and discussion

3.1 Enumeration and isolation of LAB

The viable counts of LAB in the fermented yak milk products are shown in Table 1. Total counts of LAB on BCP agar varied from 4.00 to 9.15 $\log_{10}$CFU·mL^{-1}. The highest count of LAB detected in kurut samples was 9.15 $\log_{10}$ CFU·mL^{-1}. Counts of LAB in 18 qula cheese samples ranged from 4.00 to 8.62 $\log_{10}$ CFU·g^{-1}. These findings are consistent with the results of previous studies. Duan et al. (2008) reported that the LAB counts in qula cheese were 3–7 $\log_{10}$ CFU·g^{-1}. Sun et al. (2010) reported that the LAB dominated the microbial population of kurut and the viable counts ranged from 5.74 to 10.29 $\log_{10}$ CFU·g^{-1}, with a mean value of 8.69± 0.88 $\log_{10}$ CFU·g^{-1}. Differences in the milk ripening times and transport, or differences in sampling regions could possibly contribute to the variations detected in the LAB counts.

Many isolates were obtained from the 64 yak milk products by pure culture techniques. Most of the isolates were considered as presumptive LAB by their positive gram reactions, absence of catalase, lack of motility, and cell morphology as cocci or rods, etc. In the end, a bank of 213 isolates was obtained containing 129 strains which were cocci (61% in total) and 84 strains which were rods (39% in total).

3.2 Identification of LAB

Most of the isolates were identified to species level by sequencing the 16S rRNA gene, which showed more than 99% similarity to the reference strains. The representative strains and their related type strains were chosen to construct a phylogenetic tree by the MEGA software (Fig. 1). As shown in Fig. 1, all representative strains grouped together with the corresponding type strain. However, strains IMAU80296, IMAU80323, and IMAU80441 appeared to group equally with *Lb. plantarum, Lb. paraplantarum,* and *Lb. pentosus*. In a previous study, it was reported that *Lb. plantarum* and *Lb. pentosus* have very similar 16S rRNA gene sequences that differ by only 2 bp (Ennahar et al. 2003), so the 16S rRNA gene sequence could not give discriminatory results for this group. For accurate classification of the *Lb. plantarum* group of isolates, 5 isolates *of* the *Lb. plantarum* group were differentiated by species-specific multiplex PCR assay with the primers designed on the *rec*A gene (Fig. 2). As shown in Fig. 2, isolates IMAU80296, IMAU80297, IMAU80323, IMAU80325, and IMAU80441 displayed a similar 318 bp band with that of *Lb. plantarum* ATCC 14917^{T} (share the same band size). So these five strains were subsequently identified as *Lb. plantarum*. It has been proposed that the *rec*A gene could be used as a better phylogenetic marker for the *Lb. plantarum* group (Torriani et al. 2001).

16S rRNA gene sequences of the *Lb. casei* group were extremely similar. In order to accurately identify the *Lb. casei* group containing 16 isolates, part of the *tuf* genes (850 bp) were amplified, and then digested by restriction enzyme *Hae*III. The RFLP profiles of a selection of representative isolates are shown in Fig. 3. PCR-RFLP patterns of these isolates appeared to be similar to that of *Lb. paracasei* ATCC 25302, and differences among *Lb. casei*, *Lb. zeae,* and *Lb. rhamnosus* were observed.

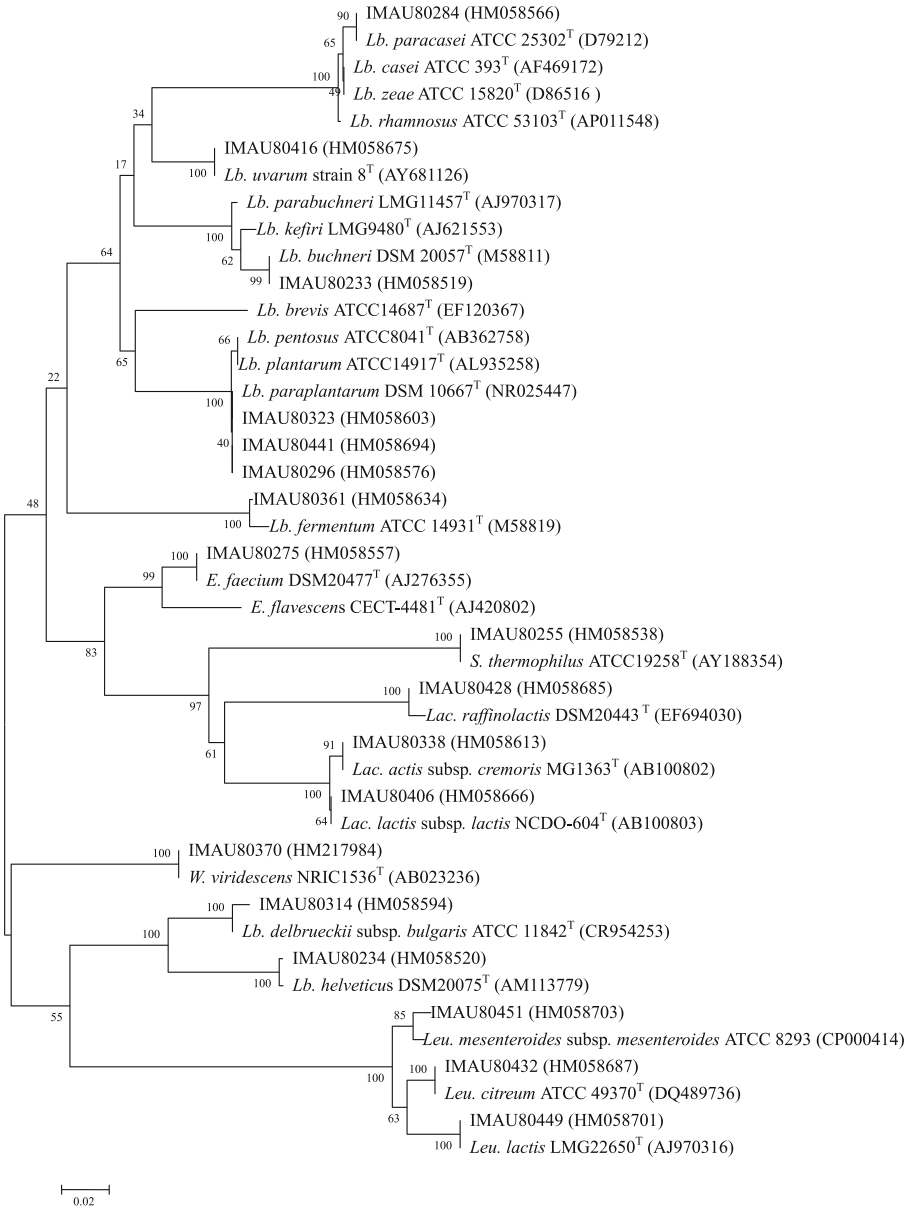

Fig. 1 Neighbor-joining tree showing the phylogenetic relationships among isolates and the type strains of related genera based on 16S rRNA gene sequences. Numbers at the branch nodes are the bootstrap values percentages (1,000 replicates). (*E.*=*Enterococcus*; *Lb.*=*Lactobacillus*; *Lac.*=*Lactococcus*; *Leu.*=*Leuconostoc*; *S.*=*Streptococcus*; *W.*=*Weissella*)

Based on the 16S rDNA sequences analysis, species-specific PCR and *tuf*-PCR-RFLP technologies, 213 isolates were classified as belonging to 6 genera (16 species): *Enterococcus* (2 strains), *Lactobacillus* (83 strains), *Lactococcus* (12 strains), *Leuconostoc* (87 strains), *Streptococcus* (28 strains), and *Weissella* (1 strain). *Leuconostoc mesenteroides* subsp. *mesenteroides* was isolated from each type of yak

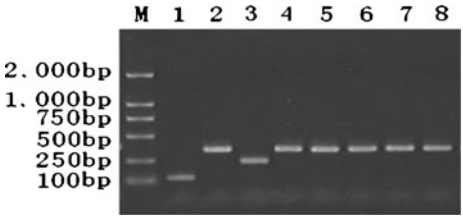

Fig. 2 Agarose gel electrophoresis of PCR amplification of *rec*A in *L. plantarum* group isolates. *1*: *L. paraplantarum* DSM10667^T; *2*: *L. plantarum* ATCC 14917^T; *3*: *L. pentosus* ATCC 8041^T; *4*: IMAU 80296 (S16-1); *5*: IMAU 80297 (S16-2). *6*: IMAU 80323 (S23-1); *7*: IMAU80325 (S23-3); *8*: IMAU80441 (S58-3); M: DL 2000 DNA markers

milk product and could be considered as a major component in the microflora (28.6% in all). *Leu. mesenteroides* has also been identified in Maasai traditional fermented milk in Kenya (Mathara et al. 2004) and in yak milk qula cheese in Qinghai, China (Duan et al. 2008). The results also showed that *Lb. helveticus* (19.2%) was the second predominant component of the microflora in all products and present in raw milk, kurut, qula, and whey samples. In previous studies, *Lb. helveticus* was found to be the major component of the LAB microflora in traditional fermented Airag and Tarag in Mongolia (Uchida et al. 2007).

Other species isolated at relatively high frequencies included *S. thermophilus*, *Leu. lactis*, *Lb. paracasei*, *Lb. delbrueckii* subsp. *bulgaricus,* and *Lac. lactis.* In another study, similar results were obtained, which showed that *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* were the predominant LAB population in kurut samples in Qinghai, China (Zhang et al. 2008). Also, some strains of *Lac. lactis and Lb. delbrueckii* subsp. *bulgaricus* have previously been isolated from traditional drinking yogurt in Iran (Azadnia and Khan Nazer 2009). Several researchers have identified *Lb. paracasei*, *Lb. fermentum,* and *Lb. plantarum* from dairy products, such as the Maasai traditional fermented milk (Mathara et al. 2004), traditional Mongolian fermented dairy products (Yu et al. 2011) and traditional Moroccan soft white cheese (Ouadghiri et al. 2005). In addition, a few other isolates were classified as *E. faecium, Lb. uvarum, Leu. citreum,* and *Lac. raffinolactis. Lb. buchneri* and *Weissella viridescens* to a lesser extent. *E. faecium* has been isolated from Maasai traditional fermented milk in Kenya (Guessas and Kihal 2004) and traditional fermented dairy products in Mongolia (Yu et al. 2011).

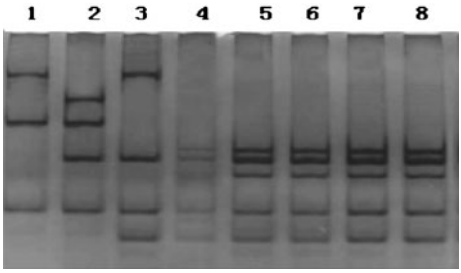

Fig. 3 *tuf*-PCR-RFLP patterns of *Lb. casei* group isolates digested with *Hae*III. *1*: *Lb. casei* ATCC 393; *2*: *Lb. zeae* ATCC 15820; *3*: *Lb. rhamnosus* ATCC 7469; *4*: *Lb. paracasei* ATCC 25302; *5*: IMAU80284 (S13-3); *6*: IMAU80291 (S15-1); *7*: IMAU80307 (S18-2); *8*: IMAU80444 (S59-2)

Table 2 Distribution of LAB species in various yak milk product samples in the Sichuan province, China

Genus	Species	Kurut (20)[a]	Raw milk (15)[a]	Qula (18)[a]	Whey (10)[a]	Butter (1)[a]	No. of LAB
Enterococcus (2 strains)	*E. faecium*	1 (1B)		1 (1A)			2 (1A+1B)
Lactobacillus (83 strains)	*Lb. buchneri*		1 (1A)				1 (1A)
	Lb. paracasei	7 (3A+4B)	1 (1A)	7 (4A+3B)	1 (1B)		16 (8A+8B)
	Lb. delbrueckii subsp. *bulgaricus*	5 (5A)		4 (3A+1B)	2 (2A)		11 (10A+1B)
	Lb. fermentum	1 (1B)		5 (1A+4B)	2 (2B)		8 (1A+7B)
	Lb. helveticus	7 (4A+3B)	4 (2A+2B)	22 (16A+6B)	8 (5A+3B)		41 (19.2%) (27A+14B)
	Lb. plantarum		5 (5A)				5 (5A)
	Lb. uvarum				1 (1B)		1 (1B)
Lactococcus (12 strains)	*Lac. lactis* subsp. *cremoris*		1 (1A)				1 (1A)
	Lac. lactis subsp. *Lactis*	5 (1A+4B)	1 (1A)	1 (1A)	1 (1A)		8 (4A+4B)
	Lac. raffinolactis		1 (1A)	1 (1B)	1 (1A)		3 (2A+1B)
Leuconostoc (87 strains)	*Leu. citreum*		2 (2B)		1 (1B)		3 (3B)
	Leu. lactis	5 (2A+3B)	13 (5A+8B)	3 (1A+2B)	2 (2B)		23 (8A+15B)
	Leu. mesenteroides subsp. *mesenteroides*	4 (4A)	29 (15A+14)	19 (10A+9B)	8 (5A+3B)	1 (1B)	61 (28.6%.) (34A+27B)
Streptococcus (28 strains)	*S. thermophilus*	20 (1A+19B)	1 (1A)	7 (3A+4B)			28 (5A+23B)
Weissella (1 strain)	*W. cibaria*			1 (1B)			1 (1B)
	Total	55 (20A+35B)	59 (33A+26B)	71 (40A+31B)	27 (14A+13B)	1 (1B)	213 (107A+106B)

E Enterococcus, *Lb Lactobacillus*, *Lac Lactococcus*, *Leu Leuconostoc*, *S Streptococcus*, *W Weissella*, A isolated on MRS agar, B isolated on M17 agar

[a] The number of samples are indicated in brackets

In this study, a greater number of species of LAB were identified than reported in previous studies (Duan et al. 2008; Gawad et al. 2010; Sun et al. 2010; Zhang et al. 2008) highlighting the fact that yak milk products in the Sichuan province contain a wide diversity of LAB species, and this archaic dairy product could be considered as a good resource for the isolation of LAB and probiotic strain selection.

3.3 Distribution of LAB in yak milk products

The distribution of the isolates in yak milk samples is shown in Table 2. *S. thermophilus* were the predominant LAB population in kurut samples. *Leu. mesenteroides* subsp. *mesenteroides* were the predominant population in raw milk samples. *Leu. mesenteroides* subsp. *mesenteroides* and *Lb. helveticus* could be considered as predominant population in qula cheese and whey samples. Also, the number of LAB species isolated from each product type is different: 9, 11, 11, 10, and 1 species of LAB for the five product types (20 kurut, 15 raw milk, 18 qula cheese, 10 whey, and 1 butter), respectively. Therefore, a wide diversity of LAB was obtained from different yak

milk products. These results further verified that these different yak milk products, including the raw milk or naturally fermented products, represent a useful and diverse resource for LAB.

LAB have a long history of safe use in fermented foods, especially in fermented dairy products (Wang et al. 2008). Actually, in raw yak milk and traditional milk products, such as cheeses, kurut, yogurt fermented milks in the pastoral area, LAB are naturally present or intentionally added for technological reasons or to generate a health benefit for the consumer (Wu et al. 2009). This paper firstly systematically analyzed the composition of LAB in various yak milk products in the Sichuan province of China, and the isolates identified may provide a useful resource for further studies involving the design and selection of starter cultures with potential for the development of commercial traditional fermented milk products.

Nucleotide sequence accession numbers HM058517–HM058580, HM058582–HM058716, HM217971–HM217975, HM217984–HM217986, and HM217994–HM217999.

Acknowledgments This research was supported by National Key Technologies R and D Program (grant no. 2009BADC1B01), the Innovation Research Team Development Program of Ministry of Education of China (grant no. IRT0967), the Earmarked fund for Modern Agro-industry Technology Research System (grant no. nycytx-0501), the Prophase Research Program of 973 Project of China (973 Program) (grant no. 2010CB134502), the Natural Science Foundation for Open Projects of Inner Mongolian (grant no. 20102010), Innovation Research Team of Inner Mongolia Agricultural University (grant no. NDTD2010-2) and the Natural Science Foundation of Inner Mongolian (grant no. 2011MS1205).

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