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## Isolation and identification of yeast in yak milk dreg of Tibet in China

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**Abstract** Yak milk dreg is a popular dairy product with a unique flavor which is frequently consumed in Tibet. The aim of this study was to investigate the occurrence and distribution of yeasts in yak milk dreg of Tibet. Ninety-one yeast colonies were

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isolated from ten yak milk dreg samples, and the isolates belonged to seven genera and ten different species based on the phenotypic characterization and analysis of ITS/5.8S ribosomal DNA (rDNA) gene. The distribution of each species among ten samples was as follows: *Kluyveromyces lactis* (6/10), *Pichia fermentans* (6/10), *Saccharomyces cerevisiae* (6/10), *Rhodotorula mucilaginosa* (6/10), *Kluyveromyces marxianus* (5/10), *Pichia kudriavzevii* (4/10), *Torulasporea delbrueckii* (2/10), *Torulasporea quercuum* (2/10), *Debaryomyces hansenii* (1/10), and *Cryptococcus albidus* (1/10). The present study reports for the first time the yeast profile in yak milk dreg products of Tibet in China, which provides useful information and yeast strain resource for further studies involving probiotic strain selection and starter culture design for the industrial production of yak milk dreg.

**Keywords** Yak milk dreg · Yeast · Isolation · Identification · Tibet

## 1 Introduction

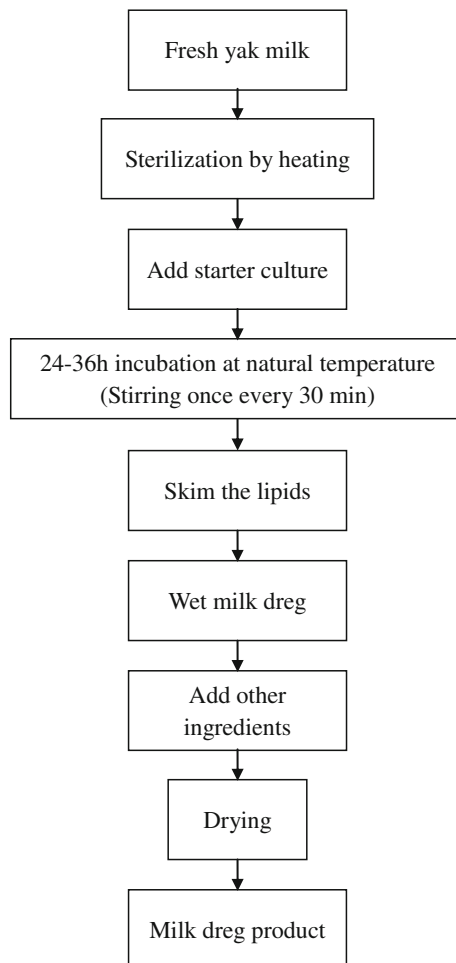
The Tibetan Plateau is located in the northwest of China belonging to the Tibetan Autonomous Region. With an average altitude of 4,000 m, it is called “the roof of the world.” The complex and highly variable topography is responsible for the distinctive altiplano climate, which is dry, has low temperature, lacks of oxygen, has low pressure, has strong sunlight, and has long exposure to the sun’s radiation (Yang and Zheng 2004). Because of the specific environment in the region, dairy products play a quite important role in Tibetan diet. The nomads of the Tibetan Plateau have been making various types of fermented dairy products using traditional methods without addition of any starters. These products are usually fermented by natural microorganism. Thus, the dairy products microflora is largely determined by the local environmental conditions (Bao et al. 2012).

Yak is a specific type of cattle living in the Qinghai-Tibetan Plateau and its surrounding regions. Compared with cow milk and goat milk, yak milk has a higher content of fat, protein, lactose, and essential minerals as well as healthy polyunsaturated fatty acids such as conjugated linoleic acid and  $\omega$ -3 fatty acids (Li et al. 2011). To extend the shelf life of yak milk, local herdsman have been making various kinds of traditional fermented yak milk products using empirical knowledge, such as Qula, milk dreg, butter, whey, etc. Yak milk dreg is a traditional fermented milk product in Tibet which is grainy, hard, yellow or white, and sweet–sour. It is prepared by fermentation of yak milk in a custom-made big jar for 1–2 days at ambient temperatures to produce acidity, alcohol, and flavor to desired level, and fat globules rise to the surface forming a cream layer. This cream is removed and domestically consumed, marketed, or manufactured into butter. Then, the fermented milk could be used for the preparation of milk dreg by filtrating and drying with the addition of slight sugar (Fig. 1). It is a kind of highly nutritious food rich in casein and has become a very popular dairy product in Tibet with a considerable increasing demand for its consumption due to its good taste and easy storage.

Earlier studies have shown that lactic acid bacteria (LAB) and yeasts are the predominant microorganisms in traditional fermented milk products (Gadaga et al. 2001). LAB have been widely reported to play a major role in fermentation and are crucial to the intrinsic properties of the fermented products, which influences the

development of the aroma, texture, and acidity of the product (Sodini et al. 2002). Yeast is also a major constituent of the microbiota of many types of fermented dairy products (Wouters et al. 2002; Bai et al. 2010). Many previous studies have shown that yeasts may contribute to the sensory characteristics of kurut, koumiss, and other fermented milk products by producing aromatic components with the capability of utilizing lactic acid as well as their proteolytic and lipolytic activities (Fleet 1990; Leclercq-Perlat et al. 2007). Moreover, the yeasts may have a beneficial influence on the growth of bacteria by increasing the pH (Sudun et al. 2013).

Thus, the isolation and identification of yeasts associated with fermented dairy products attract people's much attention. Various types of yeasts from different fermented dairy products, such as kefir, koumiss, and cheese, have been frequently reported (Bai et al. 2010; Gadaga et al. 2001). However, information on yeast in Tibet yak milk dreg has never been reported so far. The aim of this study was to investigate the distribution of yeast species in yak milk dreg collected from Tibet to provide useful information for the further industrialized production of yak milk dreg.



**Fig. 1** The traditional procedure for yak milk dreg preparation

## 2 Materials and methods

### 2.1 Collection of samples

Ten samples of yak milk dreg were offered by yak milk dreg cooperatives of Ngarzhag County. The procedure for the traditional preparation of the products was presented in Fig. 1. Each sample was aseptically transferred to a 100-mL sterile centrifuge tube, transported immediately to the laboratory, and kept in refrigerator at 4 °C before analysis.

### 2.2 Enumeration and isolation of yeasts

Each yak milk dreg sample (10 g) was homogenized with 90 mL of sterile 0.85% w/v physiological saline to make an initial dilution ( $10^{-1}$ ). Then tenfold serial dilutions ( $10^{-2}$ – $10^{-7}$ ) were prepared and 1 mL of aliquots of the appropriate dilution were inoculated into Rose Bengal agar (Landbridge, Beijing, China) plate. The plate was incubated at 28 °C for 4 days for the enumeration of yeast by colony appearance and observation of cell morphology.

For yeast isolation, 0.1 mL of each dilution was spread on YPD agar (Difco, Sparks, USA) plate, then incubated at 28 °C for 48 h. Colonies showing different appearance were randomly selected on the basis of colonies morphological differences, such as color and shape, examined microscopically, and further purified by successive streaking on YPD agar. The purified isolates were maintained at –20 °C in YPD broth containing 20% (v/v) glycerol.

### 2.3 Conventional identification

All the yeast isolates were tested by the conventional method described by Kurtzman and Fell (1998). The conventional identification included morphology, assimilation of and growth on carbon sources and nitrogen sources, as well as temperature and glucose concentration test.

### 2.4 Molecular identification

The molecular identification of the yeast strains was performed by DNA sequencing of the ITS/5.8S ribosomal DNA (rDNA). Genomic DNA was extracted using E.Z.N.A. Yeast DNA kit (Omega Bio-tek, USA) according to the manufacturer's instructions. The universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used for the amplification of 5.8S-ITS region. PCR amplification was performed in a 9600 PCR Thermo Cycler (HEMA, Zhuhai, China), using 50 µL of PCR mixture containing 0.2 µL of Taq polymerase (5 U.µL<sup>-1</sup>) (TAKARA, China), 5 µL of 10×PCR buffer (without Mg<sup>2+</sup>), 1 µL of dNTP (10 mmol.L<sup>-1</sup> each), 3 µL of MgCl<sub>2</sub> (25 mmol.L<sup>-1</sup>), 0.25 µL of forward primer (100 µmol.L<sup>-1</sup>), 0.25 µL of reverse primer (100 µmol.L<sup>-1</sup>), 1 µL of DNA template, and 39.3 µL of ddH<sub>2</sub>O. The PCR conditions were as follows: initial denaturation at 95 °C for 2 min, followed by 33 cycles of 95 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min, and a final extension of 72 °C for 8 min. Based on ITS/5.8S rDNA identification, representative isolates of yeasts were selected for sequencing of D1/D2 domain of

26S rDNA encoding gene as previously described by Bai et al. (2010). In brief, a 600 bp fragment of sequence was amplified using the primers NL1 (5'-GCATATCAAT AAGCGGAGGAAAAG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3').

The PCR reaction products were checked by electrophoresis in an agarose gel (1.0%, w/v) containing 0.5  $\mu\text{g}\cdot\text{mL}^{-1}$  of ethidium bromide. The purified products were sent to BGI Sequencing Corporation (Beijing, China) for sequencing. All the data were submitted to GenBank. Then the BLASTN program was used to assemble and compare the target sequences with the sequences available in GenBank. Subsequently, the representative strains for each species were selected and imported into MEGA version 4.0 software (Center for Evolutionary Functional Genomics, The Biodesign Institute, Arizona State University, Phoenix, AZ, USA) with their related type strains to create the 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 iterations.

## 2.5 Yeast type cultures

As control, the sequences of various type strains were retrieved from databases (CBS-KNAW Fungal Biodiversity Centre, YeastIP and American Tissue Culture Collection) and used in the analyses.

## 2.6 Frequency analysis

To study the species distribution in these samples, the frequency of occurrence of each species in all samples was calculated as a proportion of the number of samples containing the species to the total number of samples (Solieri et al. 2006).

## 2.7 Statistical analysis

All experiments were performed in duplicate, and the results were expressed as the means  $\pm$  standard deviation (SD). All microbiological counts were converted to the base

**Table 1** Quantification of yeasts in different yak milk dreg samples

	Type	Yeast ( $\log_{10}\text{CFU}\cdot\text{g}^{-1}$ )
1	Wet	6.47 $\pm$ 0.06
2	Wet	6.81 $\pm$ 0.09
3	Dry	5.41 $\pm$ 0.45
4	Dry	5.01 $\pm$ 0.10
5	Dry	5.01 $\pm$ 0.34
6	Dry	4.00 $\pm$ 0.31
7	Dry	4.22 $\pm$ 0.10
8	Dry	3.55 $\pm$ 0.13
9	Dry	3.08 $\pm$ 0.20
10	Dry	3.29 $\pm$ 0.13

ten logarithm of CFUs per gram of yak milk dreg samples ( $\log_{10}\text{CFU.g}^{-1}$ ), and from these, mean values and their SD were calculated.

### 3 Results and discussion

#### 3.1 Enumeration and isolation of yeast

The yeast populations in yak milk dreg are given in Table 1. The yeast load in all samples was 3–6  $\log_{10}\text{CFU.g}^{-1}$ , which was a little more than that of Qula or cheese previously reported (El-Sharoud et al. 2009; Tan et al. 2010). The yeast population in sample 1 and sample 2 was 6.47–6.81  $\log_{10}\text{CFU.g}^{-1}$ , which was close to that (ranging from 5 to 8  $\log_{10}\text{CFU.mL}^{-1}$ ) reported in fermented milk like suusac (traditional Kenyan fermented camel milk), Gariss (traditional Sudanese fermented camel's milk products), and koumiss of the Tibetan Plateau (Mu et al. 2012; Bai et al. 2010), and the population in other samples was 3.08–5.41  $\log_{10}\text{CFU.g}^{-1}$ . The yeast populations in samples 1 and 2 were larger than that seen in samples 3–10. In fact, sample 1 and sample 2 were wet milk dregs with a water content of about 63%, while other dry samples have a water content of about 12%. The populations in dry samples were smaller than that seen in wet samples, which corresponded to the results of Fadda et al. (2004), indicating that more microflora were working in the initial fermentation of yak milk dreg. The yeasts in dry samples 8, 9, and 10 occurred in an extremely low population (about 3  $\log_{10}\text{CFU.g}^{-1}$ ) because these three samples had been stored for months before collection and analysis, resulting in lower water content. The lower values of yeast counts could be explained by the faster decreasing of water activity ( $a_w$ ) values due to the drying process (Fadda et al. 2004).

#### 3.2 Identification and characterization of yeast isolates

Since yeasts are ubiquitous in our environment, a broad spectrum of yeasts was, as expected, found in the yak milk dreg samples analyzed. A total of 91 yeast colonies were isolated from the ten samples. All isolates were identified to species level based on phenotypic characterization and analysis of ITS/5.8S rDNA gene. The identification revealed ten species, including *Kluyveromyces lactis* ( $n=15$ ), *Kluyveromyces marxianus* ( $n=11$ ), *Pichia fermentans* ( $n=19$ ), *Rhodotorula mucilaginosa* ( $n=13$ ), *Saccharomyces cerevisiae* ( $n=18$ ), *Cryptococcus albidus* ( $n=1$ ), *Pichia kudriavzevii* ( $n=8$ ), *Debaryomyces hansenii* ( $n=1$ ), *Torulaspora delbrueckii* ( $n=2$ ), and *Torulaspora quercuum* ( $n=3$ ) (Table 2). Most ( $n=77$ ) isolates belonged to ascomycetous yeasts, while only 14 isolates were of basidiomycetous origin. The products obtained by the amplification of the ITS/5.8S rDNA region with primers ITS1 and ITS4 varied in size between 366 and 843 bp. In fact, in most cases, species of the same and related genera showed similar sizes for the amplified ITS/5.8S rDNA region. All isolates shared similarities from 99% to 100% with the sequences available in GenBank. The representative strains and their related type strains were chosen to constitute a phylogenetic tree by the MEGA software (Fig. 2). The exact lengths of the final alignment in the phylogenetic tree for *K. lactis*, *K. marxianus*, *S. cerevisiae*, *P. fermentans*, *P. kudriavzevii*, *D. hansenii*, *T. delbrueckii*, and *T. quercuum* were 719,

721, 849, 444, 507, 639, 798, and 796 bp, respectively, while those for *R. mucilaginosa* and *C. albidus* were 616 and 621 bp, respectively. As shown in Fig. 2, all representative strains grouped together with the corresponding type strain. Then 23 representative isolates from different species were selected for sequencing of D1/D2 domain of 26S rDNA encoding gene sequencing. The resulting sequences were aligned with related sequences available in the GenBank database. The identification of all the isolates based on the analysis of ITS/5.8S rDNA gene was confirmed by D1/D2 domain of 26S

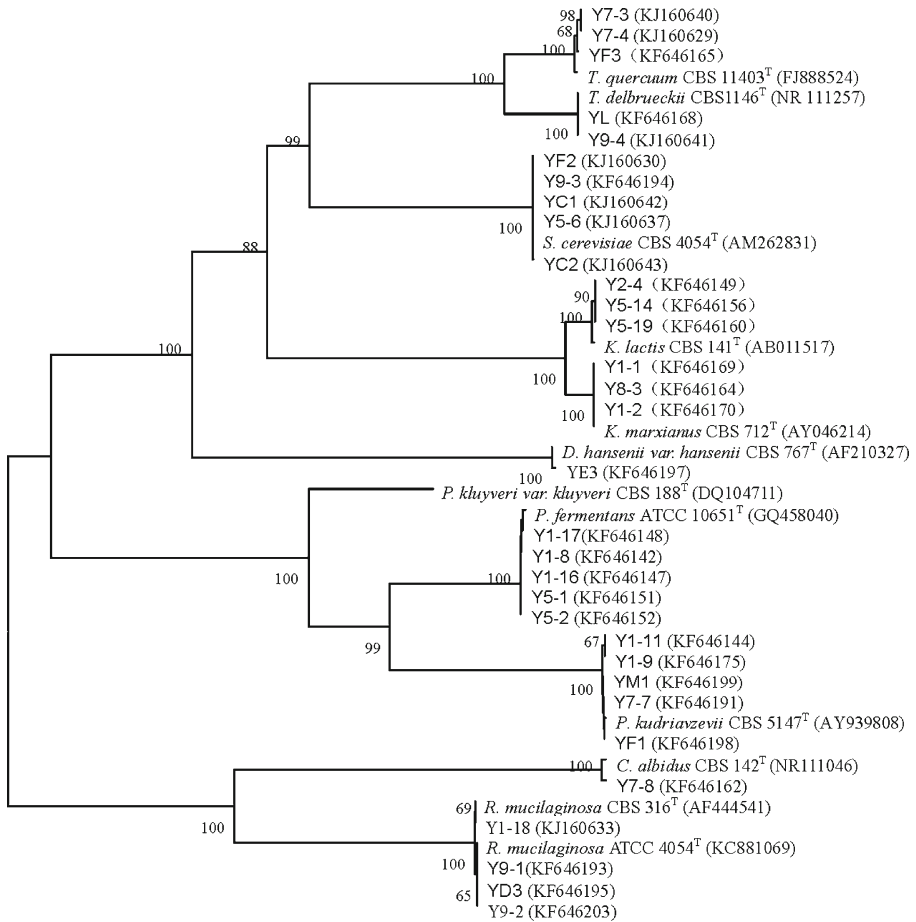
**Table 2** Physiological characteristics of yeasts isolated from yak milk dreg of Tibet

Groups	A	B	C	D	E	F	G	H	I	K
Number of isolates	19	18	15	13	11	8	2	3	1	1
Growth										
At 30 °C	19	18	14	13	11	8	2	3	1	1
At 37 °C	11	17	0	5	11	8	0	0	0	0
At 42 °C	2	7	0	0	6	8	0	0	0	0
On 50% D–G	1	0	15	0	1	0	2	2	1	0
On 60% D–G	0	0	0	0	0	0	0	0	0	0
Fermentation										
Glucose	18	18	15	0	10	8	2	3	1	0
Maltose	2	8	15	0	1	0	2	0	1	0
Sucrose	3	9	15	0	9	0	2	3	0	0
Melibiose	1	8	1	0	0	0	1	2	0	0
Lactose	2	0	15	0	9	0	0	0	0	0
Raffinose	2	9	15	0	6	0	0	1	1	0
Assimilation										
Arabinose	0	0	0	13	0	0	0	0	0	1
Xylose	19	0	15	13	0	0	0	1	1	1
Xylitol	1	17	8	0	0	0	1	1	1	0
Galactose	2	17	15	13	9	0	0	0	1	0
Sorbitol	0	0	15	13	0	0	1	2	1	0
Cellobiose	0	1	15	0	1	0	0	0	1	1
Lactose	1	0	15	0	10	0	0	0	0	0
Maltose	2	18	15	13	0	0	1	2	1	1
Sucrose	2	18	15	13	11	2	2	3	1	1
Melezitose	0	9	15	13	0	0	1	1	0	1
Raffinose	2	18	15	13	11	0	2	3	1	0
Melibiose	0	0	13	0	0	0	0	1	0	1
Ethanol	19	2	14	5	8	8	2	3	0	0
Mannitol	0	1	0	13	1	0	1	2	1	1
Succinic acid	17	2	15	13	5	8	0	0	0	1

Values represent the number of positive strains

D–G D-glucose, A *Pichia fermentans*, B *Saccharomyces cerevisiae*, C *Kluyveromyces lactis*, D *Rhodotorula mucilaginosa*, E *Kluyveromyces marxianus*, F *Pichia kudriavzevii*, G *Torulaspota delbrueckii*, H *Torulaspota quercuum*, I *Debaryomyces hansenii*, K *Cryptococcus albidus*





**Fig. 2** Neighbor-joining tree showing the phylogenetic relationships among isolates and the type strains of related genera based on ITS/5.8S rDNA sequences. Numbers at the branch nodes are the bootstrap values percent ages (1,000 replicates)

rDNA encoding gene sequencing (Table 3). All the sequences were submitted to GenBank with the accession numbers as KF646142–KF646211, KJ160625–KJ160644, and KJ183057 for the ITS/5.8 rDNA sequences and KJ183036–KJ183056 and KF646225–KF646226 for the D1/D2 sequences.

Study on the microbiota of dairy products has been traditionally relied on culturing and physiological tests, which could lead to a large degree of ambiguity, thus the results may not always reflect their real ecosystems. Therefore, in this study, the experimental results obtained were compared with the characteristics of the type strain (Kurtzman and Fell 1998). Table 2 showed the physiological and biochemical characteristics of culturable yeast strains in yak milk dreg from the Tibetan Plateau, and the results were found to be part of the false positive and negative results. However it did not affect our finding that the predominant yeasts, such as *S. cerevisiae*, *P. fermentans*, *K. marxianus*, and *K. lactis* can ferment glucose to produce carbon dioxide. *K. lactis* and *K. marxianus* were the major lactose-fermenting yeasts. *P. kudriavzevii* isolates could not use the majority of sugar, such as maltose, sucrose, lactose, and galactose. Some

**Table 3** Identification of the representative yeast isolates by analysis of the D1/D2 domains of 26S encoding gene

Representative strains	Type strains	Identity (%)	Gaps	Substitutions
Y1-16	<i>P. fermentans</i> CBS 187	99.6	0	2
Y5-1	<i>P. fermentans</i> CBS 187	99.6	0	2
Y7-9	<i>P. fermentans</i> CBS 187	99.6	0	2
YC1	<i>S. cerevisiae</i> NRRL Y-12632	100	0	0
YC2	<i>S. cerevisiae</i> NRRL Y-12632	100	0	0
YF2	<i>S. cerevisiae</i> NRRL Y-12632	100	0	0
Y2-4	<i>K. lactis</i> CBS 141	99.6	0	2
YM2	<i>K. lactis</i> CBS 141	100	0	0
Y1-18	<i>R. mucilaginosa</i> CBS 316	100	0	0
Y9-2	<i>R. mucilaginosa</i> CBS 316	100	0	0
YD3	<i>R. mucilaginosa</i> CBS 316	100	0	0
Y1-1	<i>K. marxianus</i> CBS 712	100	0	0
Y8-3	<i>K. marxianus</i> CBS 712	100	0	0
Y1-9	<i>P. kudriavzevii</i> CBS 5147	100	0	0
Y1-11	<i>P. kudriavzevii</i> CBS 5147	100	0	0
Y7-7	<i>P. kudriavzevii</i> CBS 5147	100	0	0
Y7-3	<i>Torulaspora sp.</i> CBS 11403	100	0	0
Y7-4	<i>Torulaspora sp.</i> CBS 11403	100	0	0
YF3	<i>Torulaspora sp.</i> CBS 11403	100	0	0
Y9-4	<i>T. delbrueckii</i> CBS 133	100	0	0
YL	<i>T. delbrueckii</i> CBS 133	100	0	0
Y7-8	<i>C. albidus</i> ATCC 10666	98.8	0	7
YE3	<i>D. hansenii</i> CBS 767	100	0	0

gas is mainly produced by predominant strains from fermentation process, while most yeast such as *S. cerevisiae* and *K. lactis* had the ability to assimilate galactose. Rohm et al. (1992) have reported the unreliability of the biochemical and physiological tests in the identification of yeasts from milk products by discussing the environmental selection mechanism and associations of yeast product. Besides, many tests used for characterizing ascomycetous yeasts are not suitable for identification of basidiomycetous yeasts, and the standard phenotypic tests applicable for one group of yeasts may not always useful for other groups. However, information on the biochemical activity and physiological properties is still useful in characterizing yeasts of biotechnological significance, especially in differentiating strains which are involved in the processes of fermentation.

Molecular methods allow a relatively fast and exact identification of the isolates and have formed the basis of current taxonomy of yeasts over recent years. However, physiological tests are still important as an assistant tool in the identification of the isolates. In this study, implementation of a polyphasic approach based on the sequence analysis of ITS/5.8S rDNA and phenotypic studies may represent a reliable diagnostic system for foodborne yeasts.

3.3 Frequency percentage analysis

Table 4 showed the frequencies of specific strains present in all the samples. Ten yeast species were isolated from the eight dry samples, while the wet yielded only four species. *K. lactis*, *P. fermentans*, *S. cerevisiae*, *R. mucilaginosa*, and *K. marxianus* were the most common culturable species for all samples with frequencies of 60%, 60%, 60%, 60%, and 50%, respectively. We could see that the most frequent yeast species contained both non-lactose-fermenting yeasts (like *S. cerevisiae* and *P. fermentans*) and lactose-fermenting yeasts (such as *K. marxianus* and *K. lactis*). The predominance of *K. marxianus* and *K. lactis* strains in the present study is consistent with results presented elsewhere: Rahman et al. (2009) reported on the frequent occurrence of these two species in fermented camel milk; Bai et al. (2010) indicated the dominance of strains in fermented milk collected from Tibet. The strong ability of *K. marxianus* and *K. lactis* to utilize lactose is considered to be one of the key properties contributing to their growth in dairy products (Fleet 1990), and these species probably participate in the initial milk fermentation together with the lactic acid bacteria. Besides, *K. marxianus* has been reported to contribute to flavor with the production of ethanol and acetaldehyde (Welthagen and Viljoen 1998). *P. fermentans* and *S. cerevisiae* have also been isolated from a great number of fermented milk products in previous studies (Aponte et al. 2010). *R. mucilaginosa* was scarce in traditional fermented milk in previous reports. It was not found in many dairy products, like koumiss in Xinjiang, Qinghai provinces (Mu et al. 2012), Inner Mongolia in China, and Domiati cheese in Egypt (El-Sharoud et al. 2009). In this study, *R. mucilaginosa* existed in the samples with a quite

**Table 4** Frequencies and distribution of yeast species isolated from yak milk dreg samples in Tibet

	Wet sample		Dry sample								Total		
	n=2	F (100%)	n=8								F (100%)	F (100%)	
			1	2	3	4	5	6	7	8			9
<i>P. fermentans</i> (19)	0	2	50	4	2	0	8	2	1	0	0	62.5	60
<i>P. kudriavzevii</i> (8)	0	0	0	0	2	0	4	1	1	0	0	50	40
<i>K. lactis</i> (15)	1	1	100	5	0	4	3	1	0	0	0	50	60
<i>K. marxianus</i> (11)	2	0	50	0	0	3	2	0	2	2	0	50	50
<i>S. cerevisiae</i> (18)	3	0	50	4	7	0	0	1	2	0	1	62.5	60
<i>R. mucilaginosa</i> (13)	0	0	0	6	2	0	1	1	0	1	2	75	60
<i>T. quercuum</i> (3)	0	0	0	0	1	0	0	0	2	0	0	25	20
<i>T. delbrueckii</i> (2)	0	0	0	0	1	0	0	0	0	0	1	25	20
<i>C. albidus</i> (1)	0	0	0	0	0	0	0	0	1	0	0	12.5	10
<i>D. hansenii</i> (1)	0	0	0	0	1	0	0	0	0	0	0	12.5	10

Values represent the number of strains isolated from the sample

n number of samples, F frequency percentage, which was calculated as a proportion of the number of samples containing the species to the total number of samples

high frequency. *R. mucilaginosa* is one of the most common species in foods, and it could produce pink or red pigments and cause discoloration of foods. It has been found to be from the environment, such as wall, floor, and the surface of equipment, in the production and mature period of cheddar cheese (Suzzi and Schirone 2003). *R. mucilaginosa* may play a role in flavor development, but the species has also been reported to cause spoilage in dairy products such as cheese, yoghurt, butter, and cream (Pitt and Hocking 1999). Since its presence in dairy products is supposed to be a spoilage factor, the existence of *R. mucilaginosa* does not have a functional significance in the fermentation process.

The results also showed that *P. kudriavzevii* (40%) and *T. delbrueckii* (30%) were common component of the microflora, while the frequencies of the species *D. hansenii* and *C. albidus* were quite low (10% for each). Wu et al. (2009) reported the frequent occurrence of *K. marxianus*, *S. cerevisiae*, *R. mucilaginosa*, and various *Torulaspora* species from dairy products. In a study by Aponte et al. (2010) on buffalo mozzarella cheese, *K. marxianus* and *S. cerevisiae* were the most frequently isolated yeast species followed by seven other species. Previous work has reported that *T. delbrueckii*, *P. fermentans*, and *P. kudriavzevii* are usually used in cheesemaking because of their metabolic capabilities (including proteolytic and lipolytic activities) (Spinnler et al. 2001). *D. hansenii* had been found in the Turkish white cheese with quite large quantities, and it was considered to have the potential to be used in the production of cheese in Turkey (Yalcin and Ucar 2009; Corbaci et al. 2011), but in the present study, only one *D. hansenii* was isolated from dry sample 4, indicating that it might slightly affect the fermentation of yak milk dreg. Besides, *C. albicans* was also detected with only once in one sample (sample 8). As a kind of pathogenic yeast, *C. albicans* has been rarely reported in dairy products. Though the source of the pathogenic yeasts in dairy products is not well known, it may be present during storage and contributes to the decreased sensory appeal with many other biochemical and microbiological factors (Roostita and Fleet 1996). This indicates that the traditionally fermented milk products can be a source of pathogenic microorganisms. So, it emphasizes the necessity of developing starter cultures to produce safer fermented milk products.

### 3.4 Distribution of yeast in yak milk dreg

As showed in Table 4, there were different degrees of yeast diversity in wet and dry samples. For the wet samples, only *K. lactis*, *P. fermentans*, *S. cerevisiae*, and *K. marxianus* were present. Sample 1 contained three species, while sample 2 only had two species. Among the dry samples, *R. mucilaginosa* was the most frequently isolated species, which was found in six out of the eight investigated samples. *P. fermentans* and *S. cerevisiae* were both isolated from five out of eight samples, while *P. kudriavzevii*, *K. lactis*, and *K. marxianus* were found in four samples. *T. quercuum*, *T. delbrueckii*, and *D. hansenii* all exist in sample 4, while *C. albidus* was only found in sample 8. The dry samples contained more diverse yeast floras compared with the wet samples. Considering the yeast populations, we assumed that the species *K. lactis*, *P. fermentans*, *S. cerevisiae*, and *K. marxianus* were the main microorganism involved in the initial milk fermentation processing of yak milk dreg. With the decrease of water content, most yeast were hard to

exist; therefore, in the dry samples, especially in samples 9 and 10, fewer yeast types and smaller populations were observed. However, the samples 3–8 had more species, especially sample 4 contained seven species, and sample 8 was found to have six species. Samples 3, 6, and 7 were all examined to have five species, while those of sample 5 were relatively few. In fact, the six samples were all obtained after the drying for 1–2 days from the wet yak milk dreg. So, this could be attributed to the further processing of wet yak milk dreg used for the making of final dry yak milk dreg, which involved few days of drying in air. Such conditions may allow additional yeasts to gain access to milk dreg and then form the final product (El-Sharoud et al. 2009).

Natural fermented milk products in different regions have their own specific properties. In this study, we isolated ten species of yeast; however, the species *Candida* spp. (like *Candida catenulate*, *Candida laurentii*, and *Candida zelanooides*) and *Trichosporon* spp. (like *Trichosporon beigellii* and *Trichosporon cutaneum*) which had been detected in many dairy products among Sardinia, Turkey, and other regions of Qinghai-Tibet Plateau were not detected in the present study (Corbaci et al. 2011; Fadda et al. 2004; Bai et al. 2010). Similar differences were also reported in other dairy products. For instance, the yeast population in traditional fermented cow's milk from Zimbabwe is significantly different from that of samples from different places (Gadaga et al. 2001). The differences in the yeast flora could be attributed to variations in the specific environmental conditions. Geographic location and climate had a quite important impact on the population and diversity of yeast in traditional fermented milk products. Besides, it is suggested that different kinds of milk sources were much more important than geographic varieties for microbial composition diversity in traditional fermented dairy products (Watanabe et al. 2008). The composition of fermented milk might also be influenced by preprocessing or fermentation processing, because the local habitants still adopt the diverse empirical methods of making fermented yak milk in Tibet (Wu et al. 2009).

#### 4 Conclusion

Ninety-one strains of yeast were isolated and identified by a combination of traditional and molecular techniques. *K. lactis*, *P. fermentans*, *S. cerevisiae*, *R. mucilaginosa*, and *K. marxianus* were the most common culturable species. It may suggest that the home-made milk product could be considered as a good resource for the isolation of yeast and useful for probiotic strain selection. Further studies are necessary to obtain better knowledge about the participation of these indigenous yeasts in yak milk dreg formation and to discover the species and strains that mainly affect the production and final quality of yak milk dreg. Our study provides basic data on the yeast flora associated with yak milk dreg and could be useful for developing high-quality and large-scale industrial production of yak milk dreg.

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