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Identification of yeasts isolated from Chinese light cream in Inner Mongolia

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Abstract

Aim: This study was carried out to identify twenty-one yeast strains isolated from Chinese light cream in Inner Mongolia.

Methodology: The morphological, physico-chemical characterization of yeast strains were performed. ID 32 C system was used to examine the carbohydrate fermentation. The 26S rDNA sequencing of yeasts were amplified by the primers (26SrDNA1: 5' GCA TAT CAA TAA GCG GAG GAA AAG 3'; 26SrDNA2: 5' GGT CCG TGT TTCAAGACG G 3').

Results: The phylogenetic tree was constructed. According to the results of BLAST in NCBI , twenty-one yeasts were identified as *Kluyveromyces marxianus* (9), *Pichia kudriavzevii* (8), *Saccharomyces cerevisiae* (3) and *Kluyveromyces lactis* (1) according to 26S rDNA representative amplicons sequencing. *Kluyveromyces marxianus* and *Pichia kudriavzevii* were the main yeast strains present in light cream.

Interpretation: The Chinese light cream is a traditional milk product popular in Mongolia. Some researchers pay more attention on the lactic acid bacteria, but few reports on the yeast in it. In traditional fermentation method, yeast is also contributed to the flavor of light cream. So we identified the yeast of it.

Key words : 26S rDNA, Light cream, Inner Mongolia, Yeast

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Introduction

Light cream is a popular traditional milk product in Mongolia and Xinjiang, China. Dairy products such as light cream and sour milk are usually made by traditional fermentation methods at home of Inn Mongolia. Milk is usually used to make sour milk, light cream, milk wine and other dairy products.

Recently, many yeasts have been isolated from different fermented food, such as Koumiss and cachaca (Padilla *et al.*, 2016; Mu *et al.*, 2012; Meririana *et al.*, 2009; Xiao and Yuqing, 2018). *Kluyveromyces lactis* and *Torulaspora delbrueckii* have been isolated from the canastra cheese production (Rafaela Pereira Andrade *et al.*, 2017; Sedaghatthor and Shahrajil, 2018). In Thailand, few *Candida* and *Pichia* strains have been isolated from yeast starter doughs for Chinese steamed buns (Naphatrapi, *et al.*, 2009; Zhao and Li, 2018). Most yeasts are isolated from wine (Barrajon *et al.*, 2009; Gonzalez, *et al.*, 2007; Rosalinda *et al.*, 2010; Mohammed, 2018). Recently, from dairy products have been isolated and identified *Kluyveromyces marxianus*, *Pichia fermentans*, *Pichia cactophila*, *Candida zylanoieds* were isolated form Koumiss in Inner Mongolia and Xinjiang (Xiaoxu *et al.*, 2016; Sultana *et al.*, 2018). With the development of genomics and bioinformatics, the classification of yeast is not only based on their morphological characteristics and physiological and biochemical characteristics. The 26S sequence analysis method is commonly used for the identification of yeast, and it can be more accurate to analyze the distribution of yeast strains (Visintin *et al.*, 2016; Brilhante *et al.*, 2016; Ying *et al.*, 2018).

We analyzed some lactic acid bacteria from traditional Chinese fermented food before. Some yeasts were isolated from the the Chinese light cream. We thought the yeasts maybe also give reference good taste and smear performance. So in this paper we analyzed their variety.

Materials and Methods

Sample collection: Twenty-one samples were collected from traditional light cream which were made by traditional fermentation methods in Inner Mongolia. The light cream was collected into a sterilized centrifuge tube to send to the Key Lab of Dairy Science after sealing well. All the samples were kept at -20°C for isolation.

Isolation of yeasts: Ten gram of each sample was resuspended in 90ml of sterile NaCl solution (0.9%) and homogenized three times. The suspension was diluted tenfold in NaCl solution (0.9%), and 100µl dilution were spread on the surface of YPD plates to incubate at 28°C for five days. The appearance of colony was observed, single colonies of yeasts were purified by streak plating on the YPD agar (1% yeast extract, 2% peptone, 2% glucose and 2% agar).

Phenotypic characterization: Purified yeast were inoculated into YPD broth separately and incubated at 28°C for five days. ID 32C tests were used for investigating the physico-chemical characterization of yeast by reacting with different sugars (BioMérieux, USA). The isolates were inoculated into suspension medium, 250µl mixed medium was extracted into C Medium of ID 32C test box, then 135µl mixture medium was added into each

Table 1 : Identification carbohydrate fermentation of yeast (+ means it could ferment this carbohydrate)

	GAL	ACT	SAC	NAG	LAT	RAF	MAL	SOR	XYL	GLY	MAN	LAC	GLU
S-1				+	+					+			+
S-2	+	+	+	-	+	+	0	+	?	0	+	+	+
S-3	+	+	+	0	+	+	0	+	+		+	+	+
S-4	+	+	+	0	+	+	0	+	+	0	0	+	+
S-5	0	0	0	0	+	0	0	0	0		0	0	+
S-6	+	+	+	0	+	+	0	+		0	+	+	+
S-7		0	0	+	+	0	0		0		0	0	+
S-8		+	+	0	+	+	0	+		+	+	+	+
S-9	0	0	0	0	+	0	0	+		+		0	+
S-10	+	+	+	0	+	+	0	+				+	+
S-11	+	+	+	0	+	+	0	+	+		+	+	+
S-12	+	+	+	0	+	+	0	+			+	+	+
S-13	+	0	+	0	0	+	+	0	0	0	0	0	+
S-14					+					+			+
S-15	+	+	+	0	+	+	0	+	+			+	+
S-16	+	+	+	0	+	+	0	+		0	+	+	+
S-17	+	0		0			+	0	0	0	0	0	+
S-18	+	+	+	0	+	+	0	+	+	0	+	+	+
S-19	+	+	+	0	+		0			+		+	+
S-20	+	0	0	+	+	0	0	+				0	+
S-21	+				+		+						+

Fig. 1 : Phylogenetic tree based on 26S rDNA D1/D2 domain sequence alignment. All bootstrap percentages over 50% are shown.

cupule of ID 32C incubated for 24 -48 hrs at 30°C. The reaction results were identified by ATB machine.

Identification of yeasts: The D1/D2 domains of 26S rDNA encoding gene were amplified by the primers (26SrDNA1: 5' GCA TAT CAA TAA GCG GAG GAA AAG 3'; 26SrDNA2: 5' GGT CCG TGT TTC AAG ACG G 3') (Fell *et al.* 2000). Amplified PCR of 26S rDNA products were purified. The sequence was tested by Sangon Biotech (Shanghai). The sequences obtained were aligned with known 26S rDNA gene sequences procured from the Genbank by BLAST. The generated percent homology scores

were used for the identification of yeast isolations. The sequences were aligned, and phylogenetic tree was constructed by neighbor joining method using Mega Software (version 5.0).

Results and Discussion

ID32C is the most commonly used identification system for yeast. It can identify the use of yeast for different carbohydrates, with high accuracy, but limited identification. Therefore, it is necessary to identify it with 26S (Fell *et al.*, 2000). The examination of carbohydrate fermentation were tested by the

Table 2 : Identification of 26S rDNA of yeast isolates

Strain	Identification	Homology%
S-1-5-6-7-9-14-19-20	<i>Pichia kudriavzevii</i>	99-100
S-2-3-4-10-11-12-15-16-18	<i>Kluyveromyces marxianus</i>	100
S-8	<i>Kluyveromyces lactis</i>	99
S-13-17-21	<i>Saccharomyces cerevisiae</i>	99

ID 32C system. The main phenotypic characteristics of isolates are summarized in Table 1. All strains can ferment glucose. The differences of fermentation sugars among strains were observed for ACT, SAC, NAG, LAT, RAF, MAL, SOR, XYL, GLY, MAN, LAC. The substrates of Gal, ACT, SAC, NAG, LAT, RAF, MAL, SOR, XYL, GLY, MAN and LAC are galactosum, D- galactose, actidione, saccharose, acetylglucosamine, lactic acid, D- raffinose, D-maltose, D-sorbitol, D- xylose, , D- mannitol and D- lactose.

The 26S rDNA of isolated yeasts were determined by the nucleotide sequence of D1/D2 domains. According to BLAST results, eight strains isolated S-1, S-5, S-6, S-7, S-9, S-14, S-19, S-20 were similar to *Issatchenkia orientalis*, nine strains S-2, S-3, S-4, S-10, S-11, S-12, S-15, S-16, S-18 were similar to *Kluyveromyces marxianus*, three strains S-13, S-17, S-21 were similar to *Saccharomyces cerevisiae*. One strain S-8 was similar to *Kluyveromyces lactis* (Table 2). The phylogenetic tree showed the maximum identity of each strain which was similar to the Blast results (Fig.1). Except S-12, which was more similar to *Kluyveromyces marxianus* than *Kluyveromyces lactis* when BLAST in NCBI, however in the phylogenetic tree it was closer to S-8 which belong to *Kluyveromyces lactis*.

In this study, The yeast isolated from Inner Mongolia in china, and all isolates were identified based on physiological characteristics and analysis of 26S rDNA sequences. Most of the yeast can fermented Lactose excepted S13 and S17, but S13 and S17 can fermented maltose which means these two strains can fermented the milk well with other strains. *Kluyveromyces marxianus* and *Pichia kudriavzevii* are the major yeasts isolated from the dilute cream. *Kluyveromyces marxianus* can improve the volatile flavor components in the fermentation of dilute cream (Wang et al., 2015). More experiment will be done in the future.

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