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Separation, Purification and Identification of Excellent Yeasts from the Natural Fermented Beverage of Boza

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Abstract In this study, 40 yeasts were separated and purified from five Boza samples collected in five different areas of Xinjiang Uyghur Autonomous Region. By adopting traditional morphology observation, preliminary identification, physiological and biochemical experiment, 16 excellent strains with stable fermentation characteristics were obtained, and they were confirmed with the molecular biology identification. Final results show that 12 excellent yeast strains belong to *Saccharomyces cerevisiae*, one excellent yeast strain belongs to *Pichiamembranifaciens*, and one excellent yeast strain belongs to *Pichifermentans*.

Keywords: *Boza, yeast, separation, identification*

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1. Introduction

Fermentation is an ancient and economical food processing technique that has been transmitted from one generation to another. As a simple way of food processing and an important innovation by mankind, fermentation not only leads to prolong the self-life of a fresh producing, but also improves its nutritional value, sensory properties and functional qualities via transforming the raw materials into products by biochemical modification [1]. In this context, a great variety of indigenous spontaneously fermented cereal foods, such as Turkish boza, Bulgarian bozan, Egyptian busa, Nigerian bousa, East European brascha and Kenyan busaa, etc., have been developed in different parts of the world [2,3,4]. Traditionally, boza is often prepared in traditional domestic setting from slurry containing various cereals, such as barley, oat, millet, maize, wheat and rice semolina or flour. The manufacturing process of boza involves many steps, including preparation of materials, boiling, filtration, cooling, sugar addition and fermentation by back-sloping or naturally occurring microorganisms [5].

Boza is a kind of natural fermented beverage (without any artificial additives) with low alcohol, low sugar, thick texture and refreshing taste. Its taste depends on the production method, raw materials and starter. Its taste usually is mellow, sweet, fragrant, sweet, slightly sour, and rich in nutrition. It is a pure natural pollution-free cereal fermented beverage. Boza is a national fermented beverage with high nutritional value and economic value. It can supplement all kinds of vitamins needed for human body, and helps for digestion, accelerates blood circulation, reduces hypertension, defecate and urinate, replenishes blood, and reduces fat etc. [6]. Boza is produced in many parts of Asia, the Balkan region of Europe and Northern Africa [7,8] and it is also the one of traditional fermented beverages in Xinjiang Uyghur Autonomous Region (XUAR) of China . It is greatly appreciated and widely consumed by several minority ethnic groups in some areas of XUAR, the north-western part of China.

Benefiting from recent advances in microorganism identification, several studies on the microflora of boza have been carried out in other countries through traditional phenotype-based methods [9] as well as molecular approaches [10]. These results demonstrated that the taxonomic composition of microbiota found in boza worldwide differ significantly between regions and with respect to the used raw materials, indicating the necessity of investigating the microflora of boza produced in different regions . Although it is popular in XUAR, Boza has been marginally explored so far, and thus leads to restrict the commercialization of boza due to the lack of suitable microorganisms for industrial application. Therefore, detailed investigation of Boza is substantially important to maintain biodiversity as well as quality of boza manufactured in the different region and to find new starter cultures of interest, for both industry and future research purposes.

It is obvious that boza is a complex biological ecosystem caused by the complicated microbial composition and the interactions of different fermentation processes. We also believe that boza still remains a natural and rich reservoir of biodiversity to search for "novel" strains with technological and functional properties. With the aim of overcoming problems of commercialization and food safety, the present study was carried out to separate and

purified yeast from traditional XUAR boza samples based on morphological, biochemical characteristics and molecular biology techniques, and to search for yeast strains with the optimal technological aptitude to be applied in boza manufacture. This study might fill in gaps in existing research and provide insight into the yeast diversity of traditional boza from different regions of XUAR and its technological aptitude for application in boza making.

2. Materials and Methods

2.1. Materials

Samples: 5 samples were used in total. Four kinds of naturally fermented boza were collected from Kunes County, Tikes County, Aktu county and Ghulja City in XUAR, and one were made of corn, black millet and barley mixed with natural fermentation at the laboratory of Xinjiang Agricultural University in Urumqi. The fermentation time was 1-2 days. The prepared 5 samples were stored in 4°C refrigerator for further investigations.

Culture medium were potato glucose agar (PDA), wort agar, wort liquid medium, corn meal agar medium, hydrolyzed urea medium, ester production medium, nitrate reduction medium, litmus milk medium, yeast extract medium, carbon source assimilation basic medium, nitrogen source assimilation basic medium, etc.

The reagents used during the experiments were glucose, maltose, lactose, sucrose, fructose, D-galactose, soluble starch, raffinose, melibiose, rhamnose, trehalose, L-arabinose, d-cellobiose, citric acid, urea, ammonium sulfate, KNO3; ITS primer (PAGE specification, synthesized by Beijing Dingguo Changsheng Biotechnology Co., Ltd.), 2xmix DNA polymerase (specification: 2U / ul, Beijing Dingguo Changsheng Biotechnology Co., Ltd.) NEP025-1 Recovery Kit (50T specification, Beijing Dingguo Changsheng Biotechnology Co., Ltd.).

2.2. Methods

2.2.1 Sample dilution

Under aseptic condition, 1 ml of fully shaken sample was put into a test tube containing 9 ml sterile normal saline, and a 1:10 uniform diluent was prepared. Use the sterilized pipette with gun head to draw 1 ml of 1:10 diluent, and slowly inject it into the test tube containing 9 ml sterile normal saline along the tube wall (note that the tip of the pipette should not touch the diluent in the tube). Replace the sterilized gun head, and make 6-fold incremental diluent according to the above operation sequence, so that one sterilized pipette head is used for each increment [11].

2.2.2. Yeast Separation and Purification

The sample was diluted to 10-6 with sterile normal saline, and then 10-1, 10-2, 10-3, 10-4, 10-5, 10-6 were taken respectively, and 1 ml gradient sample was coated on potato glucose agar medium, and incubated at 28°C for 48 h. Observe the individual and colony morphology, select the yeast characteristics consistent with the purification of 3-4 times. After purification, the agar medium was used for slant culture, and keep them in refrigerator at 4°C.

2.2.3. Morphological Identification

The morphological identification of yeast strains isolated from different samples mainly includes the following five tests:

2.2.3.1. Colony morphology observation

The yeast to be tested was inoculated on PDA medium and cultured at 28°C for 24 h. The color, shape, transparency, edge uniformity, smoothness and texture of single colony were observed by naked eyes [12].

2.2.3.2. Individual morphological observation

Take a small amount of bacteria from the yeast colony of the bacteria to be tested and observe the individual cell morphology with the help of microscope [12].

2.2.3.3. Asexual propagation

The tested bacteria were inoculated on Sabouraud's slant medium and cultured at 25°C for 24 hours. After methylene blue staining, microscopic examination was performed. Observe that the reproduction mode of the tested bacteria is budding reproduction or fission reproduction [13].

2.2.3.4. Pseudohyphae observation

It was inoculated on potato glucose agar or corn meal agar medium and cultured at 25-28°C for 3-5 days [13].

2.2.3.5. Morphological observation of ascospores

The aim of this study was to determine whether the yeast purified from Boza could form ascospores and the morphology and quantity of ascospores. Cultured on Sabouraud's slant medium at 25°C for 1 day, the yeast culture with vigorous growth was inoculated on the medium suitable for ascospores, cultured at 25°C for three days, and examined by microscope. The strains without ascospores were further cultured at 20°C and examined weekly for at least 6 weeks [14].

2.3.4. Physiological and Biochemical Reactions

2.3.4.1. Urea hydrolysis test

The tested yeast was inoculated on the agar slope for urea hydrolysis test and cultured at 28°C. The results were observed every day. After 5-7 days, if the agar slope was light red, the yeast could decompose urea [14].

2.2.4.2. Fat production test

Take 20 ml fat producing medium and put it into a 50 ml triangular flask. Strain to be tested is added into the flask. Culture at 25°C - 28°C for 3-5 days. The observation results show that if there is fat fragrance, it will be positive, otherwise it will be negative [11,15].

2.2.4.3. Nitrate reduction test

The yeast with vigorous growth was inoculated in

nitrate medium and cultured at 25°C for 1-7 days. The mixture of a and B (1:1) was added dropwise, and 0.1 ml of mixed solution was added every 5 ml of culture medium After the observation, red is positive. If there is no red, further inspection should be carried out. Add a little zinc powder to the above test tube. If the red color appears, it means that nitrate still exists, which is a negative reaction (nitrate in the presence of acetic acid, zinc powder reduces it to nitrite, and further forms arylhydrazine purplish red compound); if no red color is produced, it indicates that nitrate has been Reduction to ammonia and nitrogen was still positive [13].

2.2.4.4. Milk peptization test

The new milk is boiled, cooled, and centrifuged twice to remove the upper layer of fat to obtain skimmed milk. After soaking 2.5G of pistil in 100ml distilled water overnight or longer, the litmus becomes soft and easy to dissolve, and replenish water, which is called litmus liquid. 4 ml of 2.5% litmus water solution and 100 ml of skimmed milk were mixed evenly. The prepared litmus milk should be lilac color and packed into small test tubes and sterilized. After inoculating the strain to be tested and cultured at suitable temperature for 3, 5 and 7 days, the reactions of milk acid production, alkali production, coagulation, peptization and reduction were observed and recorded [15].

2.2.4.5. Sugar fermentation test

0.6% of yeast extract was separately packed into the tube containing Duchenne tube and sterilized with 0.1 MPa for 15 min. The fresh strains were put into the fermentation liquid medium and cultured at 25°C - 28°C for daily observation. If there is gas ($CO_{2)}$ at the top of the Duchenne tube, it indicates that the bacterium can ferment some sugar (glucose, sucrose, lactose, maltose, soluble starch, etc.), which is a positive reaction, otherwise it is a negative reaction [15].

2.2.4.6. Carbon source Assimilation Experiment

Using the growth map method, take 3 ml of sterile normal saline, put the tested bacteria into it, shake well, then take 1 ml of bacterial suspension into the sterile culture dish, pour into the non carbon original basic culture medium which has been melted and cooled to 45-50°C, shake well, after coagulation, turn it upside down at 28°C for 7 h, make the surface slightly dry, and then divide 6 small areas under the culture dish with a marker pen. One plot was used as the control, and the remaining five plots were labeled with carbon source for the experiment [15].

2.2.4.7. Nitrogen source Assimilation Experiment

Melt the yeast nitrogen free synthetic medium, take 4 sterile test tubes, add 5 ml of melted culture medium into each tube, and then add the tested nitrogen source (0.78% urea and ammonium sulfate) to two of them, sterilize and make slope. The four slopes were connected with the tested bacteria (2 slant tubes without nitrogen source as control), and cultured at 28°C for one week to observe the utilization of different nitrogen sources by yeast [15].

2.2.5. Identification of excellent yeasts

2.2.5.1. Extraction of yeast genome

Select the fungi from the culture medium, remove agar as far as possible, wash with distilled water; add 200 μl plant lysis buffer, grind to powder; add 600ul plant lysis buffer, 0.8 μl β - mercaptoethanol, fully mix; then water bath at 65°C for 40min, 12000rpm, 10min centrifugation, take the supernatant, add equal volume chloroform extraction, 12000rpm, 5min centrifugation; add 700μl plant binding Buffer, fully stirring, standing on the column for 5min, then centrifuging at 12000rpm for 2min, adding 500μl 80% ethanol to wash twice, 12000rpm, 1min centrifugation, standing at room temperature for 10min, volatilizing and drying; adding 40μl H_2O, standing for 5-10min, and then changing the tube, 13000rpm, 2min centrifugation, the bottom of the tube is the genome.

2.2.5.2. Amplification of target fragment

The genomic DNA extracted from yeast (Table 1) was amplified by PCR using its 1 (5 '- TCCGTAGACC TGCGG-3') and its4 (5 '- TCCTCCCTTGATTGATGC-3') as primers.

Table 1. The genomic DNA extracted from yeast

Reaction system:			
Compo			Volume
2XMix			25μL
ITS 1(10μM)			1 μL
ITS4(10μM)			1 μL
DNA mode			2μL
ddH2O			to 50 μL
Reaction conditions:			
94°C	Pre denaturation	5 min	(1~3) 30 times repeats
94°C	Denaturation	30 s	
54°C	Annealing	30 s	
72°C	Extend	50 s	
72°C	Terminal extension	10 min	

2.2.5.3. Recovery of PCR products

2% agarose gel electrophoresis, cut the target fragment, add the recovery reagent A 300ul/0.1g, 6 0°C water bath until the glue is completely dissolved, add 50ul recovery reagent solution B, mix well, put the solution in a centrifugal column, stand for 5min, 12000rpm, 1min, discard the liquid; add 500ul 80% ethanol, 12000rpm, centrifugation for 1min, discard the liquid, repeat once; 12000rpm, centrifuge for 5min, shake off the residual liquid, discard the liquid, dry in the air for 5-8min; put the centrifugal column in a new centrifugal tube, add 30ul sterilized double steam water The purified product was obtained by centrifugation at 13000 rpm for 3 min.

2.2.5.4. Sequence analysis of amplified products

The amplified products were sent to Beijing Dingguo Changsheng Biotechnology Co., Ltd. for sequencing. The DNA sequence of the strain was blast compared with the standard sequence in NCBI database http://blast.ncbi.nlm.nih.gov/Blast.cgi The similarity was analyzed.

2.2.5.5. Phylogeny

Dnaman analysis software was used for analysis.

3. Results

3.1. Results of Morphological, Physiological and Biochemical Tests of Yeasts

The traditional morphological, physiological and biochemical methods were used to identify the yeast. The test results are shown in Table 2 and Table 3. Seven strains were separated and purified from Boza collected in Kunes County of XUAR, seven strains were isolated and purified from corn, black millet and barley in Xinjiang Agricultural University Laboratory of Urumqi, and nine strains were isolated and purified from Boza of aktu county and Tikes county. There are 9 species and 8 strains which were isolated and purified from Ghulja city Boza.

Through morphological identification of yeast, 40 yeast strains were preliminarily identified through colony morphology observation, individual morphology observation, propagation mode observation, pseudohyphae observation and ascospore formation test. The results are shown in Table 3(A). The same characteristics were found in some yeasts, such as Y1 and Y22 are the same, Y8 and Y26 are the same, Y10 and Y21 are the same, strains Y16, Y17, Y18 and Y24 are the same, Y19 and Y35 are the same, Y27 and Y29 are the same, Y20 and Y32 are the same as Y40, thus Y17, Y18, y21, Y22, Y24, Y26, Y29, Y32, Y35, Y40 are excluded. We demonstrated the results of physiological and biochemical tests in Table 3 (B,C).

Table 2. The results of the yeasts Colony and Cellular shape

No.	Colony forms										Cell forms	
	Shape	Color	Bulge	Size (mm)	Transparent	Edge	Gloss	Texture	Individual form	Mycelium	Asexal reproductin	Ascospore
Y1	Round	Milky white	Low凸	3.0-4.0	Opacity	Neat	Flashlight	Sticky	Rod	Yes	Budding	No
Y2	Round	Cream	High凸	2.5-3.5	Opacity	Neat	Yes	Sticky	Round	No	Budding	No
Y3	Round	Cream	High凸	3.0~4.5	Opacity	Neat	Yes	Sticky	Elliptical	No	Budding	No
Y4	Round	Milky white	Flat	2.5~3.5	Opacity	Sawtooth	No	Sticky	Oval	No	Budding	No
Y5	Round	Milky white	High凸	1.0~3.0	Opacity	Irregular	Yes	Sticky	Round	Yes	Budding	No
Y6	Round	Milky white	Flat	1.5~2.5	Opacity	Neat	No	Sticky	Round	No	Budding	No
Y7	Round	Milky white	High凸	2.0~3.0	Translucent	Neat	Flashlight	Sticky	Round	Yes	No	Yes
Y8	Round	Milky white	Flat	1.0~3.0	Translucent	Neat	Yes	Sticky	Elliptical	Yes	No	Yes
Y9	Round	Dark yellow	High凸	1.5~3.0	Opacity	Neat	No	Sticky	Elliptical	Yes	Budding	No
Y10	Round	Milky white	Low凸	2.0~3.5	Opacity	Neat	Yes	Sticky	Round	Yes	Budding	No
Y11	Round	Milky white	Flat	1.5~3.0	Translucent	Neat	Yes	Sticky	Elliptical	No	Budding	No
Y12	Round	Dark yellow	High凸	2.0~4.0	Opacity	Neat	Yes	Sticky	Elliptical	No	Budding	No
Y13	Round	Milky white	Low凸	2.5~3.5	Opacity	Neat	Yes	Sticky	Round	Yes	Budding	No
Y14	Round	Milky white	High凸	3.0~4.0	Opacity	Neat	Yes	Sticky	Round	Yes	Budding	No
Y15	Elliptical	Milky white	Flat	3.5~4.5	Opacity	Rough	No	Sticky	Oval	No	Budding	No
Y16	Elliptical	Milky white	Flat	1.0-1.5	Opacity	Rough	No	Sticky	Elliptical	Yes	Budding	No
Y17	Elliptical	Milky white	Flat	1.0-1.5	Opacity	Rough	No	Sticky	Elliptical	Yes	Budding	No
Y18	Elliptical	Milky white	Flat	1.0~1.5	Opacity	Rough	No	Sticky	Elliptical	Yes	Budding	No
Y19	Round	Milky white	Low凸	3.0~4.0	Opacity	Neat	Yes	Sticky	Elliptical	Yes	Budding	No
Y20	Round	Cream	High凸	3.5~4.5	Opacity	Neat	Yes	Sticky	Oval	Yes	Budding	No
Y21	Round	Milky white	Low凸	2.0~3.5	Opacity	Neat	Yes	Sticky	Round	Yes	Budding	No
Y22	Round	Milky white	Low凸	3.0~4.0	Opacity	Neat	Yes	Sticky	Rod	Yes	Budding	No
Y23	Elliptical	Milky white	High凸	1.5~4.0	Translucent	Neat	Yes	Sticky	Oval	Yes	Budding	No
Y24	Elliptical	Milky white	Flat	1.0~1.5	Opacity	Rough	No	Sticky	Elliptical	Yes	Budding	No
Y25	Round	Milky white	Low凸	1.0~2.5	Opacity	Neat	Yes	Sticky	Round	Yes	Budding	No
Y26	Round	Milky white	Flat	1.0~3.0	Translucent	Neat	Yes	Sticky	Elliptical	Yes	No	Yes
Y27	Elliptical	Milky white	High凸	2.0~3.5	Opacity	Neat	No	Sticky	Round	Yes	Budding	No
Y28	Round	Milky white	High凸	2.5~5.0	Opacity	Neat	No	Sticky	Round	No	Budding	No
Y29	Elliptical	Milky white	High凸	2.0~4.0	Opacity	Neat	No	Sticky	Round	Yes	Budding	No
Y30	Elliptical	Milky white	Low凸	1.5~3.5	Opacity	Neat	Yes	Sticky	Elliptical	No	Budding	No
Y31	Round	Milky white	High凸	1.0~2.0	Opacity	Neat	Yes	Sticky	Round	Yes	Budding	No
Y32	Round	Milky white	High凸	3.5~4.5	Opacity	Neat	Yes	Sticky	Oval	Yes	Budding	No
Y33	Round	Cream	Low凸	4.0-5.0	Opacity	Neat	Yes	Sticky	Round	Yes	Budding	No
Y34	Elliptical	Milky white	Flat	2.0-3.0	Translucent	Rough	No	Sticky	Elliptical	Yes	Budding	No
Y35	Round	Milky white	Low凸	3.0-4.0	Opacity	Neat	Yes	Sticky	Elliptical	Yes	Budding	No
Y36	Round	Milky white	Low凸	3.0~5.0	Translucent	Sawtooth	Yes	Sticky	Round	Yes	Budding	No
Y37	Round	Milky white	High凸	2.0~3.0	Opacity	Sawtooth	Yes	Sticky	Oval	Yes	Budding	No
Y38	Round	White	Flat	3.0~4.5	Translucent	Neat	Yes	Sticky	Round	No	Budding	No
Y39	Elliptical	Milky white	Flat	2.0~3.0	Translucent	Neat	No	Sticky	Elliptical	No	Budding	No
Y40	Round	Cream	High凸	3.5~4.5	Opacity	Neat	Yes	Sticky	Oval	Yes	Budding	No

Table 3 (A). The results of the yeasts biochemistry appraisal

No.	Urea decomposition test	Determination of ester production	Nitrate reduction test	Peptization test of milk	Sugar fermentation test			
					Glucose	Malt dust	Lactose	Sucrose
Y1	-	+	-	+	+	-	-	-
Y2	-	+	-	+	+	-	-	+
Y3	-	+	-	+	+	+	-	+
Y4	-	+	-	+	+	+	-	+
Y5	-	+	-	+	+	+	-	+
Y6	+	+	-	+	+	+	-	-
Y7	-	+	-	+	+	+	-	+
Y8	-	+	-	+	+	+	-	+
Y9	-	+	-	+	+	+	-	+
Y10	-	+	-	+	+	+	-	+
Y11	-	+	-	+	+	+	-	+
Y12	-	+	-	+	+	+	-	-
Y13	-	+	-	+	+	+	-	+
Y14	-	+	-	+	+	+	-	D
Y15	-	+	-	+	D	-	-	-
Y16	-	+	-	+	+	-	-	-
Y19	-	+	-	+	+	-	-	-
Y20	+	+	+	+	D	-	-	-
Y23	-	+	-	+	+	+	-	+
Y25	-	+	-	+	+	+	-	+
Y27	-	+	-	+	+	+	-	D
Y28	-	+	-	+	+	+	-	+
Y30	-	+	-	+	+	+	-	+
Y31	-	+	-	+	D	+	-	D
Y33	+	+	-	+	+	+	-	+
Y34	-	+	-	+	+	-	-	-
Y36	+	+	-	+	+	+	-	+
Y37	-	+	-	+	+	+	-	-
Y38	-	+	-	+	+	+	-	+
Y39	-	+	-	+	+	+	-	+

Note: + "means positive;" - "means negative; D means delayed fermentation.

Table 3(B). Continues

No.	Sugar fermentation test								
	Fructose	D-galactose	Soluble starch	Raffinose	Honey disaccharide	Rhamnose	Trehalose	L-arabinose	D-cellobiose
Y1	-	+	-	-	-	-	D	-	-
Y2	-	-	-	+	+	-	-	-	-
Y3	+	+	-	-	-	-	-	-	-
Y4	+	+	-	+	-	-	-	-	-
Y5	+	+	-	D	-	-	-	-	-
Y6	+	+	-	+	-	-	-	-	-
Y7	-	+	-	+	-	-	-	-	-
Y8	+	+	-	-	-	-	+	-	-
Y9	+	+	-	+	-	-	-	-	-
Y10	+	+	-	+	-	-	+	-	-
Y11	+	+	-	+	-	-	-	-	-
Y12	-	-	-	-	-	-	D	-	-
Y13	D	D	-	-	-	-	+	-	-
Y14	+	+	-	-	D	-	-	-	-
Y15	-	-	-	-	-	-	-	-	+
Y16	-	-	-	-	-	-	-	-	-
Y19	-	D	-	-	-	-	-	+	+
Y20	-	+	-	-	-	-	D	-	-
Y23	-	+	-	+	-	-	+	-	-
Y25	-	+	-	D	-	-	-	-	-
Y27	+	+	-	-	-	-	-	-	-
Y28	-	-	-	-	-	-	-	-	-
Y30	D	D	-	-	-	-	-	-	-
Y31	-	+	-	+	-	-	-	+	-
Y33	+	+	-	+	-	-	-	-	-
Y34	-	-	-	-	-	-	-	-	-
Y36	+	+	-	D	-	-	-	-	-
Y37	D	+	-	-	-	-	-	-	-
Y38	+	+	-	+	+	-	-	-	-
Y39	+	+	-	+	D	-	+	-	-

Table 3 (C). Continues

No.	Carbon source Assimilation Experiment						Experiment of nitrogen source assimilation		
	Maltose	Raffinose	Soluble starch	Glucose	Lactose	Citric acid	Urea	Ammonium sulphate	KNO_3
Y1	D	D	-	D	-	-	+	+	+
Y2	+	-	-	-	-	-	+	+	D
Y3	+	-	+	+	-	-	+	D	+
Y4	D	D	-	D	-	D	+	+	+
Y5	D	D	-	D	-	-	+	+	+
Y6	+	+	-	+	-	-	+	-	-
Y7	+	+	-	+	-	-	+	+	+
Y8	+	+	-	+	-	-	+	+	+
Y9	+	+	-	+	-	-	+	D	+
Y10	-	+	-	+	-	-	+	-	+
Y11	-	+	-	+	-	+	D	D	D
Y12	D	-	-	+	-	-	+	+	+
Y13	+	-	-	+	-	-	+	+	+
Y14	+	D	-	D	-	-	D	+	D
Y15	-	-	-	+	-	-	+	+	+
Y16	-	-	-	-	-	-	+	+	+
Y19	+	+	-	+	-	-	+	+	+
Y20	-	-	-	-	-	D	+	+	+
Y23	+	+	-	+	-	-	-	+	+
Y25	+	+	-	+	-	-	-	-	-
Y27	-	D	-	D	-	-	+	+	+
Y28	+	D	-	+	-	-	+	+	+
Y30	+	+	-	D	-	-	+	+	+
Y31	-	-	-	-	-	-	+	+	+
Y33	-	-	-	+	-	-	D	D	D
Y34	-	-	+	-	-	-	+	+	+
Y36	-	-	-	+	-	-	+	+	+
Y37	+	+	+	+	-	-	+	+	+
Y38	D	+	+	+	-	-	+	+	+
Y39	+	-	-	+	-	-	+	+	+

Note: + "means positive;" - "means negative; D means delayed fermentation. 1-16 in brackets are excellent strains

Among identified yeasts from results (Table 2 and Table 3) of the traditional morphological, physiological and biochemical characteristics, 25 strains of *Saccharomyces cerevisiae*, 1 strain of *Cryptococcus*, 1 strain of *Candida* and 3 strains of *Pichi* were identified. The colonies of y1-y14, Y23, Y25, Y27, Y28, Y30, Y31, y33, y36-y39 were milky white, milky white, cream colored and dark yellow. The colony morphology was round and oval, and the cells were round and oval. The asexual reproduction was budding. Some had ascospores, and there were 1-2 round or oval ascospores in the ASCUS. There were pseudohyphae. All of them fermented glucose and sucrose Maltose, fructose, galactose, alose, soluble starch, trehalose, different lactose, soluble starch and citric acid. They were identified as *S. cerevisiae*.

Y19, whose colony were milky white or milky white, the colony shape were round, the cell were oval or lemon shape, asexual reproduction were budding, and it can form mycelium. It did not have ascospore, fermented glucose and sucrose while did not ferment maltose, lactose, fructose, starch, raffinose, melibiose, rhamnose, trehalose, different starch, lactose, and citric acid. It were identified as *C.andida.*

The colonies of Y15, Y16 and y34 were milky white or milky white, round, oval or oval cells. The asexual propagation was budding, with pseudohyphae, no ascospores and sporozoites. They fermented glucose and did not ferment. They grew on glucose yeast juice agar with ethylamine hydrochloride, and were identified as *S.rouxii*.

Strain Y20 were milky white or cream colored, round, oval or oval cells. Asexual reproduction were budding, sometimes with pseudohyphae, without ascospores and spores. It fermented galactose, sometimes slowly, did not ferment sucrose and maltose, and did not ferment melibiose, assimilates sucrose, nitrogen source assimilates (NH4) 2SO4, KNO3; alcohols assimilate ethanol and sorbitol. Hydrolysis of urea, liquefaction of gelatin, reduction of nitrate and formation of starch like substances were all negative, and were identified as *S.kluveromyces marxianus.*

3.2. Molecular Biological Identification Results of Yeast Strains

3.2.1. PCR Amplification and Electrophoresis of Some Excellent Yeast Strains

16 strains of fine yeasts with relatively stable fermentation characteristics were selected from the purified strains for molecular biological identification, including 3 strains of excellent yeasts (Y1-Y3) screened from Boza of Kunes County, 3 strains of excellent yeasts (Y4-Y6) selected from Urumqi Boza, 2 strains of excellent yeast strains (Y7-Y8) screened from aktu, four strains of excellent yeasts (Y9-Y11 and Y16) were screened from Tekes county Boza, and 4 strains of excellent yeasts (Y12-Y15) were screened from Yili Boza. According to the PCR system and conditions in 2.3.5.2, the yeast DNA was used as template. The PCR products of the strain were tested by electrophoresis with 2% agarose gel electrophoresis. After the image was stained by ethidium bromide, the gel imaging analysis system was taken, and the results were shown in Figure 1.

It can be seen from Figure 1 that the specific fragments of some excellent yeast strains appear at the position of 750 BP, and the bands are very clear, which can be preliminarily identified as yeast. The PCR products can be stored at - 20°C for standby and can be sequenced by the sequencing company.

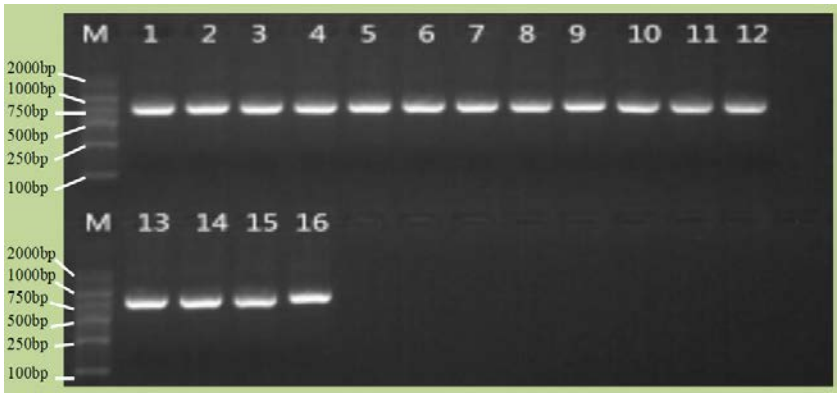

Figure 1. PCR amplified electrophoresis map of yeasts

3.2.2. DNA Sequencing Results and Storage Comparison Results of Excellent Yeast Strains

The PCR products of the amplified excellent yeast strains were sent to Beijing Dingguo Changsheng Biotechnology Co., Ltd. for sequencing. The PCR products were compared in NCBI database of international nucleic acid database, and the strains with the closest sequence were selected. The comparison results are shown in Table 4.

The results in Table 4 have shown that the similarity of the sequenced strains in the database is more than 94%, except for strains 9 and 10, which need to be further determined.

Table 4. Comparative results of excellent yeast sequencing

No.		Similarity %	Serial number	Similar strains
	2.957e+05	99	CP006441.1	*Saccharomyces cerevisiae YJM271 chromosome XII sequence*
1	2.201 e+05	99	CP006391.1	*Saccharomyces cerevisiae YJM1307 chromosome XII sequence*
	2.197 e+05	99	CP006436.1	*Saccharomyces cerevisiae YJM193 chromosome XII sequence*
	2.945e+05	99	CP006442.1	*Saccharomyces cerevisiae YJM320 chromosome XII sequence*
2	2.553e+05	99	CP006452.1	*Saccharomyces cerevisiae YJM555 chromosome XII sequence*
	2.481e+05	99	CP006430.1	*Saccharomyces cerevisiae YJM1549 chromosome XII sequence*
	2.994e+05	99	CP006441.1	*Saccharomyces cerevisiae YJM271 chromosome XII sequence*
3	2.210e+05	99	CP006391.1	*Saccharomyces cerevisiae YJM1307 chromosome XII sequence*
	2.203e+05	99	CP006436.1	*Saccharomyces cerevisiae YJM193 chromosome XII sequence*
	2.656e+05	95	CP006441.1	*Saccharomyces cerevisiae YJM271 chromosome XII sequence*
4	2.570e+05	96	CP006391.1	*Saccharomyces cerevisiae YJM320 chromosome XII sequence*
	2.228e+05	96	CP006436.1	*Saccharomyces cerevisiae YJM555 chromosome XII sequence*
	2.902e+05	98	CP006441.1	*Saccharomyces cerevisiae YJM271 chromosome XII sequence*
5	2.242 e+05	98	CP006391.1	*Saccharomyces cerevisiae YJM1307 chromosome XII sequence*
	2.105 e+05	98	CP006436.1	*Saccharomyces cerevisiae YJM193 chromosome XII sequence*
	2.845e+05	97	CP006441.1	*Saccharomyces cerevisiae YJM271 chromosome XII sequence*
6	2.242 e+05	97	CP006391.1	*Saccharomyces cerevisiae YJM1307 chromosome XII sequence*
	1.347 e+05	98	CP006467.1	*Saccharomyces cerevisiae YJM993 chromosome XII sequence*
	2.849e+05	97	CP006441.1	*Saccharomyces cerevisiae YJM271 chromosome XII sequence*
7	2.099 e+05	97	CP006391.1	*Saccharomyces cerevisiae YJM1307 chromosome XII sequence*
	1.095 e+05	97	CP006436.1	*Saccharomyces cerevisiae YJM193 chromosome XII sequence*
	2.955+05	99	CP006441.1	*Saccharomyces cerevisiae YJM271 chromosome XII sequence*
8	2.179 e+05	99	CP006391.1	*Saccharomyces cerevisiae YJM1307 chromosome XII sequence*
	1.507 e+05	99	CP006436.1	*Saccharomyces cerevisiae YJM1478 chromosome XII sequence*
	1.566e+05	84	CP006441.1	*Saccharomyces cerevisiae YJM271 chromosome XII sequence*
9	1.152e+05	84	CP006391.1	*Saccharomyces cerevisiae YJM1307 chromosome XII sequence*
	1.152 e+05	84	CP006436.1	*Saccharomyces cerevisiae YJM193 chromosome XII sequence*
	2.564+05	94	CP006441.1	*Saccharomyces cerevisiae YJM271 chromosome XII sequence*
10	1.891 e+05	94	CP006391.1	*Saccharomyces cerevisiae YJM1307 chromosome XII sequence*
	1.886e+05	94	CP006436.1	*Saccharomyces cerevisiae YJM193 chromosome XII sequence*
	1.573+05	84	CP006441.1	*Saccharomyces cerevisiae YJM271 chromosome XII sequence*
11	1.472 e+05	83	CP006424.1	*Saccharomyces cerevisiae YJM1463 chromosome XII sequence*
	1.175e+05	84	CP006391.1	*Saccharomyces cerevisiae YJM1307chromosome XII sequence*
	2. 753e +05	96	CP006441.1	*Saccharomyces cerevisiae YJM271 chromosome XII sequence*
12	2.664 c+05	96	CP006424.1	*Saccharomyces cerevisiae YJM1463 chromosome XII sequence*
	2.045e+05	96	CP006391.1	*Saccharomyces cerevisiae YJM1307 chromosome XII sequence*
	2. 860e +05	98	CP006441.1	*Saccharomyces cerevisiae YJM271 chromosome XII sequence*
13	2.795 c+05	98	CP006442.1	*Saccharomyces cerevisiae YJM320 chromosome XII sequence*
	2.729e+05	97	CP006424.1	*Saccharomyces cerevisiae YJM1463 chromosome XII sequence*
	2.879e+05	97	CP006441.1	*Saccharomyces cerevisiae YJM271 chromosome XII sequence*
14	2.122 e+05	97	CP006391.1	*Saccharomyces cerevisiae YJM1307 chromosome XII sequence*
	2.118 e+05	97	CP006436.1	*Saccharomyces cerevisiae YJM193 chromosome XII sequence*
	749	100	KF646196.1	*Pichia fermentans strain YE1*
15	743	99	KF646206.1	*Pichia fermentans strain Y1-14*
	736	99	KF646176.1	*Pichia fermentans strain Y3-5*
	769	98	DQ198951.1	*Pichia membranifaciens strain CBS 82*
16	769	99	JX188205.1	*Pichia membranifaciens strain P43C007*
	769	99	JX188207.1	*Pichia membranifaciens strain P43C010*

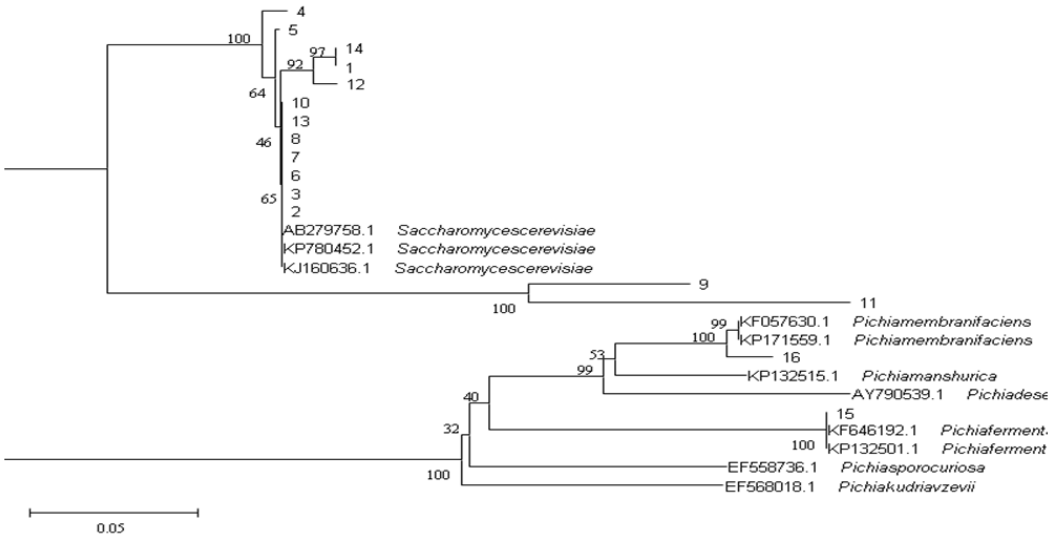

Figure 2. Phylogenetic tree

3.2.3. Phylogenetic Tree

According to the phylogenetic tree (Figure 2), the excellent strains isolated from borax were all yeasts. Among them, samples 2, 3, 6, 7, 8, 10, 13 were clustered with AB279758.1 (*Saccharomyces cerevisiae)* bases KP780452.1 (*Saccharomyces cerevisiae*) and kj160636.1 (*Saccharomyces cerevisiae*). Therefore, strains 2, 3, 6, 7, 8, 10 and 13 may be *Saccharomyces cerevisiae*, According to the physiological and biochemical characteristics, strain 15 was identified as *Saccharomyces cerevisiae*; strain 15 was identified as *Pichia fermentans* by clustering with KF646192.1 (*Pichia fermentans*) and KP132501.1 (*Pichia fermentans*), and the reliability of their homology reached 100%. Therefore, strain 15 was identified as *Pichia fermentans*. Strain 16 was clustered with KF057630.1 (*Pichia membranifaciens*) and *KP171559* (*Pichia membranifaciens*), and the reliability of their homologous relationship was more than 99%. Therefore, strain 16 was identified as *Pichia membranifaciens*. Strains Y9 and Y10 may be new yeasts and need further identification.

3.3. Discussion

XUAR, with its unique geographical environment and cultural tradition, is rooted in the long-standing cultural soil of all ethnic groups. It produces colorful and unique ethnic characteristic food. This precious resource not only contains the profound traditional customs and cultural heritage of various nationalities, but also is more and more favored and pursued by the public at home and abroad. Due to small production scale, poor conditions, unstable product quality and short shelf life. Note that it is very important to select excellent yeasts with stable fermentation characteristics in the production and storage of hybrid due to small production scale, poor conditions, unstable product quality and short shelf life. Foreign countries such as Bulgaria, Turkey reported the traditional method of separation and identification of miscellaneous, but most of the research is limited to the study of microbial components [9]. Akhberdy et al. [16] reported that different yeast genera were identified by traditional morphological and biochemical identification methods. The results showed that there were 5 strains of *Cryptococcus*, 3 strains of *Saccharomyces cerevisiae* and 4 strains of *Candida*. In this study, 40 strains of yeasts were isolated and purified from 5 kinds of samples collected from five different places of XUAR. Through the preliminary identification of morphology, physiology and biochemistry, 16 excellent strains with stable fermentation characteristics were selected. The main important discoveries in this study are summarized in the next section.

4. Conclusion

In this experimental research work by using preliminary identification of morphology, physiology and biochemistry methods, 40 strains of yeasts were separated and purified from 5 kinds of samples collected from Kunes County, Urumqi City, aktu County, Tikes county and Ghulja City of XUAR. The results showed that there were 4 excellent yeasts isolated from Ghulja Boza, 3 excellent yeasts isolated from Urumqi Boza, and 3 excellent yeasts isolated from Boza in Kunes County, 4 strains from Tikes county and 3 strains from aktu Boza. 12 of identified yeast strains belonged to *Saccharomyces cerevisiae*, 1 belonged to *Pichia membranificens* and 1 belonged to *Pichia fermentans*. According to the characteristics of excellent yeasts in different regions, the number of *Saccharomyces cerevisiae* was higher, which indicated that *S.cerevisiae* was one of the most suitable fermentation agents in XUAR. Two new yeast strains (Y9 and Y10) may be found in this study, which need further identification. Due to the unstable quality of XUAR ethnic traditional beverage, the optimization of traditional fermentation technology and the improvement of fermentation technology were carried out through the breeding and identification of the dominant strains. The significance of this study lies in selecting more suitable modern technology, developing new processing technology of miscellaneous, improving product quality, providing theoretical guidance for industrial production of miscellaneous, and creating conditions for large-scale production.

Acknowledgments

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