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# Identification of yeast species in traditional Iranian fermented products

**H. Ziaie-Juybari** ✉
**H. Rahimmian**
*Department of Plant Protection, Sari Agricultural Sciences and Natural Resources University, Mazandaran, Sari, Iran*

**F. Darvishi**
*Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran*

**A. Barzegar**
*Department of Plant Protection, Sari Agricultural Sciences and Natural Resources University, Mazandaran, Sari, Iran*

**M. Arzanlou**
*Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, Iran*

**Abstract**: Fermented foods and beverages play an important role in maintaining human health. Yeasts are one of the essential microbial groups for food fermentation and are found in fermented products. The current study aimed to investigate the diversity of yeast species from fermented foods and beverages in some regions of Iran. Samples were collected from traditional fermentation products including sourdough, kombucha, kefir, and water kefir. Initially, 38 yeast isolates were studied morphologically and biochemically, followed by M13 DNA fingerprinting. Representatives identified through DNA fingerprinting were selected for rDNA-LSU (D1/D3 regions) sequencing. As a result, six species were identified. The identified yeast species belonged to ascomycetous yeasts, namely *Saccharomyces cerevisiae*, *Pichia membranifaciens*, *P. kudriavzevii*, *P. fermentans*, *Kazachstania servazzii*, and *Kluyveromyces marxianus*. Sourdough samples showed more diversity of isolated yeast species compared to kombucha, water kefir and kefir samples. *Saccharomyces cerevisiae* was the only yeast species isolated from kombucha, kefir, and water kefir samples. The current research findings provide valuable insights into the yeast diversity of traditional fermented products.

**Keywords:** Food microbiology, Kefir, Kombucha, Probiotics, Yeast biodiversity.

## INTRODUCTION

Fermented foods and beverages have enriched the human diet for thousands of years, providing not only a means of food preservation but also a wealth of nutrients and health benefits (Tamang & Lama 2022). The use of yeasts in fermented food has a long history in different cultures worldwide due to their beneficial properties (Ilango & Antony 2021).

Traditional fermented products, such as sourdough, kefir, water kefir, and kombucha, are rich sources of diverse microorganisms including yeasts and bacteria (Guzel-Seydim et al. 2021, Lynch et al. 2021, Ferremi Leali et al. 2022).

The microbial diversity and composition of fermented foods and beverages can vary depending on geographic location, type of raw materials, and a wide range of technological parameters used in food production (Boyaci□Gunduz & Erten 2020, Korcari et al. 2020, Pino et al. 2022). Among the microorganisms involved in fermentation, yeasts are particularly prominent. They can ferment sugars into ethanol and carbon dioxide, while also producing metabolites that enhance the flavor, texture, and quality of fermented foods and beverages (Ilango & Antony 2021). Moreover, yeasts can have probiotic attributes, such as improving the intestinal microbiota, modulating the immune system and preventing infections (Ghaffari et al. 2021, Lynch et al. 2021).

Isolation and identification of yeast species from fermented foods and beverages can provide valuable insights into their diversity and potential probiotic benefits. Such findings can provide valuable information to support the development of new probiotics and other useful biotechnological products as well as enhancing the quality and safety of the existing ones (Punyauppa-Path et al. 2022).

The yeast species found in sourdough, kombucha, kefir and water kefir mainly belong to the genera *Saccharomyces*, *Kazachstania* and *Pichia*. *Saccharomyces cerevisiae* is the most widespread species found in sourdoughs, kombucha, water kefir and milk kefir (Carbonetto et al. 2018, Coelho et al. 2020, Guzel-Seydim et al. 2021). Although *S.*

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✉ Corresponding Author: E-mail: Ziaie.Hakimeh@gmail.com

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*cerevisiae* is the predominant yeast species in various fermented foods (Carbonetto et al., 2018; Guzel-Seydim et al., 2021), the diversity of yeast species in fermented products is much more complex and extensive. Recent studies, particularly in Asia (e.g., China and Thailand) and South America (e.g., Brazil and Argentina), have highlighted a rich and largely unexplored biodiversity of yeast species in traditional local fermentations (Kurtzman et al., 2015, Yurkov, 2018). This exciting discovery underscores the critical need for further exploration of understudied regions and biomes, which could potentially harbor novel yeast species with unique properties (Boekhout et al. 2022)

The high genetic diversity within yeast populations, influenced by ecological and geographical factors holds immense potential for unlocking new applications (Huys et al. 2012, Wang et al. 2012, Liu et al. 2021) For example, studies in Iran have revealed the rich microbial diversity within traditional fermented foods, highlighting the potential of some strains for industrial applications (Homayouni-Rad et al. 2020, Yazdi et al. 2022).

Traditional yeast species identification through physiological methods is time-consuming, challenging, and prone to inaccuracies. Recent advances, such as the development of a database (barcode) with gene sequences from rRNA (D1/D2) and ITS regions, have streamlined species identification without relying on physiological traits (Kurtzman, 2014). However, species differentiation in environmental samples remains difficult when a single genetic marker is insufficient. Preserving yeast cultures provides high-quality genomic data for discovering new genetic markers and understanding environmental impacts (Libkind et al., 2020; Boekhout et al., 2021). Understanding microbial communities in fermented foods is essential, as over 200 yeast species have been identified through phenotypic and genetic methods (Rué et al., 2023). Yeast domestication, shaped by ecological, biological, and cultural factors, highlights the need for better eco-friendly food systems and the protection of microbial diversity through enhanced science-society communication (De Guidi et al., 2023).

This study focuses on isolating and identifying yeast species from a range of traditional Iranian fermented foods and beverages. We examine both well-characterized fermented products like sourdough and kefir as well as less extensively sources like water kefir and kombucha. We aim to shed light on yeast diversity within these fermented foods.

## MATERIALS AND METHODS

### Sample collection

Three samples of kefir, water kefir and kombucha were collected from Zanjan, Iran. Seven samples of homemade sourdough were obtained from local home bakeries in five different regions of Iran, where bread is traditionally prepared using these sourdough starters. Two samples were also taken from bakery starter cultures in Tehran and Shoosh, Iran. The characteristics of the collected samples are summarized in Table 1.

### Isolation of yeasts

Ten grams of each sourdough sample, washed water kefir grains, milk kefir and commercial baker's yeast were mixed with 90 mL of buffered peptone water (Merk, Germany) at a concentration of 0.01M, pH 7.2 (Fraberger et al. 2020, Goktas et al. 2021). Serial 10-fold dilutions were prepared using the same diluent. Additionally, one gram of a commercially available active dry *S. cerevisiae* yeast (ADY) was dissolved in 50 mL of buffered peptone and added to the culture media.

For milk and water kefir ten grams of washed water kefir grains and milk kefir were mixed with 90 mL of buffered peptone and homogenized using a Waring blender at medium speed for five minutes (Goktas et al. 2021).

For kombucha preparation, 20 grams of Iranian tea leaves were steeped with 980 ml of freshly boiled water for approximately five minutes before being filtered through a sterilized sieve. A 10% solution of cane sugar in sterile distilled water was added to the filtered tea. Subsequently, 10 % of a previously fermented tea fungal mat was aseptically added to the freshly prepared tea. The bottles were tightly wrapped with a muslin cloth, allowing fermentation to occur for 28 days at room temperature in the dark (Ferremi et al. 2022).

A volume of 100 µl of each dilution was cultured in triplicate on yeast peptone dextrose agar (1% yeast extract, 2% Bacto-peptone, 2% glucose and 2% agar) supplemented with 10 µg/mL chloramphenicol (Mirbagheri et al. 2012). The plates were incubated at 28°C for 48 h. Yeast colonies were checked out under a stereoscopic microscope (Olympus SZX9) with 60X magnification. Individual colonies were purified based on color, surface, margin, and size by sub-culturing on YPD. The yeast isolates were suspended in 25% glycerol and stored at -80°C (Kurtzman et al. 2011).

### Identification of yeasts

The ability of yeast isolates to ferment an array of carbohydrates (sucrose, melezitose, maltose, raffinose, lactose, inulin, d-glucose, d-galactose, and cellobiose) under anaerobic conditions was examined using Durham tubes (Ghaffari et al. 2021). The

concentration of carbohydrates used was 2%, except for raffinose, which was adjusted to 4%. The fermentation abilities of the isolates were determined by monitoring the accumulation of gases in Durham tubes at frequent intervals over three weeks. Growth of the isolates in YPD broth was investigated at 4°C to 40°C for three weeks (Kurtzman et al. 2011).

**DNA extraction and fingerprinting**

DNA extraction was done as described by Hoffman and Winston (Hoffman & Winston 1987). For the selection of proper representatives for further characterization, the collected isolates were subjected to DNA fingerprinting using the M13 primer (5'-GAGGGTGGCGGTTCT) following the PCR protocol of Arzanlou & Narmani (2016). PCR products were subjected to electrophoresis at 80 V for 45 min on 1% (w:v) agarose gel containing 0.1 µg/mL ethidium bromide in 1× TAE buffer (0.4 M tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualized under UV-light. Repeatable banding patterns generated for each isolate were analyzed and consistently amplified fragments were scored for band presence (1) or absence (0) using UVIDoc software. Jaccard similarity coefficients were calculated between all isolates based on their shared banding profile. The resulting distance matrix was then subjected to an unweighted pair group method with arithmetic mean (UPGMA) clustering in PAST version 4.14 (Hammer et al. 2001). Representative isolates from each UPGMA cluster were selected for sequencing.

**PCR amplification and phylogenetic analysis**

Yeast isolates with various morphological and DNA fingerprinting patterns were further identified using the sequence data of the LSU-rRNA gene. The LSU-rDNA, including the D1/D3 regions, was amplified using the primer pair LROR (5'-ACCCGCTGAACTTAAGC) and LR5 (5'-TCCTGAGGGAAACTTCG), following the method described by Vilgalys & Hester (Vilgalys & Hester 1990). In brief, polymerase chain reaction (PCR) was conducted in a 20 µL reaction mixture, containing 50 ng of genomic DNA, 1 mM dNTPs, 1× PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.4, and 0.1% Triton X-100), 2.5mM of MgCl2, and 1 unit of Taq DNA polymerase (Sina Gene, Tehran, Iran), along with 1 µM of each primer. The PCR cycling parameters consisted of an initial denaturation step at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, primer annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. A final extension step at 72°C for 5 minutes was also included. The PCR products were electrophoresed on a 1% agarose gel, stained with 0.5 µg/mL ethidium bromide, and observed under UV light.

PCR products were sequenced using the Sanger method by Topaz Gene Company (Tehran, Iran). Forward and reverse ABI raw trace files were used to create consensus sequences using the Staden package program, version 2.0.0b9-src.tar.gz (Staden 1996). Through preliminary BLAST searches, sequences similar to those of the isolates in this study, including ex-type strains of each species, were downloaded from the NCBI GenBank database (Table 5) and aligned together with each other using the MUSCLE program implemented in MEGA7 (Kumar et al. 2016). The alignment was manually checked when necessary and all gaps were considered as missing data. MrModeltest v 2.3 (Nylander 2004) was used to select the best evolutionary model. The phylogenetic tree was generated using Bayesian inference (BI) implemented in Mr. Bayes v.3.2.1 (Ronquist & Huelsenbeck2003).

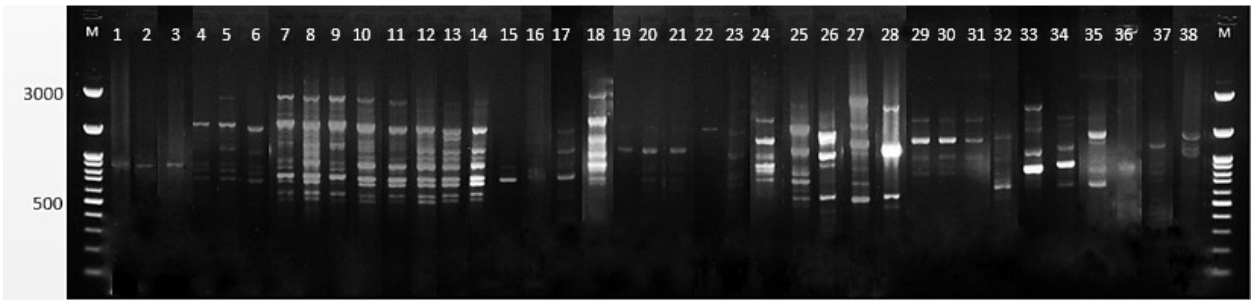


**Fig. 1.** M13 minisatellite DNA fingerprinting profiles in yeast isolates with identical fingerprint patterns. Representative isolates (1-2 per group) were selected for further characterization by DNA sequencing. For example, if isolates in lanes 1, 2, and 3 displayed the same fingerprint, a single isolate (e.g., from lane 1) would be chosen for sequencing. Lane1,Teh2/ Lane2, Sho3/ Lane 3, Sho1/ Lane 4,Sar14/ Lane 5,Sar5/ Lane 6,Kim4/ Lane 7,Sar13/ Lane 8,Sar12/ Lane 9,Sar6/ Lane 10,sho 6/ Lane 11,Kim5/ Lane 12,MK5/ Lane 13,WK2/ Lane 14,WK1/ Lane 15,Teh4/ Lane 16,MK4/ Lane 17,shi13/ Lane 18,sho5/ Lane 19,Kim1/ Lane 20, Ka2/ Lane 21,Ka1/ Lane 22,Kim3/ Lane 23,Kim2/ Lane 24,Tig9/ Lane 25,France1/ Lane 26,Mash3/ Lane 27,Kord2/ Lane 28,Kord1/ Lane 29,Tig3/ Lane 30,Tig5/ Lane 31,Tig2/ Lane 32,Mash6/ Lane 33,Mash9/ Lane 34,shi2/ Lane 35,shi1/ Lane 36,shi17/ Lane 37,sho4/ Lane 38,Mash8/ Lanes M, 100 bp DNA ladder.

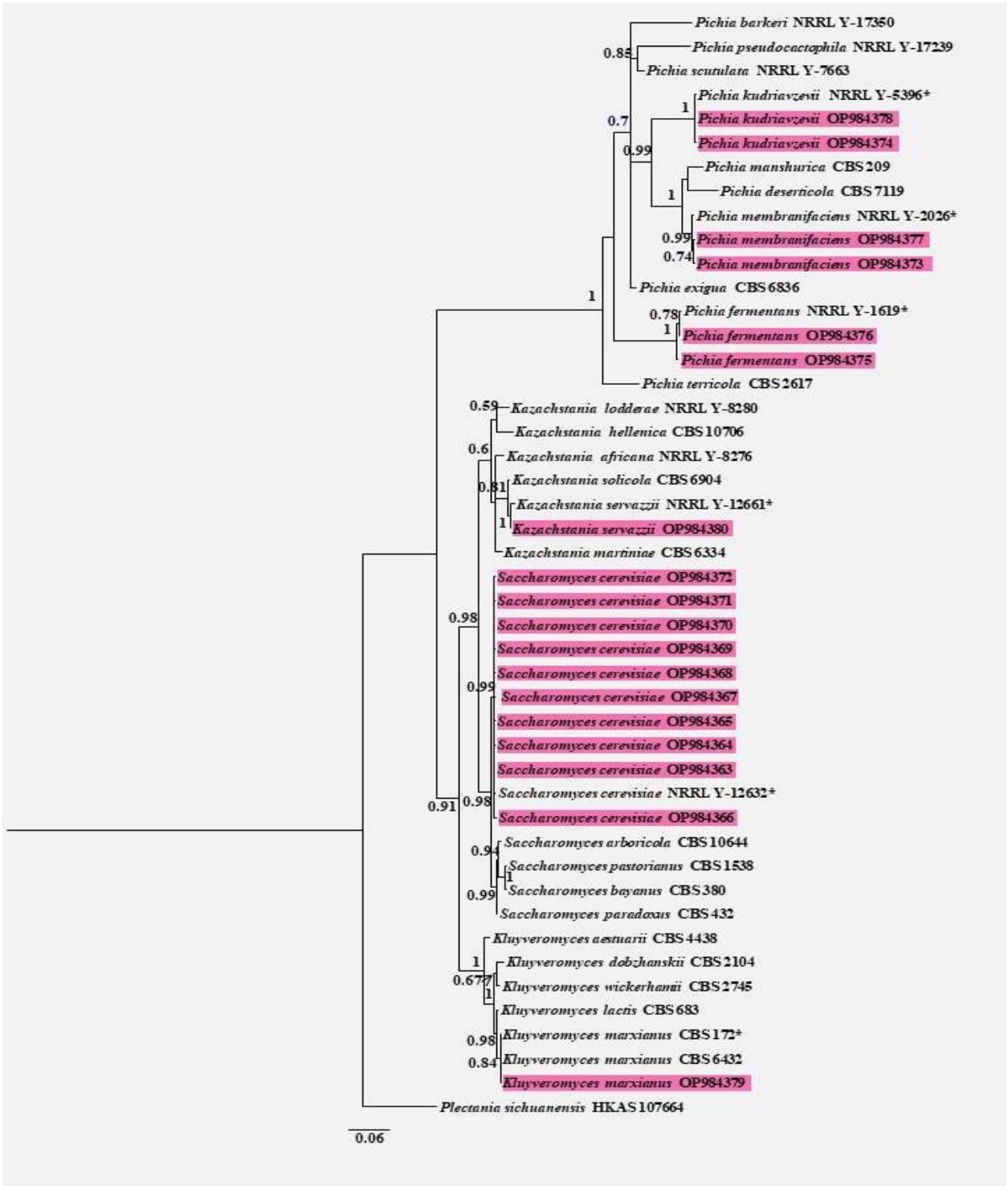


**Fig. 2.** Constructed phylogenetic tree inferred by Bayesian analysis based on GTR+I+G model recommended by MrModeltest 2.3. The tree was rooted with *Plectania sichuanensis* HKAS 107664. Posterior probability values are indicated above the branches. All isolates of this study are marked using pink color. The ex-type strains are marked with asterisks (*). The scale bar indicates 0.06 changes.

In Bayesian inference analysis, the MCMC was run with four independent chains, starting from random trees. The chains were heated, with a temperature parameter of 0.15, to facilitate efficient exploration of tree space. The MCMC was run for 1000000 generations, sampling every 1000 generations. After discarding the first 25% of sampled trees as the burn-in, consensus tree and posterior probability (PP) were determined from the remaining trees. The phylogenetic trees were printed using FigTree Ver. 1.3.1 (Rambaut & Drummond 2009). *Plectania sichuanensis* HKAS 107664 was used as the out-

group taxon. Nucleotide sequences of yeasts obtained in this study were registered in the GenBank database (Table 5).

## RESULTS

In this study, using preliminary screening of the isolates based on morphological, physiological, and M13 DNA fingerprinting, a total of 18 isolates were finally selected for sequencing and phylogenetic analysis. Phylogenetic analysis of the aligned sequences of the representative isolates contained 46 taxa (45 in-group taxa) with a total of 951 characters, comprising 321 unique site patterns. The general time reversible (GTR) substitution model with invariant sites and gamma distribution (I+G) as the best model was determined by the MrModeltest 2.3 software forthe analysis of sequences. Based on BI analyses, all selected 18 fungal isolates recovered in this study resided in six distinct clades with high posterior probability (Fig. 1) including ex-type strains of each species. The isolates MK4, Sar14, Sho3, Kim5, WK2, Kim2, Ka1, Sho4, Tig2, and Shi17, exhibited an identity higher than 98.87% with the type strain of *S. cerevisiae* (GenBank accession No.: NG042623). Isolates Kim5 and Kim2 differing by only two nucleotides, showed 98.87% and 98.98% identity with *S. cerevisiae*, respectively. The two isolates, WK2 and MK4 differing by only a single nucleotide possessed 98.87% and 99.09% identity with the type strain of *S. cerevisiae*, respectively. The isolate Mash8 showed 100% and 99% identity to the type strains *K. servazzii* NRRL Y-12661 (GenBank accession No.: NG055029) and *K. solicola* CBS 690 (GenBank accession No.: KY107950), respectively. Similar findings were observed for Mash9 and Shi2 isolates, with a 99.55% identity to the type strains of *P. fermentans* NRRL Y-16119 (GenBank accession No.: EF550234.1). Mash6 and Mash3 isolates showed 99.66% identity to the type strain of *P. membranifaciens* NRRL Y-2026 (GenBank accession No.: EU057561.1). Furthermore, Shi1 and Kord2 were determined to be *P. kudriavzevii* with a 99.78% identity to the type strain NRRL Y-5396 (GenBank accession No.: NG_055104). The similarity analysis revealed 100% identity of the isolate Kord1 to the type strains of *Kl. marxianus* CBS 712 (GenBank accession No.: KY103793), however, this isolate showed 99% similarity to the type strains of *Kl. lactis* CBS 683 (GenBank accession No.: KY108040) and just one substitution was found between these two sequences.

As a result, morphological, physiological, and phylogenetic analyses revealed that the selected fungal isolates belonged to six species: *Saccharomyces cerevisiae* (10 isolates), *Pichia kudriavzevii* (two isolates), *P. membranifaciens* (two isolates), *P. fermentans* (two isolates), *Kazachstania servazzii* (one isolate) and *Kluyveromyces marxianus* (one isolate) (Fig. 1).

All 38 yeast isolates recovered in this study were obtained from various sources, including traditional sourdough samples (seven samples), bakery starters (two samples), and single samples from kefir, water kefir, kombucha, and active dried yeast starters (Table 1). The analysis revealed that traditional sourdough starters exhibited higher microbial richness than commercial products such as milk kefir, water kefir, and kombucha. Notably, older sourdough starters demonstrated higher yeast diversity than younger starters.

Among the isolates, *S. cerevisiae* was the most prevalent species, with a total of 30 isolates identified from traditional sourdough starters (18 isolates), bakery starters (five isolates), one from commercial active dried yeast and two from each of water kefir, milk kefir and kombucha. It is the dominant yeast species in kombucha stocks, kefir, and sourdough starters. *Pichia fermentans* and *P. kudriavzevii* ranked as the second and third most common yeast species found in sourdough starters, with a total of six isolates identified.

The sourdough starters from Jiroft and Bukan exhibited a rapid development of diverse yeast communities within just 12 days. Among the identified species, *Kazachstania servazzii* and *Kluyveromyces marxianus* were isolated less frequently, with only one isolate of each detected in the starters. Tables 2, 3 and 4 present a detailed summary of the morphological and physiological characteristics of these isolates.

The fermentative capabilities of selected yeast isolates are summarized in Table 3. Most isolates demonstrated the ability to ferment glucose, galactose, and sucrose; however, lactose fermentation was exclusive to isolate Kord1 (*Kluyveromyces marxianus)*. None of the isolates exhibited the ability to ferment cellobiose, while isolates Mash3 and Mash6 from *P. membranifaciens* showed negligible fermentation activity across all tested carbohydrates. Notably Inulin fermentation was observed solely in the isolates Shi2 and Mash9 (*P. fermentans)*. All six isolates (Sho3, Sho4w, Tig2, Sar14, Kim2, Kim5) originating from Khuzestan, southern Iran, a region renowned for its ancient civilizations and historical sites, were able to use carbon sources besides cellobiose and lactose.

All yeast isolates in this study were able to grow at temperatures ranging from 4°C to 30°C, with some also tolerating higher temperatures of 37°C or 40°C. Among the isolates, *S. cerevisiae* and *K. servazzii* exhibited the broadest temperature tolerance, thriving across all tested conditions. In contrast, isolates of *P. fermentans*, *P. kudriavzevii*, and *Kl. marxianus* generally demonstrated optimal growth within the 15°C to 25°C range (Table 4).

**Table 1. Sources, locations, ages and numbers of samples collected in this study**

| Source | Location | The age of starters | Number of samples |
|---|---|---|---|
| **Water Kefir** | Zanjan, Iran | 5 years | 2 |
| **Milk Kefir** | Zanjan, Iran | 5 years | 2 |
| **Kombucha** | Zanjan, Iran | 5 years | 2 |
| **Sourdough** | Tighen, Khuzestan, Iran | About 40 years | 4 |
| (Traditional starter) | Sarcheshme, Khuzestan, Iran | About 40 years | 5 |
| | Kime, Khuzestan, Iran | About 40 years | 5 |
| | Jahrom, Shiraz, Iran | About 20 years | 4 |
| | Jiroft, Mashhad, Iran | 12 days | 4 |
| | Bukan, Urumie, Iran | 12 days | 2 |
| | Shoosh, Khuzestan, Iran | 12 days | 3 |
| **(**Bakery starter) | Tehran, Iran | 2 days | 2 |
| | Shoosh, Khuzestan, Iran | 2 days | 2 |
| **Active dried yeast** (Commercial) | Saf Levure (Marcq, France) | - | 1 |

**Table 2. The morphological characteristics of yeast isolates investigated in this study**

| Isolate | Colony on solid Media | cell shape | Growth in broth media | Species |
|---|---|---|---|---|
| WK1-2/ MK 4-5/ Ka1-2/ Tig 2-3-5-9/ Sar5-6-12-13-14/Kim 1-2-3-4-5/Shir 13-17/Teh2-4/ Sho 1-3-4-5-6/ /France1 Shi1/Kord2 | Smooth, usually flat, occasionally raised, light cream colored. Dry climbing pellicles, almost powdery, light-cream colored. | Globose, ovoid Ovoid to elongate | Sediment Turbidity and pellicle | *Saccharomyces cerevisiae* *Pichia kudriavzevii* |
| Shi2/Mash9 | Climbing pellicles, white and dull with the surface wrinkled | Ovoid to ellipsoid cylindrical | Pellicle | *P. fermentans* |
| Mash3/Mash6 | wrinkled, irregular lobed margin, climbing pellicles, pale pink colored. | ovoid to elongate | Turbidity and pellicle | *P.membranifaciens* |
| Mash8 | Smooth, waxy and glossy, striate white. | Ellipsoidal, oval globose, subglobose | Sediment | *Kazachstania servazzii* |
| Kord1 | Glossy, cream colored | Globose, ellipsoidal to cylindrical, | Pellicle | *Kluyveromyces marxianus* |

M13 DNA fingerprinting yielded unique banding patterns almost for half of the isolates (Fig. 2), with fragment sizes ranging from 500 to 3,000 bp. Based on these profiles, the isolates were placed in 16 groups.

Multiple sequence alignment of the individual LSU-rDNA sequence variants revealed a similarity of more than 95.3% between the representative isolates. An average pairwise distance of 0.125 was calculated between the individual sequences. The within-group genetic divergences of the haplotypes present in group I (*S. cerevisiae*) was 0.00039±0.00041. However, the isolates from the other three groups were very homogenous showing no genetic divergence.

## DISCUSSION

This study focused on isolating yeasts from traditional sourdough, a challenging task due to the widespread replacement of traditional starters with industrial dry yeast and the limited use of fermented beverages such as water kefir, milk kefir, and kombucha in Iran. In this study, in total of 38 isolates recovered from fermentation starters, and six yeast species were identified using a combination of biochemical tests, growth rate analysis, and sequence data.

Physiological studies showed high diversity among yeast isolates in this study so that except for cellobiose, all selected yeast isolates had different fermentation activity on the rest of the carbon sources (Table 2). All selected isolates could grow on media at lower temperatures while higher temperatures halted the growth of some isolates (Table 4). Evaluation of the growth temperature of Iranian fermentation starter yeasts unveiled: (1) optimal fermentation temperatures for distinct species, (2) ecological adaptations to diverse environments, (3) selection for specific applications

**Table 3.** Fermentative abilities of some selected yeast isolates

| Species | Isolate | Cellobiose | D-Galactose | D-Glucose | Inulin | Lactose | Raffinose | Maltose | Melezitose | Sucrose |
|---|---|---|---|---|---|---|---|---|---|---|
| *Saccharomyces cerevisiae* | WK1, WK2, MK4, MK5, Ka1, Ka2, Shi13, Shi17 | - | + | + | + | - | + | + | - | + |
| | Tig2, Tig3, Tig5, Tig9, Sar5, Sar6, Sar12, Sar13, Sar14, Kim1, Kim2, Kim3, Kim4, Kim5, Sho1, Sho3, Sho4, Sho5, Sho6, Teh2, Teh4, France1 | - | + | + | + | - | + | + | + | + |
| *Pichia kudriavzevii* | Shi1 | - | - | - | + | - | - | - | - | - |
| | Kord2 | - | - | + | + | - | - | - | - | - |
| *P. fermentans* | Shi2 | - | - | - | + | - | - | - | - | - |
| | Mash9 | - | - | - | + | - | - | - | - | - |
| *P. membranifaciens* | Mash3 | - | - | - | - | - | - | - | - | - |
| | Mash6 | - | - | - | - | - | - | - | - | - |
| *Kazachstania servazzii* | Mash8 | - | + | + | - | - | - | - | - | - |
| *Kluyveromyces marxianus* | Kord1 | - | - | + | + | + | + | - | - | + |

and (4) Temperature tolerance of yeasts at different temperatures can enhance our understanding of their potential applications.

Certain species are widespread and can be encountered in numerous if not all, fermented items. Examples include *S. cerevisiae*, *Torulaspora delbrueckii*, *Kl. marxianus*, *P. fermentans*, *Debaryomyces hansenii*, and *Brettanomyces bruxellensis*. In contrast, some species show a more distinct association, like *K. bulderi*, found exclusively in cereal products, *Starmerella bacillaris* in grape must, or *Geotrichum candidum* in fermented milk products (De Guidi et al. 2023).

In the present study, various yeast species were isolated and identified, including *S. cerevisiae*, *P. fermentans*, *P. kudriavzevii*, *P. membranifaciens*, *K. servazzii*, and *Kl. Marxianus* (Fig. 1). Of notable significance was the discovery of *K. servazzii*, which marked the first reported instance of this yeast species in sourdough. This species resided in a distinct clade and is sister to the species *K. solicola* (Fig. 1). This finding carries particular importance within the realm of microbiology and food science, as it expands our understanding of the microbial diversity in sourdough and highlights the potential influence of *K. servazzii* on sourdough fermentation processes. Previous studies have also reported the presence of *K. servazzii* in kimchi, a traditional Korean fermented vegetable dish, further highlighting its association with diverse fermentation environments (Kim et al. 2021) Further research is needed to fully understand the specific contributions of *K. servazzii* to sourdough fermentation. However, this discovery opens up new avenues for exploring the microbial diversity of

sourdough and developing innovative approaches to sourdough production.

Laureys et al. (Laureys & De Vuyst 2014) examined the genetic diversity and volatile compound profiles of yeasts in traditional water kefir fermentations, identifying a diverse array of yeasts from the genera *Saccharomyces*, *Candida*, and *Lachancea*, among others. Ferremi-Leali et al. (2022) explored the diversity of yeasts during kombucha tea fermentation and their interactions with some bacterial consortia, observing a high diversity of yeast species, including *Novacetimonas hansenii, Brettanomyces bruxellensis,* and *Zygosaccharomyces parabailii.* The results of our study in line with previous reports revealed a high diversity of yeasts in various fermented foods (Wang et al. 2012, Johansen et al. 2019, Landis et al. 2021, Ferremi Leali et al. 2022). In the present study, only *S. cerevisiae* was isolated from samples of kombucha, water kefir, and kefir. On the contrary, other studies have shown a higher variety of yeast species in these fermented beverages.

This discrepancy in findings stems from the potential variability in microbial communities within different batches, preparations, or sources of these beverages.

Results of several studies carried out for exploring the diversity and characteristics of yeasts in traditional fermented foods in Iran, are in accord with the findings of our study. Yazdi et al. (2022) investigated yeast diversity in traditional Iranian dairy products and identified *Kluyveromyces* and *Candida* as the major yeast species involved. Rahmani et al. (2022) reported the presence of *Kl. marxianus*, *S. cerevisiae*, *P. fermentans*, and *P. kudriavzevii* in traditional fermented foods of Iran. Interestingly, in the current study, *Kl. marxianus* was only isolated from a sourdough sample collected from the eastern region of western Iran. While *Kl. marxianus* has been widely reported in probiotic and fermented foods globally (Ceresino et al. 2024), its restricted occurrence in this study highlights the potential influence of regional and product-specific factors on microbial diversity.

In this study, a high sequence similarity in the predominant D1/D3 region of the 28S rDNA was revealed among different yeast isolates, with more than 95.3% sequence similarity observed between the sequences of the individual sequences. This finding is consistent with those of Laureys and De Vuyst (2014) who observed high sequence similarity among yeast isolates associated with water kefir fermentations. However, this marker may not be suitable for species-level identification in all yeast genera, as there is a high degree of genetic similarity between different species. Furthermore, our findings indicated nucleotide divergence among *S. cerevisiae* isolates, suggesting the presence of subpopulations within this species. This observation aligns with Johansen et al. (2019) who reported a high genetic diversity of *S. cerevisiae* in various fermented African indigenous foods. These results suggest that there is a high degree of genetic diversity within the *S. cerevisiae* population and that additional genetic markers may be required for accurate species-level identification. However, the phylogenetic tree constructed from the 28S rDNA nucleotide sequences of the predominant

**Table 4. The growth temperature of isolated yeasts in this study**

| Species | Isolate | Growth temperature | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 4°C | 15°C | 19°C | 21°C | 25°C | 30°C | 37°C | 40°C |
| *Saccharomyces cerevisiae* | WK1, WK2, MK4, MK5, Ka1, Ka2, Tig2, Tig3, Tig5, Tig9, Sho1, Sho3, Sho4, Sho5, Sho6, Teh2, Teh4, Sar5, Sar6, Sar12, Sar13, Sar14, Shi13, Shi17 | + | + | + | + | + | + | + | + |
| | France1, Kim1, Kim2, Kim3, Kim5 | + | + | + | + | + | + | + | - |
| | Kim4 | + | + | + | + | + | + | - | - |
| *Pichia kudriavzevii* | Shi1, Kord2 | + | + | + | + | + | + | - | - |
| *P. fermentans* | Shi2 | + | + | + | + | + | + | + | - |
| | Mash9 | + | + | + | + | + | + | - | - |
| *P.membranifaciens* | Mash3 | + | + | + | + | + | + | + | - |
| | Mash6 | + | + | + | + | + | + | + | + |
| *Kazachstania servazzii* | Mash8 | + | + | + | + | + | + | + | + |
| *Kluyveromyces marxianus* | Kord1 | + | + | + | + | + | + | + | + |

yeast variants displayed more or less six distinct groups representing different genera and species, similar to the findings of Ferremi-Leali et al. (2022) in their investigation of kombucha tea fermentation.

In our examinations, traditional sourdough starters from Khuzestan province, exhibit a longstanding tradition, having a history of over 40 years, predominantly harboring *S. cerevisiae*. Additionally, the bakery sourdough starters, aged two days from Tehran and Shoosh, Khuzestan, are dominated by *S. cerevisiae*, reflecting the common usage of this yeast in accelerated fermentation processes.

Overall, our study provides new insights into the diversity and composition of yeast species in different fermented food starters, contributing to the expanding body of literature on yeast diversity and dynamics in fermented foods. These findings underscore the potential role of yeasts in shaping the sensory and nutritional characteristics of these foods and suggest their potential applications in food biotechnology and fermentation science. Future research is warranted to explore the functional and metabolic properties of these yeast species in more depth.

**Table 5. Yeast strains and corresponding GenBank accession and culture numbers for phylogenetic analyses (Yeast isolates in this study showed in Bold font)**

| Species | GenBank accession No. | Culture accession No. |
|---|---|---|
| *Plectania sichuanensis* | MW085093.1 | HKAS 107664 |
| *Kazachstania africana* | XR_002430158.1 | NRRL Y-12661 |
| *K. hellenica* | KY107924.1 | NRRL Y-8276 |
| *K. lodderae* | KY107928.1 | CBS 10706 |
| *K. servazzii* | NG 055029.1 | NRRL Y-12661 |
| ***K. servazzii*** | **OP984380** | **Mash8** |
| *K. solicola* | KY107950.1 | CBS 6904 |
| *K. martiniae* | KY107933.1 | CBS 6334 |
| *Kluyveromyces dobzhanskii* | KY107999.1 | CBS 2104 |
| *Kl. lactis* | KY108040.1 | CBS 683 |
| *Kl. marxianus* | KY108094.1 | CBS 6432 |
| *Kl. marxianus* | KY103793 | CBS 712 |
| ***Kl. marxianus*** | **OP984379** | **Kord1** |
| *Kl. wickerhamii* | KY108113.1 | CBS:2745 |
| *Pichia barkeri* | NG_055118.1 | NRRL Y-17350 |
| *P. deserticola* | KY108789.1 | CBS 7119 |
| *P.exigua* | KY108791.1 | CBS 6836 |
| ***P. fermentans*** | EF550234.1 | NRRL Y-1619 |
| ***P. fermentans*** | **OP984376** | **Shi2** |
| ***P. fermentans*** | **OP984375** | **Mash9** |
| ***P. kudriavzevii*** | NG_055104.1 | NRRL Y-5396 |
| ***P. kudriavzevii*** | **OP984378** | **Shi1** |
| ***P. kudriavzevii*** | **OP984374** | **Kord2** |
| *P. manshurica* | KY108860.1 | CBS 209 |
| *P. membranifaciens* | EU057561.1 | NRRL Y-2026 |
| ***P. membranifaciens*** | **OP984377** | **Mash6** |
| ***P. membranifaciens*** | **OP984373** | **Mash3** |
| *P. pseudocactophila* | NG_055116.1 | NRRL Y-17239 |
| *P. scutulata* | NG_055117.1 | NRRL Y-7663 |
| *P. terricola* | KY108920.1 | CBS 2617 |
| *Plectania sichuanensis* | NG_081490.1 | HKS 107664 |
| *Saccharomyces arboricola* | NG_058391.1 | CBS 10644 |
| *S. bayanus* | KY109232.1 | CBS 380 |
| *S. cerevisiae* | NG_042623.1 | NRRL Y-12632 |
| ***S. cerevisiae*** | **OP984369** | **Ka1** |
| ***S. cerevisiae*** | **OP984363** | **MK4** |
| ***S. cerevisiae*** | **OP984367** | **WK2** |
| ***S. cerevisiae*** | **OP984365** | **Sho3** |
| ***S. cerevisiae*** | **OP984370** | **Sho4** |
| ***S. cerevisiae*** | **OP984371** | **Tig2** |
| ***S. cerevisiae*** | **OP984372** | **Shir17** |
| ***S. cerevisiae*** | **OP984364** | **Sar14** |
| ***S. cerevisiae*** | **OP984368** | **Kim2** |
| ***S. cerevisiae*** | **OP984366** | **Kim5** |
| *S. paradoxus* | KY109457.1 | CBS 432 |
| *S. pastorianus* | KY109462.1 | CBS 1538 |

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# شناسایی گونه های مخمر در محصولات تخمیری سنتی ایران

**حکیمه ضیایی جویباری[1]✉، حشمت‌الله رحیمیان[1]، فرشاد درویشی[2]، علی برزگر[1]، مهدی ارزنلو[3]**

۱- گروه گیاهپزشکی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، مازندران، ساری، ایران

۲- گروه میکروبیولوژی، دانشکده علوم زیستی، دانشگاه الزهرا، تهران، ایران

۳- گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه تبریز، تبریز، ایران

**چکیده:** غذاها و نوشیدنی‌های تخمیر شده نقش مهمی در حفظ سلامت انسان دارند. مخمرها یکی از گروه‌های میکروبی ضروری برای تخمیر مواد غذایی هستند و در محصولات تخمیر شده یافت می‌شوند. مطالعه حاضر با هدف بررسی تنوع گونه‌های مخمری از غذاها و نوشیدنی‌های تخمیری در برخی مناطق ایران انجام شد. نمونه‌ها از محصولات تخمیر سنتی شامل خمیر ترش، کامبوچا، کفیر و آب کفیر جمع‌آوری شد. در ابتدا، ۳۸ جدایه مخمر از نظر مورفولوژیکی و بیوشیمیایی مورد مطالعه قرار گرفتند و پس از آن انگشت‌نگاری DNA با کمک نشانگر M13 انجام شد. نمایندگان شناسایی شده از طریق انگشت نگاری DNA برای تعیین توالی rDNA-LSU (مناطق D1/D3) انتخاب شدند. در نتیجه شش گونه شناسایی شد. گونه‌های مخمر شناسایی شده متعلق به مخمرهای آسکومیست به نام‌های *Saccharomyces cerevisiae*، *Pichia membranifaciens*، *P. kudriavzevii*، *P. fermentans*، *Kazachstania servazzii* و *Kluyveromyces marxianus* بودند. نمونه‌های خمیر ترش تنوع بیشتری را در گونه‌های مخمر جدا شده نسبت به نمونه‌های کامبوچا، آب کفیر و کفیر نشان دادند. گونه *Saccharomyces cerevisiae* تنها گونه مخمر جدا شده از نمونه های کامبوچا، کفیر و آب کفیر بود. یافته‌های تحقیق فعلی بینش‌های ارزشمندی را در مورد تنوع مخمر محصولات تخمیر شده سنتی ارائه می‌دهد.

**کلمات کلیدی:** تنوع زیستی مخمر، کفیر، کامبوچا، پروبیوتیک‌ها، میکروبیولوژی مواد غذایی.

مکاتبه کننده: حکیمه ضیایی جویباری Email: Ziaie.Hakimeh@gmail.com

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