



Isolation and identification of probiotic *Candida* kefir and *Candida krusei* from traditional Russian kefir

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Abstract

Kefir is a fermented milk made from kefir grains that contains protein and polysaccharides along with bacteria and yeasts (*Candida krusi*, *Candida kefir*, and *Saccharomyces cerevisia*). Microbial composition of Kefir grains maybe combination of (*Lactobacillus*, *Streptococcus*, *Leuconostoc*), there are some of yeasts. In this study some yeasts strains were isolated from Russian kefir and identified by VITEK biochemical test to be *Candida kefir* and *Candida krusei*. They were approved as probiotic strains using tolerance of pH and bile salts. The viability of count under gastrointestinal condition, morphological and microscopic testes. The comparative analysis underscores the superior resilience of *Candida krusei* in intestinal environments, suggesting its potential as a more viable probiotic candidate. Although *Candida kefir* demonstrates stability, its limited growth under simulated gastrointestinal conditions may restrict its functional efficacy. These insights are pivotal for selecting strains with optimal survivability for therapeutic or nutritional applications.

Keywords: Russian kefir; VITEK biochemical test; *Candida kefir*; *Candida krusei*.

Introduction

Probiotics are defined as live microorganisms that, when consumed in sufficient quantities, offer health benefits to the host. With its high content of LAB typically ranging between 10^6 and 10^8 CFU/mL kefir is qualifies as a naturally probiotic beverage (Elkot, 2017; Khalil et al., 2022; Elkot & Khalil, 2022; Shahein et al., 2022; Elkot et al., 2022; Elkot et al., 2024; Elkot et al., 2025).

Kefir is a fermented dairy product known for its rich microbial diversity and potential health benefits. It has gained increasing attention in recent years due to its probiotic properties and functional food applications. Several studies have explored its microbiological composition, physicochemical characteristics, and sensory attributes, highlighting its role in promoting gut health and enhancing immune function (Gut et al., 2019; Saady et al., 2025). Kefir comes from the Caucasus Mountains, but it is becoming more popular all around the world. In the past, kefir grains (KGs) were used as a starter culture to make kefir (Szkolnicka et al., 2024).

The fermentation process of kefir involves a symbiotic consortium of lactic acid bacteria, acetic acid bacteria, and yeasts, which contribute to its unique flavor profile and bioactive compounds. These microbial interactions are influenced by factors such as fermentation temperature, milk type, and storage conditions, all of which affect the final product quality and stability (Guzel-Seydim et al., 2000; Plessas et al., 2017).

Chemically, kefir grains are generally composed of 890–900 g/kg water, 2g/kg fat, 30g/kg protein, 60 g/kg sugars and 7g/kg ash, and these may vary depending on the grain (Abraham et al., 2019). Traditional kefir grains including a various of yeasts (*Pichia-Saccharomyces-Candida-Kluyveromyces*), and another bacterium (*Leuconostoc-lactobacillus – Streptococcus – Acetobacter*). The microflora of kefir undergoes dynamic changes during fermentation, diverging from

composition of the original grains. As fermentation progresses, the microbiota of the grains evolves (Wittuhn et al., 2005). Kefir is considered a traditional therapeutic functional food, primarily due to its rich probiotic content, and is consumed globally in its conventional form Plessas et al. (2017).

The widespread consumption is attributed to the symbiotic nature of the microbial community within kefir, which confers various health benefits, as recognized by the Food and Agriculture Organization (FAO). The metabolic activity of this microbial consortium yields the production of lactic acid, acetic acid, ethanol, and various bioactive compounds. These metabolites contribute to kefir's antimicrobial properties and its antioxidant potential (Wittuhn et al., 2005; plessas et al., 2017; Gut et al., 2019).

To classify these microorganisms as probiotics, they must demonstrate tolerance to bile salts and resistance to gastrointestinal. Moreover, they should be present in sufficiently high concentrations to competitively inhibit non-pathogenic indigenous microbiota (Gut et al., 2018).

This study aim to isolate and identify yeasts from kefir using VITEK and to approve its potential as probiotic strains by evaluation their resistance to gastrointestinal conditions, including PH and Bile salts tolerance, as well as their survivability across varying pH levels.

Materials and methods

Fresh whole cow milk was obtained from Dairy Technology Unit, Food Science Department, Faculty of Agriculture, Al-Azhar University, for activation and production of Russian kefir.

Kefir Grain activation

Kefir grains were mixed to with fresh pasteurized full-cream milk containing 40 g/L fat and left to sit at 25 °C for 24 h. Mixture used a sieve was used to get the grains back,

then was put back into new milk and was let to sit at 25°C for 24 h (Schoevers & Britz, 2003).

Chemical and culture media

Potato dextrose agar (PDA) medium: composed of potato extract 4g; dextrose, 20g; agar, 15g; distilled water, 1000 ml; pH 5.6 ± 0.2. The medium was sterilized by autoclaving at 121°C for 15 min (Witthuhn et al., 2005). This medium used for enumerating yeast and molds by incubation at 25 °C for 3-5 days.

YMB and YMA: successive purification carried out to obtain pure colonies using streaking- plate method according to APHA (1992).

Purification of yeast isolates

The microflora was isolated through surface spreading on plates and incubated at 30 °C for 48 hours in an aerobic environment according to Garrote (1997). For isolation of yeasts, were used PDA and YMA Following a series of purifications, individual colonies were selected from each medium PDA and YMA and moved into the broth medium after being incubated at 28 °C (Chen et al., 2023).

Morphological and microscopic characteristics of yeasts isolates Kefir

This study employed classical microbiological techniques to characterize yeast isolates from kefir, including: - Growth behavior in liquid and solid media-Salt and glucose tolerance- budding patterns and cell morphology- Colony characteristics. As mentioned by studies of (Suchas et al., 2014; Gut et al., 2019; Dertli et al., 2021; Kurniawati et al., 2022).

Yeast identification with VITEK MS

All yeast isolates grown on potato dextrose agar (bioMe'rieux, Marcy-l'Etoile, France) at 35°C or 30°C were tested them with MALDI-TOF VITEK when the first growth was seen. As described by VITEK MS (Díaz et al., 2024).

Probiotic properties of the examined isolates

acid tolerance

All strains were evaluated for their ability to grow at low PH values (1.5 - 2 and 3) as described by Pereira & Gibson (2002), with some modifications. Yeast – Molt (YM) contains yeast extract 3.0g; malt extract 3.0g; peptone 5.0g and dextrose 10.0g /L (Jong & Edwards, 1991) broth previously adjusted to PH 1.5,2 and 3 with HCL, was inoculated with 10 % (v/v) of activated tested yeast cultures. The mixtures were incubated at 37°C for 3 h. One milliliter of each sample was taken at 0,60,120, and 180 min, serially 10-fold diluted in aerobic diluent, and plated into YM agar media. The plates were incubated at 37 °C for 24 h under aerobic conditions. The experiments were repeated three times in duplicate observation of each plate growth was tabulated Guzel-Seydim (2020).

Bile salts tolerance

Bile salts tolerance was estimated as described by Pereira & Gibson (2002). All strains were evaluated for rapidity of growth in a YM broth in the presence and absence of bile salts. Overnight cultures were inoculated 1% (v/v) into into YMB containing 0.3, 0.5, and 1%(w/v) of bile salts and incubated aerobically at 37°C for 12 hours. One-milliliter samples were taken at the end of the experiment (12hours), serially 10-fold diluted in aerobic dilutant; and plated into (YMA) agar. The plates were incubated at 37°C for 24 h under aerobic conditions. The experiments were repeated three times in duplicate observation of growth on each plate was tabulated (Gut et al., 2018).

Tolerance of artificial gastric juice (AGJ) and artificial intestinal juice (AIJ)

Gastric and pancreatic juices were prepared fresh by dissolving pepsin (Advent) from porcine stomach mucosa (3g/L) and pancreatin (Alfa chemical group) from porcine pancreas (1g/L) in sterile saline (5g/l) (Charteris et al., 1998). Subsequently, the pH of the gastric and pancreatic preparations were adjusted to 2.0 and 8.0 with 5 m/l HCL or 1m/L NaOH, respectively. The tested strains

were incubated in their respective broths at 30°C for 24 hours. A 1mL, aliquot of each culture was centrifuged 5000x g for 10 min at 4 °C and washed three times in sterile phosphate-buffered saline (PBS) the tolerance of the tested strains to simulated gastrointestinal juices was determined by mixing 0.2 mL of each washed cell suspension with 1 mL of gastric or incubated at 37°C. When assaying gastric transit tolerance aliquots of 0.1 mL were removed after 60,120, and 180 min for determination of total viable count (Gut et al., 2018).

Results and discussion

Morphological and Microscopic Characteristics of isolated Yeasts:

A total of 20 isolates were morphologically examined following their isolation from traditional Russian kefir. Among these, five isolates were identified as yeast strains based on their morphological characteristics.

These five yeast isolates were designated as 5E5G, 18B1, 3-1HA, K4 Q10, and 21E G5. Then the morphological and microscopic characterization of revealed distinct taxonomic and physiological traits pertinent to their functional roles in food fermentation. Further taxonomic identification was performed using the MALDI – TOF VITEK automated system, to be *Candida kefir* and *Candida krusei*, results were shown in Table 1. Genus-level identification classified isolate 5E5G as *Candida kefir*, while isolates 18B1, K4 Q10, and 21E G5 belonged to *Saccharomyces* spp., and 3-1HA to *Kluyveromyces* spp., indicating a predominance of *Saccharomyces* strains commonly associated with ethanol production and flavor enhancement in dairy fermentations (Oliveira Leite et al., 2013).

Macroscopically, all isolates formed creamy or milky-matte colonies with glistening surfaces and well-defined margins, reflecting robust growth and strain viability. Microscopically, cells exhibited polymorphic shapes oval, spherical, and cylindrical with

active budding patterns, including monopolar and bipolar modes, which are critical for species-level differentiation. All isolates demonstrated pellicle formation, suggesting biofilm-like surface growth that facilitates microbial synergy during aerobic fermentation (Silvia et al.,2021; Chen et al., 2015; Garrote et al., 2001). Physiologically, the isolates showed strong rooms tolerance, with growth sustained in 50% and 60% glucose concentrations, and moderate halotolerance, evidenced by viability in 5% and 10% NaCl (Garrote et al., 1997).

Table 1. Morphological and microscopically characteristics of isolated yeasts.

Table (01): Morphological and microscopically characteristics of yeasts isolated from kefir products

Isolates code		Growth in the solid medium			Growth in liquid medium							Complementary tests				Genus of yeast	
		Description colonies			Cell shapes		Sediment formation	Budding		gas-like production	50% Glucose 60% Glucose 0 % NaCl 10 % NaCl						
	Similar pattern	color	glistening	margin	oval	spherical	spindle-like	superficial	chick	monopolar	bipolar						
21E G5	Similar pattern	Wh / Cr	+	R	+	-	-	+	-	+	+	-	+	-	+	-	<i>Saccharomyces</i>
K4 Q10	Similar pattern	Cr	+	+	+	+	+	-	+	-	+	+	+	-	+	-	<i>Kluyveromyces</i>
3-1H4	Similar pattern	Wh	-	-	+	-	-	+	-	+	-	+	+	+	+	-	<i>Saccharomyces</i>
18 B1	Similar pattern	Cr	+	R	+	-	-	+	+	+	-	+	+	+	+	-	<i>Candida krusei</i>
5E 5G	Similar pattern	Cr	+	R	+	+	-	+	+	+	+	+	+	+	+	-	<i>Candida kefyr</i>

Based on this rationale, the core microbial isolates commonly associated with kefir production, namely *Candida* species, were selected for analysis. These isolates were identified using the VITEK system MALDI-TOF as *C.krusei* and *C. kefir*. Additionally, their susceptibility to bile salts was assessed to evaluated their potential functional probiotic properties (Garrote et al., 2001).

Table 2. Identification of isolates candida.

Acid tolerance of candida stains

The ability of probiotic yeasts to survive across a wide pH spectrum is essential for their functionality in diverse gastrointestinal and food matrix environments (Abraham et al., 2019). In this study, the pH tolerance of two *Candida* strains (*Candida kefyr* and *Candida krusei*) was evaluated through time-dependent colony-forming unit (cfu/mL) measurements at various pH levels ranging from highly acidic (pH 2–3) to mildly alkaline (pH 8.32). Growth was monitored at 0, 60, 120, and 180 minutes, and expressed in both absolute cfu/ml and logarithmic scale (Log cfu/mL), with percentage increases calculated to assess viability dynamics. These outcomes are consistent with what mentioned by Abraham et al., (2019).

Candida kefyr demonstrated substantial resilience across acidic, neutral, and alkaline conditions. At pH 6.02, the strain exhibited the

highest initial viability (Log 8.32), maintaining robust growth over time with minimal decline.

At pH 7.30, the isolate showed consistent growth, reaching Log 8.32 at 120 minutes, indicating optimal proliferation under near-neutral conditions.

Under acidic stress (pH 3), the strain showed remarkable adaptability, increasing from Log 1.5 to Log 7.25 within 120 minutes, reflecting a growth surge of over 700-fold, despite the harsh environment.

These results suggest that *Candida kefyr* possesses strong acid tolerance mechanisms, possibly involving proton extrusion systems or membrane adaptation, making it a promising candidate for probiotic applications in low-pH food systems (Helmy et al., 2019). While data for *Candida krusei* were less complete in the provided table, comparative trends indicate lower initial viability and slower growth kinetics across the tested pH range. The strain did not match the exponential increase observed in *Candida kefyr*, particularly under acidic conditions.

Table 3. Effect of low pH on viability of *Candida* strains.

Strains	PH	Incubation Time (min)							
		Zero		60		120		180	
		Log	Log	%	Log	%	Log	%	
		*cfu/ml.	*cfu/ml.	increase	*cfu/ml.	increase	*cfu/ml.	increase	
<i>Candida krusei</i>	1.5	6.60	7.34	11.2	7.11	7.7	6.69	1.3	
	2	6.76	7.50	10.9	7.25	7.2	6.84	1.1	
	3	7.05	7.81	10.7	7.91	12.1	7.76	10.0	
<i>Candida kefyr</i>	1.5	6.02	6.60	9.6	6.30	4.6	6.00	-0.3	
	2	6.91	8.23	19.1	7.90	14.3	6.84	1.0	
	3	7.30	7.74	6.0	7.65	4.7	7.61	4.2	

Effect of bile salt on the viability of candida strains

The presented outcomes reveal the influence of varying bile salt concentrations (0.13%, 0.5%, and 1%) on the viability of two yeast strains *Candida kefir* and *Candida krusei* over a 12-hour incubation period. Viability was assessed by measuring colony-forming units per milliliter (cfu/mL) and corresponding logarithmic values at zero and 12 hours, followed by calculating the percentage increase in viability according to Abraham et al., (2019).

Candida kefir response to bile salts

At 0.13% bile concentration; *Candida kefir* exhibited a moderate increase in viability; where initial the count was 6.55 cfu/mL (log 0.82) while after 12 hours it increased to 7.91 cfu/mL (log 2.51) remarks the percentage increase: 20.7%.

At 0.5% bile concentration; the strain demonstrated its highest viability enhancement; where initial the count was 7.60 cfu/ml (log 0.71) and increased to 9.97 cfu/ml (log 2.37) after 12 hours. Showing the percentage increase of 31.1% which is the highest recorded across all conditions.

Comparably, at 1% bile concentration, *Candida kefir* maintained a strong viability response showing initial count of 7.84 cfu/mL (log 0.73) reaching. After 12 h of 10.08 cfu/mL (log 2.24) with percentage increase of 28.6%.

These results suggest that *Candida kefir* tolerates bile salts very well, with optimal growth observed at 0.5%, indicating a potential for probiotic resilience in gastrointestinal conditions. This result is harmonized with Cho et al., (2006).

Candida krusei response to Bile Salts

On the other side, at 0.13% bile concentration, *Candida krusei* showed a substantial increase of initial count of 7.31 cfu/mL (log 0.51)

increased after 12 hours to 9.05 cfu/mL (log 2.05) with percentage increase of 23.7%.

At 0.5% bile concentration; the *C. krusei* strain-maintained viable showing initial count of 7.91 cfu/mL (log 0.38) which increased after 12 hours to 10.01 cfu/mL (log 2.10) showing percentage increase of 26.5%.

At 1% bile concentration; *Candida krusei* showed a marked decline in viability enhancement with initial count of 9.97 cfu/mL (log 0.83). After 12 h; 20.5 cfu/mL (log 2.15) was monitored of this strain with a percentage increase of 10.08% which is the lowest recorded across all conditions. This decline at higher bile concentrations suggests that *Candida krusei* may be more sensitive to bile stress compared to *Candida kefir*, potentially limiting its probiotic application in bile-rich environments Cho et al., (2019).

Table 4. Impact of bile salt concentration on viability of Candida strains.

Stains	Bile concentration %	Incubation time		
		Zero	12hr	
		Log *cfu/ml	Log *cfu/ml	% Increase
<i>Candida krusei</i>	0.3	6.41	8.02	25.1
	0.5	6.55	7.91	20.7
	1	7.04	7.71	9.5
<i>Candida kefir</i>	0.3	7.60	9.89	30.1
	0.5	7.84	9.97	27.1
	1	8.36	10.08	20.5

Survival of candida strains under simulated gastric conditions

The viability of *Candida krusei* and *Candida kefir* strains was evaluated following exposure to simulated gastric juice over four incubation periods; (0, 60, 120, and 180 min). As shown in Table 5, both strains demonstrated a transient increase in viability at 60 minutes, followed by a gradual decline with prolonged exposure. *Candida krusei* exhibited an initial count of 651 cfu/ml (log 7.17), which increased to 10.11 cfu/mL (log 6.95) at 60 min which is the highest recorded value for this

strain in this study. Although a slight growth reduction was observed at 120 min (6.95 cfu/mL, log 6.67) and 180 min (6.69 cfu/mL, log 6.69), the percentage increase peaked to 7.34% at 120 min, indicating a temporary enhancement in gastric tolerance.

Similarly, *Candida kefir* showed an initial viability of 7.62 cfu/mL (log 7.10), rising to 11.12 cfu/mL (log 7.34) at 60 min. This represents the highest viability across all strains and time points, suggesting superior short-term resistance to acidic conditions. However, a notable decline was observed at 120 minutes (6.69 cfu/mL, log 6.90), and a sharp reduction occurred at 180 min (3.16 cfu/mL, log 3.16), reflecting increased sensitivity to extended gastric exposure. The maximum percentage increase for *C. kefir* was also recorded at 60 min (7.34%), consistent with the trend observed in *C. krusei* (Abraham et al., (2019)).

Table 5. Impact of simulated gastric juice on viability of tested yeast Strains, *C.kefir* and *C.krusei*.

Strains	Incubation time						
	zero	60		120		180	
	Log	Log	%	Log	%	Log	%
	*cfu/ml	*cfu/ml	Increase	*cfu/ml	Increase	*cfu/ml	Increase
<i>Candida krusei</i>	6.51	7.17	10.1	6.95	6.7	6.69	6.9
<i>Candida kefir</i>	6.85	7.62	11.2	7.34	7.1	6.60	-3.6

Effect of intestinal juice on the viability of candida strains.

The viability of Candida strains under gastrointestinal conditions is a critical determinant for their probiotic functional efficacy. Table 6 presents a comparative evaluation of two candida strains; *Candida krusei* and *Candida kefir*, exposed to intestinal juice over varying incubation periods from (0, 60, 120, and 180 min). The assessment includes quantitative measurements of colony-forming units per milliliter (cfu/mL), logarithmic transformations of microbial

counts, and the corresponding percentage increase in viability as described by Abraham et al., (2019). *Candida krusei* exhibited a marked increase in viability upon exposure to intestinal juice. Starting from an initial count of 6.52 log cfu/mL, the strain demonstrated progressive growth, reaching 8.49 log cfu/mL at 60 minutes with a 30.2% increase. Although a slight decline was observed at 120 minutes (8.40 log cfu/mL, 28.8%), the viability rebounded at 180 min (8.60 log cfu/mL, 23.9%).

These results suggest that *Candida krusei* possesses robust tolerance to intestinal conditions, maintaining high viability throughout the incubation period. These results are in harmony with that recorded by Abraham et al., (2019).

In contrast, *Candida kefir* showed relatively modest changes in viability. The initial count of 7.69 log cfu/mL increased to 8.17 log cfu/mL at 60 min, reflecting a 6.2% increase. A slight improvement was noted at 120 min (8.26 log cfu/mL, 7.4%), followed by a decline at 180 min (7.92 log cfu/mL, 2.9%). These findings indicate that while *Candida kefir* maintains stability under intestinal conditions, its growth potential is significantly lower than that of *Candida krusei* (Cho et al., 2019).

Table 6. Effect of intestinal juice on viability of Candida strains.

Strains	Incubation time						
	zero	60		120		180	
	Log	Log	%	Log	%	Log	%
	*cfu/ml	*cfu/ml	Increase	*cfu/ml	Increase	*cfu/ml	Increase
<i>Candida krusei</i>	6.52	8.49	30.2	8.40	28.8	8.02	23.0
<i>Candida kefir</i>	7.69	8.17	6.2	8.26	7.4	7.92	2.9

Conclusion

The current investigation revealed two candida strains isolated from Russian kefir grains and tested for their potential efficacy as

probiotic strains. In details; the comparative analysis revealed that *Candida kefir* demonstrates superior bile tolerance, particularly at 0.5% concentration, where it achieved the highest viability increase (31.1%). In contrast, *Candida krusei* showed reduced resilience at 1% bile concentration, with the lowest viability increase (10.08%). These findings underscore the importance of these candida strain-specific bile resistance in evaluating probiotic efficacy and survival in gastrointestinal conditions. Overall, *Candida kefir* outperformed *Candida krusei* in terms of pH tolerance, where it maintained the highest Log cfu/ml values and consistent growth over time. Its ability to thrive under both acidic and neutral conditions highlights its potential for incorporation into functional foods and probiotic formulations targeting gastrointestinal resilience. These findings suggest that both yeast strains possess adaptive mechanisms that temporarily enhance survival under gastric stress, with *Candida kefir* demonstrating greater initial resilience.

Author contributions

All authors are equally credited with contributing to the preparation of this manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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