Brazilian Journal of Microbiology (2025) 56:1765–1773
https://doi.org/10.1007/s42770-025-01728-4

MEDICINE AND PUBLIC HEALTH - RESEARCH PAPER

Identification of additional probiotic attributes in yeasts isolated from tarhana fermentation

Belgin Erdem[1] · **Halil İbrahim Kaya**[2] · **Şener Tulumoğlu**[3] · **Ebru Coteli**[1] · **Ömer Şimşek**[4]

Received: 6 January 2024 / Accepted: 24 June 2025 / Published online: 9 July 2025

Abstract

Fermented foods constitute a valuable source of probiotics for both bacteria and yeasts. To date, however, there has been a limited amount of research conducted on *Saccharomyces cerevisiae*, *Kazakhstania servazzi*, *Kluyveromyces marxianus* and *Torulaspora delbrueckii* isolated from tarhana. The objective of the research is to identify additional probiotic characteristics other than the leavening activity exhibited by yeasts that were previously isolated from tarhana fermentation. In this study, yeasts were subjected to subsequent acid and bile salt tolerance, bile salt hydrolysis, antagonistic activity, aggregation activity (auto-aggregation and co-aggregation), cholesterol assimilation, folate production, biofilm production, and hemolysis activity. *S. cerevisiae* PCF122, *S. cerevisiae* PCF107, and *S. cerevisiae* PCF134 strains grew at pH 2 and 2,5 but remained at pH 3. Except for *S. cerevisiae* PCF115 and *T. delbrueckii* PCF150, all yeasts were found to be 0,5% and 1,0% oxalate tolerant. All yeasts hydrolyze oxalate (bile salt), but only *S. cerevisiae* PCF115 and *T. delbrueckii* PCF150 produced EPS. Yeasts also exhibited significant amounts of autoaggregation (46–87%). After 24 and 48 h incubation, all strains assimilated cholesterol at rates ranging from 10,4% to 87,5% and 10,6–91%, respectively. The highest folate production was determined at *S. cerevisiae* PCF108 (56 µg/mL) and the lowest was at *S. cerevisiae* PCF110 and *K. marxianus* PFC120 (18 µg/mL). In conclusion, yeasts that existed in tarhana fermentation showed cholesterol assimilation, folate production, and aggregation activity which are additional probiotic attributes that would have consumer health promotion, beside these yeast leaven the tarhana dough.

Keywords Probiotic yeasts · *S. cerevisiae* · *K. servazzi* · *K. marxianus* · Cholesterol assimilation

Introduction

Yeasts have gained interest in probiotics due to their wide metabolic activities although bacterial species such as *Lactobacillus* and *Bifidobacterium* are generally known and preferred as probiotics in the world. In addition to lactic acid bacteria, probiotics belonging to yeasts and molds are also being studied for their potential role in fermentation, flavor production, and food ripening [1, 2]. However, there are limited studies on yeasts as probiotics derived from local and traditional fermented foods [3]. Recently, fermented foods become popular for probiotic intake [4].

Tarhana is a traditional fermented food manufactured by fermenting the dough prepared using wheat flour, yogurt, sourdough, various vegetables, and spices (tomato, red pepper, onion, peppermint, salt, etc.) and then drying and grinding [5]. Tarhana, which is also used as the first food for babies, can be consumed mostly as soup, or as snacks depending on different consumption habits [6]. Industrial

Communicated by Rosane Freitas Schwan

✉ Halil İbrahim Kaya
drhikaya@gmail.com

[1] Departments of Medical Services and Techniques, Health Services Vocational College, University of Kırşehir Ahi Evran, Kırşehir, Türkiye, Turkey

[2] Department of Hotel, Restaurant and Catering, University of Bayburt, Bayburt 69010, Türkiye, Turkey

[3] Clinic of Medical Microbiology, Dr. Behcet Uz Pediatric Diseases and Surgery Training and Research Hospital, İzmir, Türkiye, Turkey

[4] Department of Food Engineering, University of Yıldız Technical, Istanbul, Türkiye, Turkey

production of tarhana is also increasing due to increased consumer demand for instant soups. DNA-based studies applied to explore tarhana LAB composition revealed that homofermentative species dominate, but heterofermentative species are also found. On the other hand, it has been reported that the yeast species responsible for tarhana dough fermentation mostly belong to the *Pichia*, *Candida*, *Kluyveromyces*, and *Kazachstania* genera [7]. *Saccharomyces cerevisiae* was also emphasized as the main yeast species that ferments the tarhana dough by Settanni et al. [8].

Yeasts have probiotic potential, as well as lactic acid bacteria in fermented foods. Although there are many studies on the presence and function of yeasts in fermented beverages and fermented foods, research on the presence of probiotic yeasts in fermented foods has become widespread in recent years [9]. Numerous investigations show some yeasts, as well such as *Debaryomyces hansenii*, *T. delbrueckii*, *Kluyveromyces lactis*, *Yarrowia lipolytica*, *K. marxianus*, and *Kluyveromyces lodderae*, can tolerate the stomach conditions, survive in the intestines of humans, and were effective against to infections of the gut [10–12]. *S. cerevisiae* var. *boulardii is* a commercially used probiotic supplement [9].

Probiotic yeast cultures provide a variety of beneficial impacts on the human body; this includes the synthesis of antioxidant and antimicrobial agents in addition to a decrease in the cholesterol in the blood levels [13–15]. Although they make up a small proportion of the intestinal microflora, they have been reported to be crucial for the physiological function of the gastrointestinal tract [16, 17]. In recent years, cholesterol assimilation ability has been determined in yeasts other than bacteria [18, 19]. *Pichia fermentans*, *Pichia kudriavzevii*, *S. cerevisiae*, *K. lactis*, *Cryptococcus humicola*, *Candida valida*, and *Candida kefyr* were found to be the yeast species in which cholesterol assimilation was detected [20–22].

Folates are involved in nucleotide synthesis and metabolism. Folic acid cannot be synthesized by humans or animals, so it must be supplemented. Folic acid deficiency is the primary cause of micronutrient deficiency, affecting billions of people worldwide. Folic acid supplementation has become mandatory in many countries, leading to an increased need for the vitamin [23]. Because of its role in such important metabolic pathways, it can cause a range of syndromes and diseases, from megaloblastic anemia to cardiovascular problems and, in the case of deficiency, cancer [24]. Yeasts are known to produce vitamins such as folate as a fermentation product like bacteria [25]. However, there are very few studies on probiotic yeast diversity in fermented foods.

The objective of this research is to identify probiotic potential exhibited by yeasts that were previously isolated from the process of tarhana fermentation. These include exploring aggregation activity (both auto and co-aggregation), cholesterol assimilation, and folate production, as well as evaluating their tolerance to acid and bile salts, antagonistic effects, and bile salt hydrolysis activities.

Materials and methods

Microorganisms and media

Culture-dependent studies of yeast strains obtained during tarhana fermentation using LSU, ITS-5.8 S and 28 S rDNA sequencing revealed that they were isolated from more than one group. The yeast strains used in the study were obtained from Pamukkale University Food Engineering Culture Collection (PCF107, PCF108, PCF110, PCF112, PCF115, PCF119, PCF120, PCF122, PCF134, and PCF150) and 6 isolates were similar to *S. cerevisiae* (96.73–100%), 2 isolates to *K. marxianus* (99.04 and 100%), 1 isolate to *K. servazzi* (100%) and 1 isolate to *T. delbrueckii* (99.84%) strains in a previous study [7]. These isolates were registered in the NCBI database before being used in our study. PUFECC code and NCBI database registration numbers are given respectively (PFC 107-PP038306, PFC 108-PP038307, PFC 110-PP038308, PFC 112-PP003906, PFC 115-PP038309, PFC 119-PP003907, PFC 120-PP038310, PFC 122-PP038311, PFC 134-PP038312, PFC 150-PP038313).

Escherichia coli ATCC 259,225, *Staphylococcus aureus* ATCC 29,213, vancomycin-resistant *S. aureus* 316, *Pseudomonas aeruginosa* ATCC 278,853, vancomycin-resistant *Enterococcus feacalis* 461, *Candida albicans* ATCC90028, and *Candida parapsilosis* ATCC 22,019 indicator strains were obtained from the Culture Collection of the Clinical Microbiology Laboratory of Dr. Behçet Uz Children's Diseases and Surgery Training and Research Hospital.

Sabouraud dextrose agar (SDA) or broth (SDB) and Trypticase Soy agar (TSA) or broth (TSB) media (Merck, Darmstadt, Germany) were used for bacterial growth. All yeasts and indicator microorganisms were stored at -80 °C in a medium containing 15% glycerol (Merck, Darmstadt, Germany).

Determination of yeast growth at various temperatures

The effect of different temperatures on yeast growth was examined by introducing 100 µl of active yeast suspensions (10^9 CFU/mL, for each isolate, SD broth, 30–35 °C for 24–48 h) to SD water and incubation for 24, 72 h at 30, 37, and 37 °C + 5% CO_2. 100 µL of culture was inoculated into SD broth medium (log 9,5 CFU/mL) in 5 mL tubes. They were kept separately in 30 °C, 37 °C, and 37 °C + 5%

CO_2 environments for 24, and 72 h. After incubation, each yeast isolate's optical density was measured blindly against the corresponding broth medium at 600 nm in a spectrophotometer (Specord, UV/Visible Spectrophotometer, Analytic Jena, Jena, Germany). Cultures grown at 37 °C with 5% CO_2, similar to the human gastrointestinal tract, were used for research.

Screening for probiotic potential of yeast isolates

Determination of acid tolerance of yeasts

To determine the tolerance of yeasts to acids, the pH of the SD broth medium was adjusted to 2.0, 2.5, and 3.0 with 3 N HCl (Merck, Darmstadt, Germany) and sterilized in the autoclave. Different pH-adjusted media were inoculated in triplicate with 100 µL of active yeast culture (log 10 CFU/mL) and incubated for 4 h at 37 °C with 5% CO_2. At the end of the period, the cultures were cultivated on SD agar by smear inoculation at 37 °C 5% CO_2 and the number of viable yeasts was determined by colony counting (log CFU/mL).

Determination of bile salt tolerance and hydrolysis of yeasts

SD broth medium was adjusted with Oxcall (Merck, Darmstadt, Germany) at 0.3%, 0.5%, and 1.0% and sterilized in the autoclave. 100 µl of active yeast cultures (log 10 cfu/ml) were inoculated into pH-adjusted media and incubated at 37 °C 5% CO_2 for 4 h. At the end of the period, the cultures were inoculated as a smear on SD agar and grown at 37 °C 5% CO_2. Growth colonies were counted, and the number of viable yeasts (log cfu/ml) was determined [26].

The agar plates method was used to perform bile salt hydrolysis. The growth medium was SD agar with 0.5% bile salts (including 50% taurodeoxycholate) (Sigma-Aldrich, St. Louis, Missouri, USA) and 0.37 g/L $CaCl_2$ added as supplements. A spot containing new yeast cultures (24 h) was left on the medium's surface with 2 µl each, and it was then incubated in triplicate for 72 h at 37 °C with 5% CO_2. According to Fadda et al. [27], the precipitation zone around colonies demonstrated yeast activity in bile salt hydrolase.

Determination of biofilm (EPS) production by yeasts

To determine biofilm production, a single colony of pure culture of each yeast strain was inoculated onto the SD agar medium. Subsequently, single colonies were inoculated into 5 mL of SD broth medium containing 20% glucose (Sigma-Aldrich, St. Louis, Missouri, USA) and incubated at 37 °C with 5% CO_2 for 24 h. After the incubation period, cultures were diluted to 1/10, and 200 µl was incubated in a 96-well microplate (Sigma-Aldrich, St. Louis, Missouri, USA) for 24 h at 37 °C with 5% CO_2 broth medium was then added, and the wells were washed three times with distilled water. 100 µl of a 1% violet crystal solution (Sigma-Aldrich, St. Louis, Missouri, USA) was added to the wells and incubated for 15 min at 25 °C. 200 µl of ethanol/acetone (40:10) (Sigma-Aldrich, St. Louis, Missouri, USA) was poured into the wells after the holes had been cleaned three times with distilled water. After waiting for ten minutes, the dye was allowed to dissolve. Sterile SD broth was used as a negative control. For the measurement of yeast biofilm, the plate was read in a microplate reader (Microplate reader, BioTek ELx808, Agilent Technologies, California, USA) with a wavelength of 540 nm. All biofilm forming tests were performed in triplicate, and the results were averaged. Based on the ODS of bacterial biofilms, all strains were classified as non-adherent (0), weakly adherent (+), moderately adherent (++), or strongly adherent (+++). For the microtiter-plate test, the cut-off OD (ODC) was defined as three standard deviations above the mean OD of the negative control. The following strains were classified as follows, $OD \leq OD_C$ non-adherent, $OD_C < OD \leq 2\times OD_C$ weakly adherent, $2\times OD_C < OD \leq 4\times OD_C$ moderately adherent, $4\times OD_C < OD$ strongly adherent [28].

Determination of antagonistic activity of yeasts

The antimicrobial activity of yeast strains was evaluated using the agar-well diffusion method [29]. Yeast strains were grown in SD broth for 24 h at 37 °C with 5% CO_2 and centrifuged at 4000 g at 4 °C for 15 min. The indicator microorganism (*E. coli* ATCC 259225, *S.aureus* ATCC 29213, vancomycin-resistant *S. aureus* 316, *P. aeuroginosa* ATCC 278853, vancomycin-resistant *E. feacalis* 461, *C. albicans* ATCC90028 and *C. parapsilosis* ATCC ATCC 22019.) was spread on SD agar. A sterile cork borer was then used to drill 8 mm diameter holes in SD agar. 100 µL of supernatants were placed in wells and the plates were incubated in a 5% CO_2 incubator at 37ºC for 24 h. The zones formed around the wells were measured in mm.

Determination of hemolysis activity of yeasts

Streak plate cultures were obtained from overnight active yeast cultures grown in a 5% sheep blood medium (Merck, Darmstadt, Germany). They were incubated at 37 °C with 5% CO_2 for 24 h. At the end of the incubation period, hemolysis around the colonies was evaluated as α, β, and non-hemolysis. The hemolytic activity was assessed by considering the hemolytic zone size using the same methodology as for the

colony zone area estimation. *S. aureus* ATCC 29,213 strain was used as control bacteria.

Determination of aggregation-related activities

Determination of auto-aggregation of yeasts

Determination of the percentage of auto-aggregation, yeasts strains were cultured on SD agar plates at 37 °C in 5% CO_2 for 24 h. After incubation, cultures were centrifuged at 4000 g for 15 min at 4 °C cells were harvested, then cells washed twice in a phosphate buffer solution (130 mM Sodium chloride in 10 mM Sodium phosphate, pH 7.2 PPB) (Sigma-Aldrich, St. Louis, Missouri, USA). The washed yeast cells were then adjusted to 1 McF in tubes containing 4 mL PPB. To allow auto-aggregation, the yeast cells were vortexed and incubated at room temperature for 10 min. Density measurements were performed with a densimeter at 550 nm at 0 and 4 h. The percentage of auto-aggregation of yeast cells was determined using the method of Kos et al. [30].

% Auto-aggregation = A_0-B_1 / $A_0 \times 100$,

A_0: 0-hour measurement (first),

B_1 4-hours measurement (final).

Determination of co-aggregation of yeasts

Determination of the percentage of co-aggregation, yeasts strains were cultured on SD agar plates at 37 °C in 5% CO2, and indicator bacteria *E. coli* ATCC 259,225, *S. aureus* ATCC 29,213 were cultured in Blood media at 37 °C for 24 h. The method of preparation of cell suspensions for co-aggregation was the same as for the auto-aggregation assay. 2 mL of yeast cells adjusted to 1 McF, and 2 mL of indicator bacterial cells were transferred into 4 mL tubes and mixed. Then vortexed for 10 s for co-aggregation and incubated at room temperature. Density measurements were performed with a densimeter at 550 nm at 0 and 4 h. Co-aggregation percentage was calculated by the given formula [31].

% Co-aggregation = C_0-D_1 / $C_0 \times 100$.

C_0: 0 h (Yeast + indicator bacteria) measurement (first).

D_1: 4 h (Yeast + indicator bacteria) measurement (final).

Determination of cholesterol assimilation of yeasts

Active yeasts were inoculated into an SD broth medium containing 0.3% oxcall. They were incubated for 24 h under 5% CO_2 conditions. Samples were centrifuged at 5000 g for 10 min. The cholesterol content of the supernatant was determined using an enzymatic kit (Synchron® Systems, Beckman Coulter, Minnesota, USA) and a Unicell DxC800 chemistry analyzer (Beckman Coulter, Minnesota, USA) [32].

Determination of folate production of yeasts

Overnight active yeast cultures were inoculated into 5 mL SD broth medium and incubated at 37 C, 5% CO_2 for 24 h. After centrifugation at 5000 rpm, 2 mL of the supernatant was taken and measured at 600 nm in an Abbott, Architect, i1000sr immunoassay analyzer (İllinoi, USA), and folate content was determined as ng/dL [33].

Statistical analysis

Results obtained from the present study were compared with one-way ANOVA analysis and Tukey test using the Minitab 16 (Minitab Inc., Coventry, UK) package program. All analyses were performed in triplicate. Differences were considered significant at $P < 0.05$.

Results and discussion

Yeasts grow at different temperatures and conditions

Yeast cells were grown in SD broth at three distinct temperatures for 24 and 72 h. Under these conditions, 30 °C and 37 °C had the highest and lowest growth rates, respectively. The growth results of yeast isolates at different temperatures and conditions are presented in Table 1. At the end of 72 h, all isolates showed a decreasing growth at 30 °C, 37 °C, and 37 °C-5% CO_2 conditions. All isolates grew at 30 °C, 37 °C and 37 °C-5% CO2 except *K. servazzi* PFC112 and *T. delbrueckii* PFC150, which did not grow at 37 °C, 5% CO_2.

All isolates reached minimum viability levels after 24 h incubation. *S. cerevisiae* strains PCF115 and PCF122 had low survival when incubated at 30 °C, 37 °C and 37 °C, 5% CO_2, while *K. marxianus* strains PCF119 and PCF120 showed the highest growth after 72 h, at 37 °C, *K. marxianus* PCF119 and PCF120 strains had higher survival rates than *S. cerevisiae* PCF107, PCF108, PCF134, and PCF110 strains.

According to a similar study investigation on probiotic yeasts, *K. marxianus* S97 demonstrated the highest growth level (7.83 log CFU/mL) over 96 h, whereas *S. cerevisiae* S34 had the lowest survival rate (7.51 log CFU/mL) throughout the same period when incubated at 37 °C [33]. Talukder et al. [34] reported that four *S. cerevisiae*, which they isolated from sugarcane juice, showed high growth at 37 °C, and 42 °C.

Table 1 Growth of yeasts under different conditions

Yeast isolates	24 h, log CFU/mL			72 h, log CFU/mL		
	30 °C	37 °C	37 °C, %5 CO_2	30 °C	37 °C	37 °C, %5 CO_2
S. cerevisiae PFC107	$7,11^{Ba}$	$7,19^{Ba}$	$7,13^{Ca}$	$6,12^{Db}$	$5,15^{Bc}$	$4,26^{Dd}$
S. cerevisiae PFC108	$7,32^{Ba}$	$7,25^{Ba}$	$7,22^{BCa}$	$6,56^{Ca}$	$5,23^{Bb}$	$4,35^{CDc}$
S. cerevisiae PFC110	$7,12^{Ba}$	$7,14^{Ba}$	$7,32^{Ba}$	$6,00^{Eb}$	$4,22^{Dc}$	$4,26^{Dc}$
K. servazzi PFC112	$5,34^{Ea}$	$4,14^{Eb}$	$0,00^{Fd}$	$4,34^{Ib}$	$3,41^{Fc}$	$0,00^{Ed}$
S. cerevisiae PFC115	$6,14^{Ca}$	$6,01^{Ca}$	$6,10^{aEa}$	$5,40^{Gb}$	$4,10^{DEc}$	$4,40^{Cc}$
K. marxianus PFC119	$7,96^{Aa}$	$7,76^{Aa}$	$7,75^{Aa}$	$7,28^{Aa}$	$6,01^{Ab}$	$6,15^{Ab}$
K. marxianus PFC120	$7,93^{Aa}$	$7,88^{Aa}$	$7,67^{Aa}$	$7,24^{Aa}$	$6,06^{Ab}$	$6,22^{Ab}$
S. cerevisiae PFC122	$6,31^{Ca}$	$6,31^{Ca}$	$6,31^{Da}$	$5,71^{Fa}$	$4,64^{Cb}$	$4,57^{Bb}$
S. cerevisiae PFC134	$7,21^{Ba}$	$7,17^{Ba}$	$7,18^{Ca}$	$6,89^{Ba}$	$5,13^{Bb}$	$4,56^{Bc}$
T. delbrueckii PFC150	$5,67^{Da}$	$5,21^{Da}$	$0,00^{Fc}$	$4,52^{Hb}$	$4,02^{Eb}$	$0,00^{Ec}$

Different small letters indicate significantly difference ($p<0.05$) at each temperatures and large letters indicate significantly difference ($p<0.05$) at each strains. The table shows mean values

Table 2 Acid tolerance of yeasts at pH 2, 2.5, and 3

Yeasts isolates	Initial log CFU/mL	pH 2,0 log CFU/mL	pH 2,5 log CFU/mL	pH 3,0 log CFU/mL
S. cerevisiae PFC107	$10,04^{a}$	$9,65^{a}$	$9,37^{a}$	$9,71^{a}$
S. cerevisiae PFC108	$9,72^{a}$	$0,00^{b}$	$0,00^{b}$	$9,60^{a}$
S. cerevisiae PFC110	$9,82^{a}$	$0,00^{b}$	$0,00^{b}$	$9,22^{a}$
K. servazzi PFC112	$9,88^{a}$	$0,00^{b}$	$0,00^{b}$	$9,32^{a}$
S. cerevisiae PFC115	$9,57^{a}$	$0,00^{b}$	$0,00^{b}$	$9,42^{a}$
K. marxianus PFC119	$9,91^{a}$	$0,00^{c}$	$0,00^{c}$	$9,61^{b}$
K. marxianus PFC120	$10,01^{a}$	$0,00^{c}$	$0,00^{c}$	$9,52^{b}$
S. cerevisiae PFC122	$10,10^{a}$	$9,66^{a}$	$9,70^{a}$	$9,52^{a}$
S. cerevisiae PFC134	$9,60^{a}$	$7,65^{b}$	$8,81^{ab}$	$9,53^{a}$
T. delbrueckii PFC150	$9,28^{a}$	$0,00^{b}$	$0,00^{b}$	$9,40^{a}$

Different letters indicate significantly different ($p<0.05$) at each isolate. The table shows mean values

The probiotic potential of yeast isolates

Acid tolerance of yeasts

Only *S. cerevisiae* strains PCF107, 122, and 134 can grow at pH 2 and 2.5. All yeasts were found to grow at pH 3. In the study conducted by Sanlıdere Aloglu et al. [22], it was determined that 8 out of 10 food-derived yeast strains could grow as a result of incubation at pH 2.5 for 3 h at 37 °C. In other similar studies, it has been reported that yeasts generally grow well at pH 3 and above [21, 35]. In our study, it was determined that all strains showed good growth at pH 3.

Oxalate intolerance and bile salt hydrolysis of yeasts

As microorganisms pass through the small intestine, they encounter around 0.3% bile salts and the majority of them are inhibited, while the resistant ones pass into the large intestine. At the same time, cholesterol is reduced to bile salts. Probiotic microorganisms are expected to exhibit resistance to bile to survive and function effectively in the gastrointestinal tract [36]. Table 3 shows the tolerance of yeasts to different levels of bile salts. All strains showed growth at 0.3% oxal. Except for *S. cerevisiae* PCF115 and *T. delbrueckii* PCF150, all yeasts were tolerant to 0.5% and 1.0% oxalate.

Syal and Vohra [19] and Şanlıdere Aloğlu et al. [22] reported that yeasts isolated from foods were tolerant to 0.3% oxal concentration. As supported by the research results, yeasts are generally known to be tolerant to 0.3% bile salt. In our study, it was found that all yeasts were resistant to oxal at 0.3% and all yeasts except two were resistant to oxal at 0.5 and 1% concentrations. Probiotic microorganisms that are resistant to high bile salts both protect their vitality and contribute to cholesterol assimilation. All yeasts hydrolyze oxalate (bile salt). By hydrolyzing bile salts, yeasts protect from the toxic effect of bile in the large intestine [36].

Determination of yeast biofilm (EPS) growth by microplate method

Exopolysaccharides (EPS), which include proteins, polysaccharides, and nucleic acids, increase bacterial adhesion, and aggregation and form exoskeletons that promote the development of biofilms [37]. EPS production protects the

Table 3 Oxalate intolerance and bile salt hydrolysis of yeasts

Yeasts isolates	Initial log CFU/mL	%0,3 log CFU/mL	%0,5 log CFU/mL	%1,0 log CFU/mL	Bile salt hydrolysis activity
S. cerevisiae PFC107	9,73[a]	9,97[a]	8,99[a]	9,59[a]	+
S. cerevisiae PFC108	9,74[a]	9,92[a]	9,68[a]	9,85[a]	+
S. cerevisiae PFC110	9,65[a]	9,46[a]	9,62[a]	9,52[a]	+
K. servazzi PFC112	9,51[a]	9,63[a]	9,39[a]	9,34[a]	+
S. cerevisiae PFC115	4,62[a]	9,34[a]	0,00[b]	0,00[b]	+
K. marxianus PFC119	9,47[a]	9,30[a]	9,49[a]	9,58[a]	+
K. marxianus PFC120	9,69[a]	9,85[a]	9,47[a]	9,68[a]	+
S. cerevisiae PFC122	9,44[a]	9,59[a]	9,26[a]	9,30[a]	+
S. cerevisiae PFC134	9,80[a]	10,02[a]	9,26[a]	9,88[a]	+
T. delbrueckii PFC150	4,62[a]	8,98[a]	0,00[b]	0,00[b]	+

Different letters indicate significantly different ($p < 0.05$) at each isolate. The table shows mean values

Table 4 Biofilm formation of yeast species in the microtiter-plate test

Yeast isolates	No adherence	Weak adherence	Moderate adherence	Strong adherence
S. cerevisiae PFC107	–	–	++	–
S. cerevisiae PFC108	–	–	–	–
S. cerevisiae PFC110	–	+	–	–
K. servazzi PFC112	–	–	–	–
S. cerevisiae PFC115	–	–	–	+++
K. marxianus PFC119	–	–	–	–
K. marxianus PFC120	–	–	–	–
S. cerevisiae PFC122	–	–	++	–
S. cerevisiae PFC134	–	+	–	–
T. delbrueckii PFC150	–	–	–	+++

microorganism from the external environment and inhibits other microorganisms by adhering to them. A research group [38] reported that *K. marxianus* and *P. kudriavzevii* yeasts isolated from dairy products synthesized EPS and their isolation was carried out. In contrast, in a similar study, Fekri et al. [39] found that *K. marxianus*, *K. lactis* and *K. aestuarii* yeast strains isolated from traditional sourdough produced more EPS than industrial bread yeast (*S. cerevisiae*).

Biofilm production rates of yeasts are given in Table 4. In our study, it was determined that *S. cerevisiae* PCF115 and *T. delbrueckii* PCF150 yeasts produced biofilms at a high rate (+++), *S. cerevisiae* PCF122 and PCF107 yeasts produced biofilms at a medium rate (+++) and *S. cerevisiae* PFC 110 and PCF134 yeasts produced biofilms at a low rate (+). It was determined that other yeasts did not produce EPS.

Six strains (PCF122, PCF107, PCF108, PCF134, PCF119, PCF120, and PCF122) produced biofilm after 24 h, with a density of stationary cells that ranged from 2.54 log CFU/cm^2 to 4.63 log CFU/cm^2. This feature was observed in Table 4, as a different indirect indicator of the ability to adhere to the gut. After 72 h, biofilm was produced by isolates of PCF110, PCF115, and PCF150. Regarding the other probiotics features, yeasts exhibited resistance to the tested drugs but lacked any antibacterial activity against bacteria (Data not shown).

Researchers have stated that in an environment rich or low in sugar, the adhesion of yeast to the surface and the resulting biofilms are important. It has been reported that biofilms occur as a result of the metabolism of various types of organisms [40].

Antagonistic activity of yeasts

None of the yeasts we studied showed any antagonistic effect against indicator microorganisms, *E. coli* ATCC 259,225, *S.aureus* ATCC 29,213, vancomycin-resistant *S. aureus* 316, *P. aeuroginosa* ATCC 278,853, vancomycin-resistant *E. feacalis* 461, *C. albicans* ATCC90028 and *C. parapsilosis* ATCC ATCC 22,019. In a study by Fadahunsi and Olubodun [29], it was reported that *Kluyveromyces phaffii* had very low antimicrobial activity (5 mm) against *Salmonella* sp. It also had no antimicrobial activity against the other three pathogens. Meanwhile, *S. cerevisiae* 003 had low (6 mm) antimicrobial activity against *Vibrio cholerae* and moderate antimicrobial activity against *Salmonella* sp. (13 mm). It did not have any antimicrobial activity against *Campylobacter jejuni* and *Listeria monocytogenes*.

Table 5 Autoaggregation and co-aggregation activity of yeasts

Yeasts isolates	Effective autoaggregation (%)	Co-aggregation (%) E. coli ATCC259225	Co-aggregation (%) S. aureus ATCC29213
S. cerevisiae PFC107	76,00^{bc}	52,67^{a}	31,67^{c}
S. cerevisiae PFC108	44,67^{d}	27,30^{e}	25,67^{cd}
S. cerevisiae PFC110	82,67^{abc}	39,67^{bc}	45,67^{ab}
K. servazzi PFC112	43,92^{abc}	32,77^{d}	19,33^{d}
S. cerevisiae PFC115	45,78^{bc}	30,33^{de}	31,67^{c}
K. marxianus PFC119	56,67^{a}	32,33^{de}	50,67^{a}
K. marxianus PFC120	56,22^{ab}	35,33^{cd}	48,67^{ab}
S. cerevisiae PFC122	49,00^{c}	41,67^{b}	31,67^{c}
S. cerevisiae PFC134	38,36^{c}	16,40^{f}	25,33^{cd}
T. delbrueckii PFC150	53,78^{abc}	42,00^{b}	41,00^{b}

Different letters indicate significantly different ($p<0.05$) at each isolate. The table shows mean values

Table 6 Cholesterol assimilation and folate production by yeasts

Yeasts	24 h Absorption of cholesterol(%)	48 h Absorption of cholesterol (%)	Folate Production (µg/mL)
S. cerevisiae PFC107	37,50^{a}	38,33^{a}	24,00^{d}
S. cerevisiae PFC108	22,50^{a}	28,33^{b}	56,67^{a}
S. cerevisiae PFC110	85,50^{a}	88,33^{a}	17,00^{e}
K. servazzi PFC112	50,50^{a}	66,00^{a}	23,33^{d}
S. cerevisiae PFC115	51,39^{a}	70,67^{b}	21,00^{de}
K. marxianus PFC119	67,61^{a}	87,00^{a}	29,33^{c}
K. marxianus PFC120	47,61^{a}	63,00^{a}	17,67^{e}
S. cerevisiae PFC122	16,78^{a}	10,87^{a}	30,00^{c}
S. cerevisiae PFC134	73,72^{a}	90,33^{a}	44,00^{b}
T. delbrueckii PFC150	43,39^{a}	53,67^{a}	23,67^{d}

Different letters indicate significantly different ($p<0.05$) at each isolate. The table shows mean values

Hemolytic activity of yeasts

Hemolytic activity is one of the pathogenic properties of microorganisms. It is an undesirable feature in probiotic microorganisms [41]. In our study, none of the yeast strains showed hemolytic activity on 5% sheep blood Mueller-Hinton Agar.

Aggregation activity of yeasts

Aggregation, cell adhesion and dominant colonisation are important criteria for probiotic microorganisms. Aggregation occurs in two ways, auto-aggregation and co-aggregation [36].

Auto-aggregation

Aggregation is the collapse of microorganisms among themselves (auto-aggregation) or by forming a group with another organism (co-aggregation). It continues its effectiveness to protect themselves with auto-aggregation. With co-aggregation, it clings to other microorganisms and prevents colonization by allowing them to collapse [36].

As seen in Table 5, yeasts showed high levels of auto-aggregation (46–87%). *S. cerevisiae* PCF108 (46%) and *K. marxianus* PCF119 (87%) strains showed the lowest and highest aggregation ability. In a study by Syal and Vohra [19], it was determined that the aggregation ability of yeasts isolated from traditional foods ranged between 47 and 97% at the end of 3 h.

Co-aggregation

It was found that yeasts formed different levels of coaggregation against *E. coli* ATCC 259,225 and *S. aureus* ATCC 29,213 (*E. coli* ATCC 259225 16.2-40% and *S. aureus* ATCC 29213 20-50%) (Table 5). Compared to our study, a study showed that 12 different *S. cerevisiae* and *S. boulardi*. strains had low co-aggregation against *E. coli* EPEC, *Salmonella Enteritidis* and *L. monocytogenes* pathogens [42]. In general, yeast auto-aggregation percentages are reported to be higher than co-aggregation. This is thought to be due to the high specific gravity of yeasts.

Cholesterol assimilation

Probiotic yeasts contribute to the reduction of cholesterol in the environment by assimilation. This helps to reduce and prevent coronary heart disease. Table 6. shows the cholesterol assimilation percentages (%) of yeasts. All 10 strains showed cholesterol assimilation between 10.4 and 87.5% in 24 h and 10.6–91% in 48 h of incubation. No significant difference was observed in the cholesterol removal of yeasts at 24 and 48 h of incubation. Psomas et al. [20] determined the cholesterol assimilation amount of yeasts as 83.4% at 24 h and 90.4% at 48 h in 0.3% oxsal and cholesterol-enhanced YEPS medium. Kourelis et al. [18] found that food- and human-derived *S. cerevisiae* 832 and *S. cerevisiae* 952 yeasts reduced cholesterol by 4% and 60%, respectively, within 24 h in the YEGP medium. Chen et al. [21] in their study, found that all of the yeasts obtained from raw cow's milk reduced cholesterol by 3.6–44% within 72 h.

Şanlıdere Aloğlu et al. [22] reported that food-derived yeasts assimilated cholesterol between 43.12% and 73.3%. *K. marxianus* K1 and M3 strains were reported to be highly cholesterol-reducing yeasts [43]. It has been reported that

cholesterol removal and folate production of yeasts are completely strain and time-dependent.

Folate production

The amounts of folate synthesized by yeasts were determined in the range of 18–56 µg/mL (Table 6). The highest producer was *S. cerevisiae* PCF108 (56 µg/ml) and the lowest producer was *S. cerevisiae* PCF110 (18 µg/ml). Cholesterol removal and folate production of yeasts vary depending on the strain and time. Folate production in *S. cerevisiae* was also increased by adjusting the medium composition and reached 360 g/L [44]. Hijortmo et al. [45] found that total folate synthesis varied greatly among yeasts, 4000-14,500 µg/100 g dry matter.

Conclusions

This study revealed that some yeasts isolated from tarhana fermentation have the potential to tolerate gastrointestinal conditions. As a result of these findings, these specific strains will contribute as beneficial microorganisms in food products with their probiotic potential. This study identified diverse probiotic capabilities of yeast strains that not only support primary digestive functions but also reveal distinctive secondary properties. Specifically, *S. cerevisiae* PFC107, *S. cerevisiae* PFC108, S. cerevisiae PFC110 and *S. cerevisiae* PFC122 were distinguished by their impressive ability to produce folate and adsorb cholesterol. The importance of the properties of these yeasts, including cholesterol assimilation, folate synthesis, and aggregation, was emphasized in this study. Beyond gut health, these additional probiotic properties extend the benefits of probiotics. Furthermore, when used as supplemental cultures or consumed in tarhana, these yeasts could effectively serve as postbiotics.

Author contributions All authors contributed to the conception and design of the study. Şener Tulumoğlu and Belgin Erdem carried out materials planning, data collection, and analysis. Halil İbrahim Kaya, Belgin Erdem, Ömer Şimşek, and Ebru Coteli wrote the draft and finalized the manuscript. All authors read and approved the final version of the manuscript.

Funding No funding was provided for this study.

Declarations

Ethics approval This article does not include any studies with human participants.

Conflict of interest The authors declare that there are no conflicts of interest.

References

1. Jespersen L (2002) Occurrence and taxonomic characteristics of strains of Saccharomyces cerevisiae predominant in African Indigenous fermented foods and beverages. FEMS Yeast Res 3:191–200. https://doi.org/10.1016/S1567-1356(02)00185-X
2. Roberts IN, Oliver SG (2011) The Yin and Yang of yeast: biodiversity research and systems biology as complementary forces driving innovation in biotechnology. Biotechnol Lett 33:477–487. https://doi.org/10.1007/s10529-010-0482-7
3. Simões LA, Ferreira I, Melo DS, Lopes LA, Magnani M, Schwan RF, Dias DR (2021) Probiotic properties of yeasts isolated from Brazilian fermented table olives. J Appl Microbiol 131(4):1983–1997. https://doi.org/10.1111/jam.15065
4. Ilango S, Antony U (2021) Probiotic microorganisms from non-dairy traditional fermented foods. Trends Food Sci Technol 118:617–638. https://doi.org/10.1016/j.tifs.2021.05.034
5. Anonymous (2002) TS 2282 Tarhana standard. Turkish Standards Institute, Ankara
6. Erbas M, Uslu MK, Erbas MO, Certel M (2006) Effects of fermentation and storage on the organic and fatty acid contents of tarhana, a Turkish fermented cereal food. J Food Compos Anal 19(4):294–301. https://doi.org/10.1016/j.jfca.2004.12.002
7. Özel S, Sabanoğlu S, Çon AH, Şimşek Ö (2015) Diversity and stability of yeast species during the fermentation of Tarhana. Food Biotechnol 29(1):117–129. https://doi.org/10.1080/08905436.2014.996895
8. Settanni L, Tanguler H, Moschetti G, Reale S, Gargano V, Erten H (2011) Evolution of fermenting microbiota in Tarhana produced under controlled technological conditions. Food Microbiol 28(7):1367–1373
9. Tamang JP, Lama S (2022) Probiotic properties of yeasts in traditional fermented foods and beverages. J Appl Microbiol 132(5):3533–3542. https://doi.org/10.1111/jam.15467
10. Chen WB, Han YF, Jong SC et al (2000) Isolation, purification, and characterization of a killer protein from *Schwanniomyces occidentalis*. Appl Environ Microbiol 66:5348–5352. https://doi.org/10.1128/AEM.66.12.5348-5352.2000
11. Kumura H, Tanoue Y, Tsukahara M et al (2004) Screening of dairy yeast strains for probiotic applications. J Dairy Sci 87:4050–4056. https://doi.org/10.1128/AEM.66.12.5348-5352.2000
12. Psani M, Kotzekidou P (2006) Technological characteristics of yeast strains and their potential as starter adjuncts in Greek-style black Olive fermentation. World J Microbiol Biotechnol 22:1329–1336. https://doi.org/10.1007/s11274-006-9180-y
13. Aidoo KE, Nout MJR, Sarkar PK (2006) Occurrence and function of yeasts in Asian Indigenous fermented foods. FEMS Yeast Res 6:30–39. https://doi.org/10.1111/j.1567-1364.2005.00015.x
14. Gil-Rodríguez AM, Carrascosa AV, Requena T (2015) Yeasts in foods and beverages: in vitro characterisation of probiotic traits. Food Sci Technol 64:1156–1162. https://doi.org/10.1016/j.lwt.2015.07.042
15. Ogunremi OR, Sanni AI, Agrawal R (2015) Probiotic potentials of yeasts isolated from some cereal-based Nigerian traditional fermented food products. J Appl Microbiol 119:797–808. https://doi.org/10.1111/jam.12875
16. Gatesoupe FJ (2007) Live yeasts in the gut: natural occurrence, dietary introduction, and their effects on fish health and development. Aquac 267:20–30. https://doi.org/10.1016/j.aquaculture.2007.01.005
17. Boonanuntanasarn S, Ditthab K, Jangprai A, Nakharuthai C (2019) Effects of microencapsulated *Saccharomyces cerevisiae* on growth, hematological indices, blood chemical, and immune parameters and intestinal morphology in striped

catfish, *Pangasianodon hypophthalmus*. Probiotics Antimicro Prot 11:427–437. https://doi.org/10.1007/s12602-018-9404-0
18. Kourelis A, Kotzamanidis C, Litopoulou-Tzanetaki E, Scouras ZG, Tzanetakis N, Yiangou M (2010) Preliminary probiotic selection of dairy and human yeast strains. J Biol Res Thessalon 13:93–104
19. Syal P, Vohra A (2013) Probiotic potential of yeasts isolated from traditional Indian fermented foods. Int J Microbiol Res 5:390–398. https://doi.org/10.9735/0975-5276.5.2.390-398
20. Psomas EI, Fletouris DJ, Litopoulou-Tzanetaki E, Tzanetakis N (2003) Assimilation of cholesterol by yeast strains isolated from infant feces and feta cheese. J Dairy Sci 86:3416–3422. https://doi.org/10.3168/jds.S0022-0302(03)73945-9
21. Chen LS, Ma Y, Maubois JL et al (2010) Screening for the potential probiotic yeast strains from Raw milk to assimilate cholesterol. Dairy Sci Technol 90:537–548. https://doi.org/10.1051/dst/2010001
22. Şanlidere Aloğlu H, Demir Özer E, Öner Z (2016) Assimilation of cholesterol and probiotic characterisation of yeast strains isolated from Raw milk and fermented foods. Int J Dairy Technol 69(1):63–70
23. Serrano-Amatriain C, Ledesma-Amaro R, López-Nicolás R, Ros G, Jiménez A, Revuelta JL (2016) Folic acid production by engineered *Ashbya gossypii*. Metab Eng 38:473–482. https://doi.org/10.1016/j.ymben.2016.10.011
24. Strobbe S, Van Der Straeten D (2017) Folate biofortification in food crops. Curr Opin Biotechnol 44:202–211. https://doi.org/10.1016/j.copbio.2016.12.003
25. Czarnowska-Kujawska M, Paszczyk B (2021) Changes in the folate content and fatty acid profile in fermented milk produced with different starter cultures during storage. Molecules 26(19):6063
26. Maragkoudakis PA, Zoumpopoulou G, Miaris C, Kalantzopoulos G, Pot B, Tsakalidou E (2006) Probiotic potential of Lactobacillus strains isolated from dairy products. Int Dairy J 16:189–199. https://doi.org/10.1016/j.idairyj.2005.02.009
27. Fadda ME, Mossa V, Deplano M, Pisano MB, Cosentino S (2017) In vitro screening of *Kluyveromyces* strains isolated from Fiore Sardo cheese for potential use as probiotics. LWT-Food Sci Technol 75:100–106. https://doi.org/10.1016/j.lwt.2016.08.020
28. Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M (2000) A modified microtiter-plate test for quantification of Staphylococcal biofilm formation. J Microbiol Methods 40:175–179. https://doi.org/10.1016/s0167-7012(00)00122-6
29. Fadahunsi IF, Olubodun S (2021) Antagonistic pattern of yeast species against some selected food-borne pathogens. Bull Natl Res Centre 45(1):1–19. https://doi.org/10.1186/s42269-020-00482-x
30. Kos BVZE, Šušković J, Vuković S, Šimpraga M, Frece J, Matošić S (2003) Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. J Appl Microbiol 94:981–987. https://doi.org/10.1046/j.1365-2672.2003.01915.x
31. Todorov SD, Furtado DN, Saad SMI, Tome E, Franco BDGM (2011) Potential beneficial properties of Bacteriocin-Producing·Lactic acid Bacteria isolated from smoked salmon. J Appl Microbiol 110:971–986. https://doi.org/10.1111/j.1365-2672.2011.04950.x
32. Tulumoğlu Ş, Kaya Hİ, Şimşek Ö (2014) Probiotic characteristics of *Lactobacillus fermentum* strains isolated from Tulum cheese. Anaerobe 30:120–125. https://doi.org/10.1016/j.anaerobe.2014.09.015
33. Moradi R, Nosrati R, Zare H, Tahmasebi T, Saderi H, Owlia P (2018) Screening and characterization of *in-vitro* probiotic criteria of *Saccharomyces* and *Kluyveromyces* strains. Iran J Microbiol 10:123–131
34. Talukder AA, Easmin F, Siraje AM, Mamoru Y (2016) Thermotolerant yeasts capable of producing bioethanol: isolation from natural fermented sources, identification and characterization. Biotechnol Biotechnol Equip 30:1106–1114. https://doi.org/10.1080/13102818.2016.1228477
35. Psomas E, Andrighetto C, Litopoulou-Tzanetaki E, Lombardi A, Tzanetakis N (2001) Some probiotic properties of yeast isolates from infant faeces and feta cheese. Int J Food Microbiol 69:125–133. https://doi.org/10.1016/s0168-1605(01)00580-3
36. Alkalbani NS, Osaili TM, Olaimat AA, Liu AN, Shah S, Apostolopoulos NP V., Ayyash MM (2022) Assessment of yeasts as potential probiotics: A review of Gastrointestinal tract conditions and investigation methods. J Fungi 8(4):365. https://doi.org/10.3390/jof8040365
37. Jayathilake PG, Jana S, Rushton S, Swailes D, Bridgens B, Curtis T et al (2017) Extracellular polymeric substance production and aggregated bacteria colonization influence the competition of microbes in biofilms. Front Microbiol 8:1865. https://doi.org/10.3389/fmicb.2017.01865
38. Rahbar Saadat Y, Yari Khosroushahi A, Movassaghpour AA, Talebi M, Pourghassem Gargari B (2020) Modulatory role of exopolysaccharides of *Kluyveromyces Marxianus* and *Pichia kudriavzevii* as probiotic yeasts from dairy products in human colon cancer cells. J Funct Foods 64:103675. https://doi.org/10.1016/j.jff.2019.103675
39. Fekri A, Torbati M, Yari Khosrowshahi A, Bagherpour Shamloo H, Azadmard-Damirchi S (2020) Functional effects of phytatedegrading,probiotic lactic acid bacteria and yeast strains isolated from Iranian traditional sourdough on the technological and nutritional properties of whole wheat bread. Food Chem 306:125620. https://doi.org/10.1016/j.foodchem.2019.125620
40. Tek EL, Sundstrom JF, Gardner JM, Oliver SG, Jiranek V (2018) Evaluation of the ability of commercial wine yeasts to form biofilms (mats) and adhere to plastic: implications for the microbiota of the winery environment. FEMS Microbiol Ecol 94:1–12. https://doi.org/10.1093/femsec/fix188
41. Ramos Monge IM, Poveda Colado JM (2021) Safety evaluation of yeasts with probiotic potential. Front Nutr 8:659328. https://doi.org/10.3389/fnut.2021.659328
42. Menezes AGT, Melo DDS, Ramos CL, Moreira SI, Alves E, Schwan RF (2020) Yeasts isolated from Brazilian fermented foods in the protection against infection by pathogenic food bacteria. Microb Pathog 140:103969. https://doi.org/10.1016/j.micpath.2020.103969
43. Liu H, Xie YH, Xiong LX, Dong RT, Pan CL, Teng GX, Zhang HX (2012) Effect and mechanism of cholesterol-lowering by Kluyveromyces from Tibetan Kefir. Adv Mat Res
44. Hijortmo S, Patring J, Andlid T (2008) Growth rate and medium composition strongly affect folate content in *Saccharomyces cerevisiae*. Int J Food Microbiol 123:93–100. https://doi.org/10.1016/j.ijfoodmicro.2007.12.004
45. Hjortmo S, Jelena Jastrebova J, Andlid T (2005) Inherent biodiversity of folate content and composition in yeasts. Trends Food Sci Technol 16:311–316. https://doi.org/10.1016/j.tifs.2005.03.014