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AKADÉMIAI KIADÓ

# Investigation of probiotic potential of yeasts isolated from sourdoughs from different regions of Turkey

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**ABSTRACT**

In this study, 14 yeast cultures from 62 isolates from traditional sourdoughs collected from 6 different regions of Turkey were selected by FT-IR identification and characterised to reveal their probiotic properties. Four yeast strains were genotypically identified and compared with FT-IR identification. In all analyses, it was observed that mostly *Saccaromyces cerevisiae* strain exhibited high hydrophobicity, auto-aggregation feature, and all yeast isolates in this study showed tolerance to 0.3%, even salt concentration. In addition, all yeast strains were susceptible to anti-yeasts agents, although they were resistant to all antibiotics used in the study. All selected yeast isolates exhibited high antimicrobial activity against the *Staphylococcus aureus*. In conclusion, this study investigated the potential probiotic properties of yeast strains isolated from sourdough.

**KEYWORDS**

sourdough, yeast, FT-IR identification, probiotic properties

## 1. INTRODUCTION

One of the important sources of different types of microorganisms is traditional sourdough. The microbial ecosystem of sourdough contains safe lactic acid bacteria (LAB) and yeast

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strains (Fekri et al., 2020). The only dominant yeast in sourdough is *Saccharomyces cerevisiae*. Therefore, LAB diversity is greater than of the yeast microbiota (De Vuyst et al., 2009). Apart from *Saccharomyces cerevisiae*, there are *Kazachstania humilis*, *Wickerhamomyces anomalus*, *Torulaspora delbrueckii*, *Kazachstania exigua*, *Pichia kudriavzevii*, and *Candida glabrata* species in sourdough (De Vuyst et al., 2016). More than 80 bacteria and 20 yeast strains have been isolated and identified from traditional sourdoughs, and some species have been described as probiotic (Fekri et al., 2020). Probiotics are defined as living microorganisms having a positive effect on the health of the host when consumed in sufficient quantities. These positive effects can be antimicrobial production, modulation of immune response, resistance to food antigens, assimilation of cholesterol, and prevention of autoimmunity (Paraschiv et al., 2011). Although the criteria for the selection of probiotic microorganisms are still in discussion, two conditions have been accepted for their selection, considering the mechanism of action. These are the ability to survive in the gastrointestinal environment and have at least one positive effect (Martins et al., 2005; Morelli, 2007). Since most of the studies focused on the probiotic properties of LABs, the main purpose of this study was to investigate the probiotic potential of 14 yeast strains isolated from traditional sourdough and the originality of the study lays in sourdough samples collected from 6 different regions of Turkey.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Sourdough samples were collected from villages of 6 regions of Turkey (Bozcaada, Cyprus, Ankara/Pursaklar, Bursa/Osmangazi, Bilecik, and Manisa/Kırkağaç), and the source of most of the samples was wheat sourdough.

### 2.2. Methods

***2.2.1. Isolation of yeast strains.*** To start the yeast isolation process, 10 g of sourdough was weighed and 90 mL of FTS (sterile saline solution (0.85% NaCl) was added. Then, it was homogenised for 5 min in stomacher. Dilutions were spread onto PDA (Potato dextrose agar) containing 10% tartaric acid, and the Petri dishes were incubated aerobically at 25–30 °C for 48–72 h. Colonies that developed were taken into PDB broth, incubated in the same way, and finally purified colonies were stocked in 40% glycerol at –80 °C.

***2.2.2. Identification of yeast isolates by FT-IR.*** FTIR profile of yeast isolates was determined according to the method of Karaman et al. (2018).

#### *2.2.3. Determination of probiotic properties*

***2.2.3.1. Resistance to antifungal discs.*** In order to determine the antifungal sensitivity of 14 yeast strains isolated from sourdough, the yeasts were allowed to grow in YPD broth at 30 °C for 24 h. After incubation, from the $10^{-2}$ dilution step, aliquots were spread onto YPD agar. Amphotericin B (AMB, 20 µg), clotrimazole (CLT, 10 µg), flucanazole (FLU, 25 µg),

ketoconazole (KTC, 10 µg), and nystatin (NY, 100 U) discs were placed onto the inoculated agar and the plates were incubated at 30 °C for 24 h.

***2.2.3.2. Determination of antimicrobial activity.*** Determination of antibacterial activity of yeast strains was done according to Demirbaş et al. (2017) and tested against *L. monocytogenes* ATCC 13932, *B. cereus* ATCC 11788, *S. typhimurium* ATCC 0402, *E. coli* ATCC 25922, and *S. aureus* ATCC 25923 pathogens. The pathogenic bacteria used in the study were obtained from Istanbul Acibadem University.

***2.2.3.3. Determination of autoaggregation and hydrophobicity properties.*** Autoaggregation and hydrophobicity abilities were determined according to Gut et al. (2019).

***2.2.3.4. Low pH and resistance to bile salt.*** The resistance of yeast cultures to low pH and bile salt was studied according to the method by İspirli et al. (2015).

***2.2.4. Statistical analysis.*** Experiments were carried out twice, and one-way ANOVA method was applied using JMP version 9 program for all data analysis. $P < 0.05$ was considered statistically significant by using Tukey's test for mean comparisons.

## 3. RESULTS AND DISCUSSION

### 3.1. Microbiological analysis

The count of yeasts in sourdoughs is given in Fig. 1. A total of 62 yeasts were isolated. It was observed that there was a significant difference in the yeast count of the sourdough samples collected from the specified regions. This change occurred between 5.44 and 7.08 (log CFU $g^{-1}$). Arici et al. (2018) isolated yeast strains from traditional Turkish sourdough samples and found yeast numbers in sourdough between 3.78 and 6.28 (log CFU $g^{-1}$). In another study, Petkova et al. (2021) isolated a total of 106 yeasts from 12 Bulgarian sourdoughs and recorded that the count of yeasts ranged from 4.15 to 9.99 (log CFU $g^{-1}$). Our results are in agreement with these

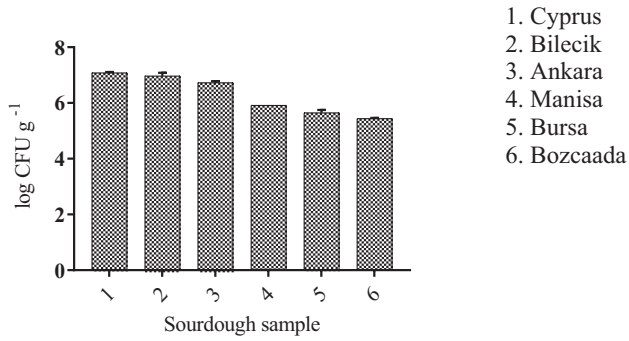


*Fig. 1.* Cell numbers of yeasts isolated from sour dough collected from 6 different locations (log CFU $g^{-1}$)

findings. This diversity may depend on factors such as flour and water type, environment, temperature, or fermentation time (Palla et al., 2020).

## 3.2. Identification of yeast strains by FTIR (Fourier-transform infrared spectroscopy) analysis

One of the powerful techniques for characterising the chemical composition of very complex niches such as microorganisms is FT-IR. It has been emphasised that this technique is successful in the identification of moulds and yeasts in various quality control areas (Fischer et al., 2006). Microbiological FT-IR typing is fast, effective, reagent-free, applicable to all microorganisms, and requires little amount of biomass (Essendoubi et al., 2007). In this study, the 14 isolated yeast strains were quickly identified by FT-IR. Cluster analyses were compared using different spectral regions (3030–2830, 1350–1200, 900–700 $cm^{-1}$) using the second derivative of the original spectra as input (Arici et al., 2018). The 14 yeast strains were separated into 4 different groups in the dendogram (Fig. 2). It was observed that *Saccharomyces, Pichia*, and *Debaryomyces* species had their own clusters. Studies have shown that the identification of microorganisms with FTIR has an accuracy rate of over 90% (Goktas et al., 2021). In order to

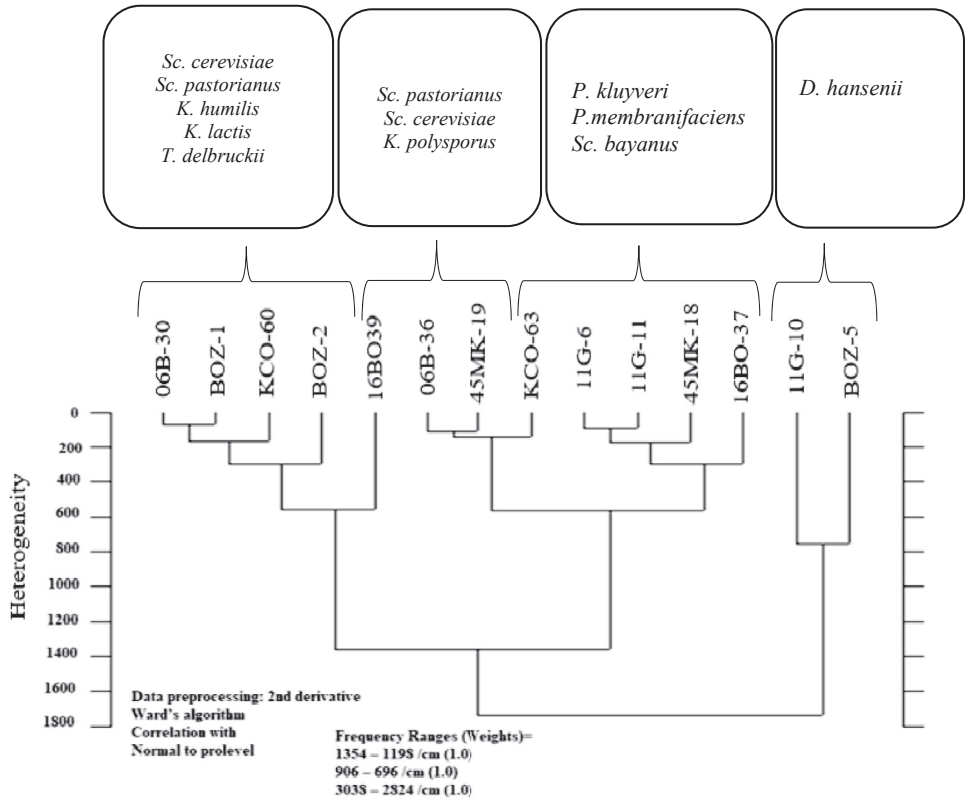


*Fig. 2.* Dendogram of yeast strains based on FT-IR analysis

determine the accuracy of the identification in our study, the 26s gene region of 4 yeast strains from the 14 yeast species was determined and sent for sequencing, and the results were found to be similar to that of the FT-IR.

## 3.3. Evaluation of autoaggregation and hydrophobicity abilities

Autoaggregation is a microscopic observation of cluster formation and depends on inorganic or extracellular characteristics of the cells. It is an important feature in regulating the formation of biofilm and colonisation of the colon (Kathade et al., 2020). In this study, the autoaggregation abilities of 14 yeast cultures after 2 h were determined, the highest autoaggregation value was observed in *Kluyveromyces lactis* BOZ-2 strain (96.36 ± 0.14%) (Fig. 3). Similar to our results, Guluarte et al. (2019) found in their study that the *K. lactis* yeast strain had more than 87% autoaggregation capability. It was observed that different yeast strains have different autoaggregation abilities. This may be due to their different cell structures or cellular aggregates, and aggregates increase the surface for attaching to the intestines (Hsu and Chou, 2021). Another important point in our study was that *D. hansenii* and *Saccharomyces* species had approximately the same autoaggregation values among themselves, and the values of *S. cerevisiae* strains showed similar results to findings of Speranza et al. (2020).

Hydrophobicity tests of yeast strains were tested against diethyl ether and *n*-hexadecane as hydrocarbon. Cell surface hydrophobicity is required for the colonisation of the digestive system and refers to the measurement of the attachment of hydrocarbons to cells. It also demonstrates the ability of LAB and yeast strains to adhere to intestinal epithelial cells and prevent colonisation of pathogens (Fekri et al., 2020). In our study, the highest hydrophobicity values of yeast strains against n-hexadecane and diethyl ether hydrocarbons were determined for *Sc. pastorianus* BOZ-1 (43.7%) and *Sc. pastorianus* 06B-36 (44.8%) (Fig. 4).

## 3.4. Antifungal sensitivity

Another important parameter for determining the probiotic properties of yeasts is the determination of their antifungal resistance capabilities. *K. humilis* KCO-60 strain showed the least resistance against all antifungals. Nystatin had the best activity against all yeast cultures. The

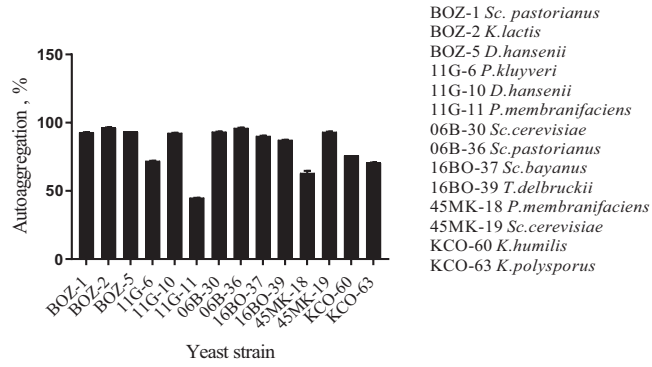


*Fig. 3.* Autoaggregation percent of selected yeast cultures isolated from sourdough

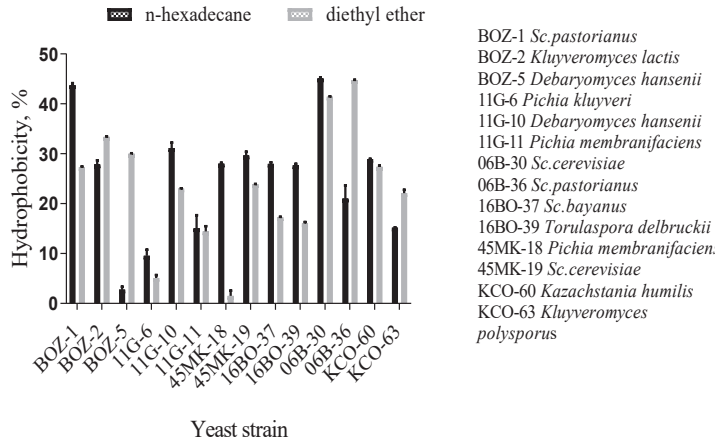


*Fig. 4.* Percentage of hydrophobicity of selected yeast cultures isolated from sourdough

*Pichia membranifaciens* 45MK-18 strain showed the highest resistance against antifungals. *S. cerevisiae* strains were found to be susceptible to almost all antifungals that were used in this study (Table 1).

*Table 1.* Antibiotic/antiyeast sensitivity of yeast strains

| Strains | KTC | FLU | AMB | CLT | NY |
|---|---|---|---|---|---|
| BOZ-1 | $1.0^{B}$ | $8.5^{AB}$ | $3.5^{ABC}$ | $2.5^{B}$ | $5.5^{BC}$ |
| BOZ-2 | – | $3.5^{C}$ | $3.5^{ABC}$ | – | $5.5^{BC}$ |
| BOZ-5 | – | $8.5^{AB}$ | $4.5^{AB}$ | – | $5.5^{BC}$ |
| 11G-6 | – | – | $1.1^{C}$ | $1.5^{B}$ | $3.5^{C}$ |
| 11G-10 | – | – | $2.7^{ABC}$ | – | $4.5^{C}$ |
| 11G-11 | – | $8.5^{AB}$ | $3.1^{ABC}$ | $13.0^{A}$ | $5.5^{BC}$ |
| 06B-30 | – | $9.5^{AB}$ | $3.5^{ABC}$ | $4.5^{B}$ | $4.5^{C}$ |
| 06B-36 | $1.5^{AB}$ | $7.5^{B}$ | $5.0^{A}$ | – | $4.5^{C}$ |
| 16BO-37 | – | $8.5^{AB}$ | $4.5^{AB}$ | $1.5^{B}$ | $5.5^{BC}$ |
| 16BO-39 | – | $9.5^{AB}$ | $2.5^{ABC}$ | – | $4.5^{C}$ |
| 45MK-18 | – | – | $1.1^{C}$ | – | $3.5^{C}$ |
| 45MK-19 | $1.0^{B}$ | $11^{A}$ | $2.2^{BC}$ | – | $5.5^{BC}$ |
| KCO-60 | $3.5^{A}$ | $9.5^{AB}$ | $4.5^{AB}$ | $2.5^{B}$ | $7.5^{AB}$ |
| KCO-63 | – | $3.5^{C}$ | $3.5^{ABC}$ | – | $9.5^{A}$ |

AMB: Amphotericin B, CLT: Clotrimazole, FLU: Fluconazole, KTC: Ketoconazole, NY: Nystatin. *Sc. pastorianus* BOZ-1, *K. lactis* BOZ-2, *D. hansenii* BOZ-5, *P. kluyveri* 11G-6, *D. hansenii* 11G-10, *P. membranifaciens* 11G-11, *Sc. cerevisiae* 06B-30, *Sc. pastorianus* 06B-36, *Sc. bayanus* 16BO-37, *T. delbruckii* 16BO-39, *P. membranifaciens* 45MK-18, *Sc. cerevisiae* 45MK-19, *K. humilis* KCO-60 and *K. polysporus* KCO-63 (results are given as the average diameter of the clearing zones around antifungal disks of two parallels, given in mm).
Means with different letters in the same column are significantly different based on a one-way ANOVA with Tukey's test ($P < 0.05$).

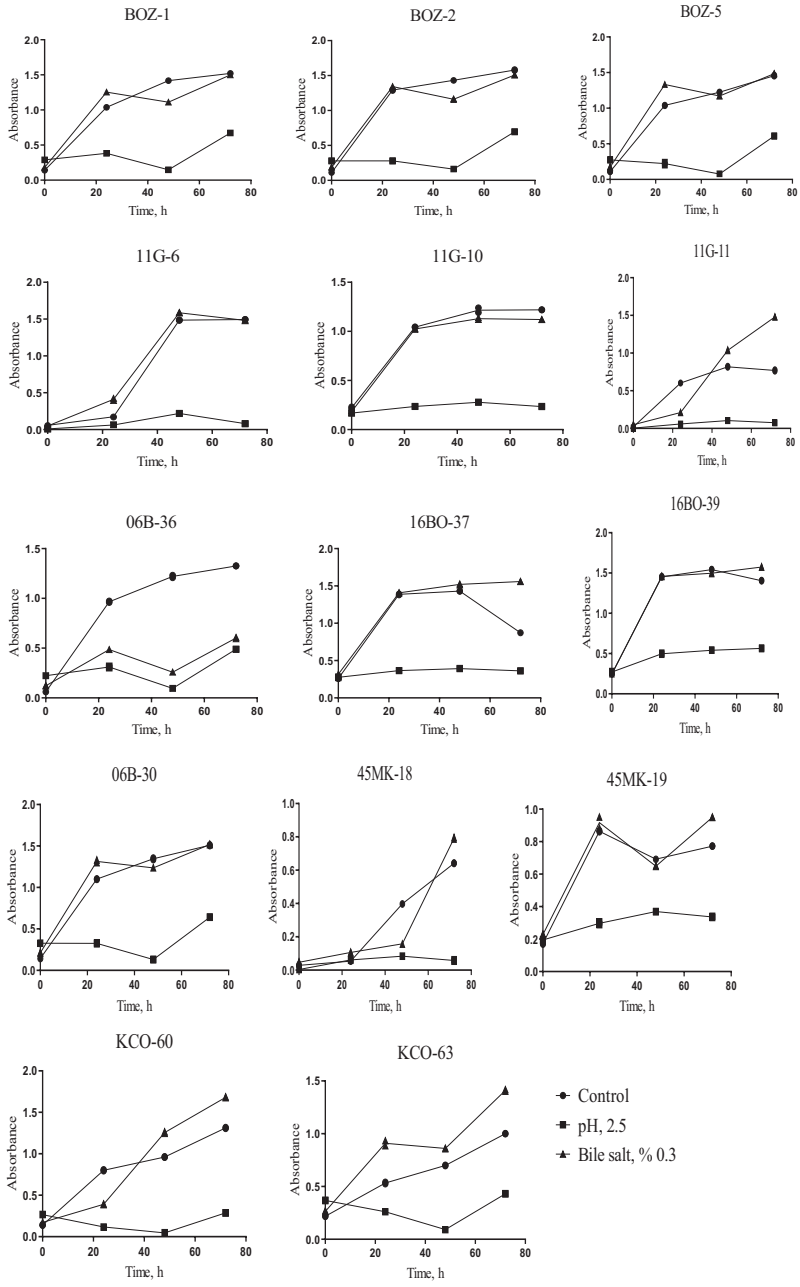


*Fig. 5.* The tolerance to low pH and bile resistance of yeast strains *Sc. pastorianus* BOZ-1, *K. lactis* BOZ-2, *D. hansenii* BOZ-5, *P. kluyveri* 11G-6, *D. hansenii* 11G-10, *P. membranifaciens* 11G-11, *Sc. cerevisiae* 06B-30, *Sc. pastorianus* 06B-36, *Sc. bayanus* 16BO-37, *T. delbruckii* 16BO-39, *P. membranifaciens* 45MK-18, *Sc. cerevisiae* 45MK-19, *K. humilis* KCO-60, and *K. polysporus* KCO-63 measured at 0, 12, 48, and 72 h

### 3.5. Determination of antimicrobial activity

Antimicrobial activity through peptides and organic acids plays a key role in probiotic properties (Offei et al., 2019). The antagonism of bacteria by yeasts is dependent on food composition, organic acids and ethanol production, or release of antimicrobial components such as mycosin (Hatoum et al., 2012). It was observed that not all yeasts in our study provided inhibition against pathogenic bacteria, though all yeasts except *Pichia kluyveri* 11G-6 isolate showed antibacterial activity against *S. aureus*, although the results were very low in mm. These results were similar to the findings of Andrade et al. (2019). These data suggest that the probiotic effect of yeasts against pathogens may be related to other mechanisms associated with the physical elimination of pathogenic bacteria.

### 3.6. Low pH and bile salt

Another important factor in the selection of probiotic microorganisms is survival in transit through the gastrointestinal tract. This vitality plays an important role in some beneficial properties (Diosma et al., 2014). The pH of the stomach varies from 2.5 to 3.5, which is lethal for many microorganisms. Several yeast species have been reported to survive under acidic conditions (pH 1.5) (Ogunremi et al., 2015). In this study, survival of yeast cultures at 0, 24, 48, and 72 h at pH 2.5 were evaluated. Our findings revealed that yeast species demonstrated strain specific characteristics for their survival at pH 2.5 during the 72 h of incubation period. For instance, no growth was observed for strains 11G-6, 11G-10, 11G-11, 45MK-18, 45MK-19, 16BO-37, and 16BO-39, whereas strains BOZ1, BOZ2, BOZ5, 06B-36, 06B-30, KCO-60, and KCO-63 showed an adaptation phase up to 48 h and then demonstrated a growth phase until the end of the test period of 72 h (Fig. 5). In terms of bile salt resistance, all yeast strains showed resistance to 0.3% bile salt in the first 24 h. Bile is a lipid emulsifying agent released into the duodenum after food intake, and since it has antimicrobial activity, probiotics must have a bile tolerance or exclusion mechanism to survive in the intestinal environment (Ogunremi et al., 2015).

## 4. CONCLUSIONS

Probiotics are important for both food and pharmaceutical industries. To find more yeasts with probiotic properties, strains were isolated from Turkish sourdoughs, and these yeast isolates were identified by FT-IR followed by genotypic confirmation. Fourteen yeast isolates were selected, and their potential as probiotics were investigated. Marked differences were seen among the isolates. The probiotic character of *Saccharomyces cerevisiae* strains among all selected yeast isolates was pronounced. In general, it can be said that the probiotic potential of some of the selected strains deserves further investigation.

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