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Evaluation of Probiotic, Antifungal, and Antioxidant Characteristics of *Pichia Kudriavzevii* Isolated from Sprouted Chickpea Sourdough

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HIGHLIGHTS

- The predominant yeast isolated from sprouted chickpea sourdough was characterized.
- The isolate showed proper survivability under simulated gastrointestinal conditions.
- Antibacterial and co-aggregation activities of the isolate were also approved.
- The isolate exhibited proper antifungal effect on Aspergillus flavus in the overlay bioassay.

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Abbreviations
CFU=Colony Forming Units
DPPH=DiPhenyl PicrylHydrazyl
PCR=Polymerase Chain Reaction
PTCC=Persian Type Culture Collection
SGI=Simulated GastroIntestinal
YGC=Yeast Extract Glucose Chloramphenicol

ABSTRACT

Background: It is important to evaluate the probiotic and functional characteristics of yeasts isolated from naturally fermented dough with complex microbial communities to provide proper microbial cultures. Probiotic yeasts have been studied less extensively than probiotic bacteria, despite their unique characteristics, such as the inability to transfer antibiotic resistance genes.

Methods: In the present study, the predominant yeast isolated from naturally sprouted chickpea dough, which harbors complex microbial communities, was identified using Polymerase Chain Reaction (PCR). Hemolytic activity and survival in the Simulated GastroIntestinal (SGI) conditions by yeast were investigated. Then, auto-aggregation, hydrophobicity, biofilm formation, and co-aggregation characteristics were measured using the absorbance technique. Furthermore, antibacterial effects (through the co-culture method), antioxidant activity (via examining DiPhenyl PicrylHydrazyl [DPPH] radical scavenging ability), and antifungal effect (using the overlay technique) were evaluated. The obtained data were analyzed statistically using SPSS version 20 and compared using one-way analysis of variance. Means were compared using the least significant difference test at a significance level of $p<0.05$.

Results: Sequencing of the PCR products led to the identification of *Pichia kudriavzevii* (as the predominant yeast isolated from naturally fermented sprouted chickpea dough). This isolate lacked hemolytic activity. Its survival rate under SGI conditions was 64.75%, and its auto-aggregation, hydrophobicity, and biofilm formation ability were 75.00%, 74.21%, and 4 Log Colony Forming Units (CFU)/cm^2, respectively. Moreover, the yeast isolate exhibited high antibacterial and co-aggregation ability with *Listeria monocytogenes*. In addition, *P. kudriavzevii* was resistant to ketoconazole, potassium sorbate, and calcium propionate, and showed strong antifungal activity against *Aspergillus flavus* as well as antioxidant activity (67.94% DPPH scavenging ability).

Conclusion: Accordingly, the isolate possesses suitable characteristics for use as a probiotic culture in food products, with special potential applications.

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Introduction

According to the World Health Organization (WHO), probiotics are live microorganisms that confer health benefits to the host when consumed in adequate amounts. The most important capabilities of probiotic are associated with their effects on the modulation of the gut microbiota and the immune system. Yeasts are eukaryotic microorganisms capable of producing a variety of enzymes and metabolites, thereby enhancing the nutritional value of products (Sadeghi *et al.*, 2022; Shruthi *et al.*, 2022). Over the past decade, many probiotics yeasts, such as *Saccharomyces*, *Debaryomyces*, *Hanseniaspora*, *Pichia*, *Meyerozyma*, and *Torulaspora*, have been identified in food and environmental habitats (Sadeghi *et al*., 2023; Staniszewski and Kordowska-Wiater, 2021). For example, after investigating the health-promoting functional attributes and antioxidant activity of *Pichia kudriavzevii* isolated from kefir, Maione *et al.* (2024) reported that the isolate had favorable health-promoting capabilities that make it a promising candidate for functional foods. Furthermore, Lata *et al*. (2022) isolated *P. kudriavzevii* from pickled mango and investigated its probiotic traits. These researchers concluded that the yeast isolate had the ability to tolerate high concentrations of bile salt and showed good viability in the Simulated GastroIntestinal (SGI) conditions. Probiotics can survive in the human SGI tract under unfavorable conditions (in the presence of digestive enzymes, pancreatic juice, and low pH) and contribute to the host's health by regulating gut microbiota and attaching to intestinal epithelial cells (Fijan, 2014). Greppi *et al.* (2017) evaluated the health-promoting functional attributes of *P. kudriavzevii* strains and reported that the survival of these yeasts after treatment in SGI conditions was between 11-45% and their auto-aggregation rate was equal to 12-40%.

The use of sprouted legumes as fermentation substrates is significant from various aspects. The sprouting process stimulates enzymatic activity, leading to the breakdown of polysaccharides and proteins while increasing the levels of dietary fibers, amino acids, and polyphenolic compounds (Hung *et al.*, 2012; Setia *et al.*, 2019). Perri *et al*. (2020) examined the effect of the sprouting process on the diversity of yeasts and Lactic Acid Bacteria (LAB) in legumes, cereals, and pseudo-cereals. Their findings indicated that the yeast population in the tested samples increased after the sprouting process. Moreover, the results of the aforementioned study showed that a high percentage of the fungal population in sprouted peas and sprouted lentils belongs to *Glomeromycota*. To date, several reports have described the health-promoting functional attributes of yeasts isolated from sprouted legumes. For example, Falfán-Cortés *et al*. (2022) reported that a selected strain from sprouted beans exhibited the ability to survive in the presence of 1% bile salt and at pH 3, and demonstrated the highest antimicrobial effect against *Salmonella Typhimurium*. Hsiung *et al.* (2021) isolated and identified yeasts from various fermented foods and studied their health-promoting functional attributes. Their results indicated that the isolated yeasts were able to survive in SGI conditions and had high auto-aggregation capabilities.

Each fermented ecosystem is a unique resource for the isolation of potential probiotic strains. So far, there has been no report on the use of fermented sprouted chickpea for the isolation of probiotic yeasts. Therefore, the objective of this study was to isolate the predominant yeast from the aforementioned fermented dough and investigate its probiotic, antifungal, and antioxidant properties.

Materials and methods

Materials

All chemical reagents and microbial media used in the present study were of analytical grade. The studied foodborne bacteria and fungi were also supplied from the Persian Type Culture Collection (PTCC). Xylen (1.08661.2500, Merck, Germany), DiPhenyl PicrylHydrazyl (DPPH; 1898-66-4, Sigma, USA), bile salts (MFCD00130648, Sigma, USA), pepsin (9001-75-6, Sigma, USA), and pancreatin (8049-47-6, Sigma, USA) from porcine were also used.

Preparation of the substrate and sprouting process

After preparing chickpeas, the sprouting process was carried out according to Donkor *et al.* (2012) for five days, and then the sprouted seeds were dried at 55 °C and milled.

Preparation of fermented sprouted chickpea (naturally fermented dough)

The naturally fermented dough was prepared with a dough yield (ratio of dough to flour ×100) of 160 and incubated for 24 h at 28 °C (Perri *et al*., 2020).

Isolation of the predominant yeast

After preparing serial ten-fold dilutions of the naturally fermented sprouted chickpea dough and surface plating on Yeast Extract Glucose Chloramphenicol (YGC) agar, the predominant yeast was isolated and counted. The morphology of the yeast was also examined (Palla *et al.*, 2019).

Molecular identification of the predominant yeast

The DNA of the predominant isolate was extracted using a commercial kit (GeneAll, South Korea). In brief, yeast cells (overnight culture, ~10^6 cells) were harvested and subjected to enzymatic–mechanical lyses. Proteins were

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removed with proteinase K. RNA was eliminated by RNase A treatment. DNA was also purified using purification column of the kit according to the manufacturer's instructions. The isolate was then identified using Polymerase Chain Reaction (PCR) with Internal Transcribed Spacer (ITS) primers, including ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) in accordance with White *et al.* (1990). The amplification reagents were including 1.0 U Taq DNA polymerase, 1× reaction buffer, 1.5 mM $MgCl_2$ for final runs, 0.20 mM dNTPs, 0.4 µM each ITS1 and ITS4 primers, and 10–50 ng template DNA. DNA concentration and purity were verified by NanoDrop (A260/A280 1.8–2.0; A260/A230 >2.0). The thermal-cycling included initial denaturation at 95 °C for 3 min; then 30 cycles of denaturation at 95 °C for 30 s; annealing at 55 °C for 30 s; extension at 72 °C for 45 s; and a final extension at 72 °C for 5 min. Consequently, the PCR product was sequenced (Pishgam Company, Iran) after agarose gel electrophoresis. Each PCR run included a negative control (no-template control) to exclude contamination and a positive control (genomic DNA from a reference yeast strain previously verified by ITS sequencing) to confirm assay performance. Finally, the maximum-likelihood tree with bootstrap support (figure 2) was constructed to illustrate the evolutionary relationship of the isolate according to Tamura *et al.* (2013).

Examination of carbohydrate fermentation profile

After preparing an oxidative-fermentative medium containing a 10% solution of each carbohydrate (glucose, sucrose, raffinose, mannitol, lactose, and xylose), the yeast isolate was inoculated into the medium and incubated for 24 h. Finally, the color change of the medium indicated the fermentation of the sugars used (Lata *et al.*, 2022).

Hemolytic activity assessment

For this purpose, the predominant isolate was cultured on blood agar containing 5% sheep blood and incubated at 37 °C for 24 h. Then, the formation of halos and color changes in the culture medium were examined (Bonatsou *et al.*, 2018).

Survival ability of the predominant yeast in SGI conditions

The survival rate of the yeast under SGI conditions was assessed after culturing on Brain Heart Infusion (BHI) broth and adjusting the isolate population (10^6 Colony Forming Units (CFU)/ml). Its ability to withstand gastric (pH 2, 0.1% pepsin) and i-ntestinal (pH 8, 0.3% bile salts and 0.1% pancreatin) conditions were examined for separate 1.5 h at 37 °C (Rolim *et al.*, 2015).

Investigation of adhesion capabilities

In the present study, the auto-aggregation capability using absorbance method at OD = 600 nm (Gil-Rodriguez, Carrascosa and Requena, 2015), hydrophobicity using xylene (Fadda *et al.*, 2017), and biofilm formation based on attachment to glass slides (Speranza, Corbo, and Sinigaglia, 2011) were assessed.

Assessment of antibacterial effects

For this purpose, chromogenic culture media (Chromagar, France) specific to each foodborne bacterium, including *Staphylococcus aureus* PTCC 1112, *Escherichia coli* PTCC 1399, *Listeria monocytogenes* PTCC 1298, and *Salmonella enterica* PTCC 1709 were used to investigate the inhibitory effects of the predominant isolate through co-culturing (Zarali *et al.*, 2023).

Assessment of co-aggregation capability

After adjusting the yeast population (10^6 CFU/ml), a mixture of the yeast suspension and each studied foodborne bacterium was prepared with the same volume and population. After four h of incubation, the OD of the suspensions was recorded by a spectrophotometer (PGI, UK) at a wavelength of 600 nm, and the co-aggregation capability was calculated using the following equation:

$$\text{Co-aggregation } (\%)=[(A_p+A_y)/2-(A_{mix})/(A_p+A_y)/2]\times 100$$

Where, A_p is the absorbance of each foodborne bacterium suspension, A_y is the absorbance of yeast suspension, and A_{mix} is the absorbance of the mixture of yeast and foodborne bacterium suspension (Maione *et al.*, 2024).

Assessment of antimycotic resistance

The disc diffusion bioassay was used to investigate the anti-mycotic susceptibility of the yeast isolate. A mixture of overnight-cultured yeast (10^8 CFU/ml) and YGC agar was poured into plates. Then, the discs were placed on the plates, and the diameter of the inhibition zone was determined after 24 h incubation at 28 °C (Rahimi *et al.*, 2024).

Evaluation of antioxidant capacity

After adjusting the yeast population (10^6 CFU/ml) and centrifuging (Centurion, UK) at 12,000 rpm for 5 min, the ability of the yeast isolate to inhibit DPPH radicals was evaluated. The DPPH solution was added to the suspension, and after 30 min, it was centrifuged again (12,000 rpm for 5 min), and absorbance was measured at a wavelength of 517 nm. Finally, the antioxidant capacity of the isolate was determined using the following equation:

$$\text{Antioxidant activity } (\%)=[1-(A_s/A_b)]\times 100$$

Where, A_s and A_b represent the absorbance of the sample and the absorbance of the control sample, respectively (Gil-Rodriguez, Carrascosa and Requena, 2015).

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Antifungal activity of the isolate

For this purpose, a dual-layer cultivation method (overlay bioassay) was used against *Aspergillus flavus* PTCC 5006. Initially, yeast was cultured as two parallel lines on YGC agar, and after 48 h of incubation, fungal spores (10^4 spores/ml) were mixed with Potato Dextrose Agar (PDA) culture medium and poured onto the plates. The plates were incubated at 28 °C until the control sample (without yeast isolate) covered the entire surface of the plate (Ruggirello *et al.*, 2019).

Statistical analysis

The results obtained were analyzed using one-way analysis of variance, and means were compared using the Least Significant Difference (LSD) test at a significance level of $p<0.05$. All tests were performed in triplicate, and SPSS version 20 and Microsoft Office Excel 2016 software were used for data analysis and drawing the charts, respectively.

Results and discussion

Identification of the predominant yeast isolate

Based on the images obtained from the gel electrophoresis of the PCR products (Figure 1), the specific amplification of the target sequence of the yeast isolate was confirmed. According to the sequencing results of the PCR products and their comparison with data from the National Center for Biotechnology Information (NCBI) database, the yeast *P. kudriavzevii* HSP04 was identified with a similarity of 96% as the predominant yeast isolated from naturally fermented sprouted chickpea dough.

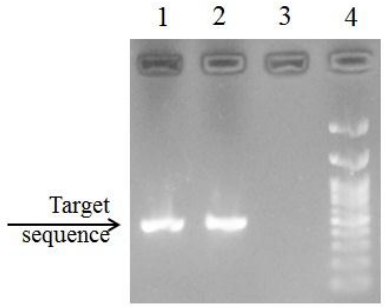

Figure 1: Gel electrophoresis of the Polymerase Chain Reaction (PCR) products obtained from amplification of the target sequence (the Internal Transcribed Spacer [ITS] region) of the predominant yeast isolated from naturally fermented sprouted chickpea dough with complex microbial communities (lane 1) compared to the positive and negative control samples (lane 2 and 3, respectively) and 100 bp DNA ladder (lane 4). The expected Internal Transcribed Spacer (ITS) amplicon for yeast is ~600 bp.

In studies with similar substrates, *Debaryomyces* was the predominant yeast in sprouted chickpea flour (Perri *et al.*, 2020), and *Saccharomyces cerevisiae* was predominant in naturally fermented chickpea dough (Munch-Andersen *et al.*, 2024). Given the similarity of the substrate in these studies compared to the present research, the predominant microbial flora differs, which can be attributed to the effects of the sprouting and fermentation processes. The fermentation process creates stress, alters the dough's Water Activity (Aw), and the back-slopping process changes the pH, thereby modifying the microbial flora of naturally fermented dough with complex microbial communities. Moreover, the creation of an acidic environment during back-slopping reduces the likelihood of yeast presence and allows Lactic Acid Bacteria (LAB) to dominate (Munch-Andersen *et al.*, 2024; Perri *et al.*, 2020).

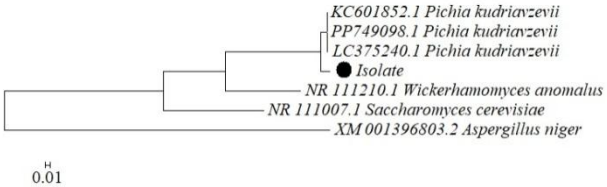

Figure 2: The maximum-likelihood tree with bootstrap support to illustrate the evolutionary position of the *Pichia kudriavzevii* isolate. The bar indicates 1% differentiation and *Aspergillus niger* was used as an out-group.

Carbohydrate fermentation profile

The carbohydrate fermentation profile of the predominant yeast also indicated the genus *Pichia*, as it could only ferment glucose among the studied carbohydrates. In the research by Lata *et al.* (2022), the results of the carbohydrate fermentation pattern by *P. kudriavzevii* Y33 were entirely in line with the current study. The mentioned yeast could ferment glucose but was unable to ferment lactose, sucrose, xylose, raffinose, and mannitol. Examining the carbohydrate fermentation pattern is a method for the preliminary identification of yeasts. Yeasts typically ferment simple carbohydrates like glucose easily, but they require specific enzymes to ferment more complex carbohydrates. For example, *Kluyveromyces lactis*, due to its lactase enzyme, is capable of fermenting lactose (Varela *et al.*, 2019).

Hemolytic activity

In this research, it was found that the predominant yeast isolate lacks hemolytic activity, meaning that the yeast does not have the ability to destroy red blood cells and create a clear area in the blood-supplemented medium. Considering the importance of probiotic safety, this finding is an important factor for using this yeast in food and probiotic products. In this regard, Fernández-Pacheco *et al.* (2021) also reported that 20 tested yeast strains lacked hemolytic activity in blood-containing media. Furthermore, in another study, hemolysis was not observed with *Pichia* (Ganapathiwar and Bhukya, 2023). Evaluating the hemolytic activity of yeasts is known as one of the most

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important assays in the field of probiotic safety. The ability of hemolysis or destruction of red blood cells can potentially lead to pathogenicity. If the yeast can create a clear area or color change in the blood supplemented medium, it indicates the yeast ability to destroy red blood cells and subsequently cause potential problems in human health (Sadeghi *et al.*, 2022).

Survival in SGI conditions

In this study, *P. kudriavzevii* isolate showed a survival rate of 75.64±3.96% under SGI conditions. Previous research has shown that *P. kudriavzevii* has over 50% survival capability in SGI conditions (Ganapathiwar and Bhukya, 2023). Recent studies have demonstrated that *P. kudriavzevii* can resist acid and bile salt and has a high survival rate in SGI conditions (Maione *et al.*, 2024; Alvarez *et al.*, 2023). The findings of Menezes *et al.* (2020) indicated that out of 116 yeast isolates, 36 isolates were able to survive in SGI conditions (pH = 2, 0.3% bile salt at 37 °C). These results are a prerequisite for probiotic microorganisms and they are consistent with findings previously reported for potential probiotic strains. A survival rate of about 70% in SGI conditions is considered suitable for probiotics.

Auto-aggregation, hydrophobicity, and biofilm formation capabilities

In the present study, the auto-aggregation, hydrophobicity, and biofilm formation values of *P. kudriavzevii* isolate were 75.00±5.58%, 74.21±1.11%, and 4 log CFU/cm², respectively. In a similar study, Lata *et al.* (2022) reported that the auto-aggregation and hydrophobicity of *P. kudriavzevii* Y33 were 72.45% and 16.60%, respectively. Alkalbani *et al.* (2022) also investigated the auto-aggregation capability of the studied yeasts and reported that the auto-aggregation rate of yeasts varied between 50.7 and 85.8%. Additionally, the results of Fernandez-Pacheco *et al.* (2018) showed that the biofilm formation of 10 yeast strains with an initial population of 10^6 CFU/ml after 72 h of incubation ranged from 3.5 to 5.25 log CFU/cm². Perricone *et al.* (2014) concluded that the biofilm formation ability of the studied yeasts after 4 days of incubation was between 1.75-5.10 log CFU/cm^2. In another study comparing various probiotic yeasts, it was found that *P. kudriavzevii* had higher hydrophobic properties than other yeasts, resulting in better attachment and biofilm formation capabilities (Alvarez *et al.*, 2023). Probiotics with about 30-40% hydrophobicity can interact with mucus and establish at least one short, unstable connection (Ilavenil *et al.*, 2016). Intestinal colonization by microorganisms often leads to the formation of biofilm through adhesion to the intestinal mucosa, and these phenomena (adhesion and biofilm formation) are positively correlated (Arevalo-Villena *et al.*, 2017). By forming a biofilm and providing a physical barrier against microbial invaders, hydrophobic yeasts can help maintain the balance of the gut microbiome and the overall health of the host (Maione *et al.*, 2024).

Antibacterial and co-aggregation capabilities of the yeast isolate

The results of the antibacterial and co-aggregation capabilities of the yeast isolate are shown in Table 1. Accordingly, the inhibitory activity against *L. monocytogenes* was significantly (p<0.05) higher than that against other studied bacteria. Additionally, while the highest co-aggregation rate was observed with *E. coli*, there was no significant difference compared to *L. monocytogenes*. In a study, the co-aggregation of *P. kudriavzevii* with *L. monocytogenes*, *E. coli*, and *Salmonella* was reported to be about 80% (Maione *et al.*, 2024). Reports have confirmed a correlation between antimicrobial effects and co-aggregation ability. The mechanism of antibacterial and aggregation potential of *P. kudriavzevii* depends on various factors. The production of metabolites that neutralize bacterial toxins and the modulation of the host cell's signaling pathway in the pro-inflammatory response during bacterial infection are among these modes of action (Maione *et al.*, 2024). Bacterial pathogens rapidly bind to the mannan on the yeast cell-wall instead of attaching to enterocytes, and upon agglutination by yeast, they can be removed from the host's SGI tract (Lata *et al*., 2022). Probiotic yeasts prevent the attachment of pathogens to epithelial cells by competing for binding sites on intestinal cells and producing polysaccharides, thereby exerting their antibacterial effects. Additionally, yeasts provide protective roles by directly attaching to pathogenic bacteria and preventing their interaction with the host (Alvarez *et al*., 2023; Rahimi *et al*., 2024).

Table 1: Antibacterial and co-aggregation capabilities of *Pichia kudriavzevii* isolate with the studied foodborne bacteria.

Foodborne bacteria	Co-aggregation (%)	Antibacterial effect (%)
Escherichia coli	64.24±3.74 ^{a}	64.55±3.58 ^{b}
Staphylococcus aureus	51.99±3.43 ^{b}	70.77±0.91 ^{b}
Listeria monocytogenes	59.27±1.29 ^{ab}	79.38±2.91 ^{a}
Salmonella enterica	60.84±1.90 ^{a}	52.42±2.49 ^{c}

The different letters in each column indicate significant difference at p<0.05.

Antimycotic susceptibility

According to the results obtained, the *P. kudriavzevii* studied in this research was resistant to ketoconazole, potassium sorbate, and calcium propionate, while it was sensitive to itraconazole (with 21.70±0.62 mm diameter of

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inhibition) and natamycin (with 24.56±1.69 mm diameter of inhibition). In a study, *P. kudriavzevii* was found to be sensitive to ketoconazole and itraconazole (Ganapathiwar and Bhukya, 2023). In another study, *P. kudriavzevii* Y1 was sensitive to ketoconazole and resistant to itraconazole (Maione *et al.*, 2024). Thus, yeasts exhibit antimycotic susceptibilities that are strain-dependent and, because they cannot transfer resistance genes, they have more practical applications compared to probiotic bacteria (Maione *et al.*, 2024). Furthermore, the resistance of the yeast studied in the present study towards potassium sorbate and calcium propionate allows its use in products containing these preservatives. Additionally, resistance to ketoconazole enables the effective simultaneous use of the yeast in patients undergoing ketoconazole treatment.

Antioxidant activity of the isolate

In the present study, the ability of *P. kudriavzevii* isolate to inhibit DPPH free radicals was equal to 67.94±2.93%. According to a report by Gil-Rodriguez, Carrascosa and Requena (2015), antioxidant activity levels of yeasts ranging from 20% to 30% indicate low activity, 30% to 40% indicate good activity, 40% to 50% indicate very good activity, and over 50% indicate excellent activity. Based on this classification, the yeast isolate studied in the present research demonstrates excellent antioxidant activity, making it a probiotic yeast with practical capabilities. In the study by Wulan *et al.* (2021), the antioxidant activity of strains of *P. kudriavzevii* ranged from 59.67% to 68.51%. Due to the production of various antioxidant compounds, yeasts can play an effective role in scavenging of free radicals. Free radicals are unstable molecules that can damage cells with harmful chemical reactions and cause oxidative stress in the body. Oxidative stress is one of the main causes of cell damage and various diseases. Antioxidant activity helps mitigate the harmful effects of free radicals in the body and represents an important advantage for host health. The mechanism of free radical scavenging may be attributed to β-glucans, α-mannans, mannoproteins, lipids, and cell-wall proteins of the yeast (Aydın *et al.*, 2024).

Antifungal activity of the isolate

As shown in Figure 3, the antifungal effect of the *P. kudriavzevii* isolate after four days on *A. flavus* was 44.19±2.02%. In another study, *P. kudriavzevii* demonstrated a growth inhibition capability of 33.88% against *A. flavus* (Shahryari *et al.*, 2022). The results of another study showed that the probiotic *S. cerevisiae* had effective antifungal properties against *A. flavus* and was able to reduce the growth of this fungus between 8.9-56.8% (Ruggirello *et al.*, 2019). Considering that *A. flavus* is one of the pathogenic and dangerous fungi for humans and can cause serious problems including lung infections, the use of yeasts as a preventive and therapeutic solution can be used against such infections is promising. Antioxidant compounds in yeast can not only maintain cell health by reducing the effects of free radicals, but also act as a defense against harmful microorganisms such as fungi. Meanwhile, some antioxidant compounds have direct effects on the metabolism of fungi and prevent their growth or reproduction through disrupting their metabolic pathways (Khan *et al.*, 2011). In this regard, due to its excellent antioxidant activity, *P. kudriavzevii* not only can help reduce the activity of fungi, but also prevent the occurrence of fungal spoilage in food products and can be used as a natural treatment option against fungal infections.

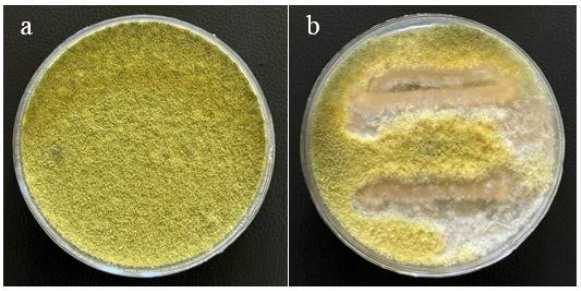

Figure 3: Antifungal activity of *Pichia kudriavzevii* isolate against *Aspergillus flavus* (b) compared to the control sample (a).

Conclusion

In the present study, *P. kudriavzevii* was identified as the predominant yeast species in naturally fermented dough prepared from sprouted chickpea, which contained a complex microbial community. A detailed evaluation of this isolate revealed that it exhibited no hemolytic activity, indicating that it does not harm red blood cells and is safe for potential applications in the food industry. In addition, the isolate demonstrated strong adhesion properties and was able to survive under SGI conditions, suggesting its potential resilience and functionality as a probiotic. Notably, *P. kudriavzevii* also displayed significant antibacterial activity and co-aggregation ability against *L. monocytogenes*, further highlighting its beneficial probiotic attributes.

Beyond its probiotic potential, the isolate showed remarkable antioxidant and antifungal activities. The observed antifungal effects may be attributed to the yeast's ability to produce antioxidants and reduce oxidative stress —mechanisms that are particularly valuable in preventing fungal infections. Such infections in the food and agricultural sectors can lead to severe consequences, including decreased product quality, economic losses, and adverse health effects for humans and animals. Therefore, the application of this yeast as a protective culture in food products could enhance both food safety and quality while

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reducing the risk of fungal contamination.

Overall, the findings of the present study demonstrate that *P. kudriavzevii* possesses multiple advantageous properties, making it a promising candidate for probiotic and protective applications. By leveraging its functional traits, this yeast could be used to develop innovative and sustainable strategies to improve food quality, ensure public health, and address challenges related to fungal contamination. Consequently, the results provide a foundation for the optimal use of yeasts across various industrial sectors and support their potential role in advancing food security and safety.

Author contributions

A.S. and S.M.J. supervised the research, reviewed the manuscript, and editing; F.H., M.E. and K.T.H. accomplished the data analysis and wrote the manuscript as well. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Ethical consideration

Not applicable.

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