Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Evaluation of probiotic, antifungal, and antioxidant properties of the predominant yeast isolated from acorn sourdough

Hossein Purabdolah[1], Alireza Sadeghi[1*], Seid Mahdi Jafari[1,2], Maryam Ebrahimi[3], Hoda Shahiri Tabarestani[1]

1-Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
2-Halal Research Center of IRI, Iran Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran.
3- Food, Drug and Natural Products Health Research Center, Golestan University of Medical Sciences, Gorgan, Iran.

ARTICLE INFO

Article History:

Received: 2024/11/12
Accepted: 2025/05/11

Keywords:

Acorn sourdough,
yeast isolate,
probiotic properties,
antifungal effect,
antioxidant activity.

DOI: 10.22034/FSCT.22.160.162.

*Corresponding Author E-Mail:
sadeghi.gau@gmail.com;
asadeghi@gau.ac.ir

ABSTRACT

There is always the possibility of encountering probiotic yeasts with functional capabilities in natural habitats that have been less studied. In the present study, the predominant yeast from acorn sourdough was isolated and identified using PCR. The probiotic properties of the isolate, as well as its antifungal and antioxidant activities were also investigated. Sequencing results of PCR products led to the identification of *Pichia kudriavzevii* as the predominant yeast isolate. The survival rate of the isolate in simulated gastrointestinal conditions was 91.93%. The auto-aggregation ability of the isolate was equal to 84.65%, and its hydrophobicity against hexane and xylene was 35.15% and 21.70%, respectively. The antibacterial activity of *P. kudriavzevii* studied in this research against *Listeria monocytogenes* was 85.58%, which was significantly (P<0.05) higher than other studied foodborne bacteria. However, the co-aggregation ability of the yeast isolate against tested pathogens showed no significant difference. Furthermore, the isolate showed no hemolytic activity, and it was resistant to all tested antibiotics, but showed relative sensitivity to the antimycotic agents including itraconazole, ketoconazole, and natamycin, while being resistant to potassium sorbate. The antifungal activity of the isolate against *A. flavus* was also confirmed, with antioxidant activity measured at 78.67%. Accordingly, *P. kudriavzevii* yeast isolate can be introduced as a suitable candidate for use as a probiotic and/or protective culture in fermentation industries.

[DOI: 10.22034/FSCT.22.160.162]

1- Introduction

Probiotics are live microorganisms that positively affect human health when consumed in an adequate population. Some of the benefits of probiotics include improved digestion, maintaining gut microbiome balance, modulation of the immune system, reducing symptoms of gastrointestinal diseases, and even lowering the risk of various infections [1,2]. Probiotic microorganisms include lactic acid bacteria and yeasts. Unlike probiotic bacteria, the probiotic properties of yeasts have been less studied. These eukaryotic microorganisms offer potential benefits such as antimicrobial activity, resistance to acid and bile salts, adhesion to the mucosal surfaces of the digestive tract, antioxidant properties, and the ability to reduce fungal biohazards. One key advantage of yeasts over probiotic bacteria is their resistance to antibiotics and the absence of gene transfer for resistance, which allows them to remain active during antibiotic treatment and help restore gut microbial flora without the risk of transferring antibiotic resistance genes, as sometimes observed with probiotic bacteria [3,4].

To date, research has been conducted on isolating probiotic yeasts from non-dairy substrates, particularly fermented cereals and pseudo-cereals. For example, Shahryari et al. [5] reported that the isolated yeast from buckwheat sourdough demonstrated good survival in simulated gastrointestinal (SGI) conditions, and in addition to suitable adhesion and hydrophobicity, significantly inhibited foodborne pathogens. Shruthi et al. [6] also isolated 73 yeasts from traditional fermented foods in India and screened them based on antimicrobial activity, selecting 10 yeasts for further probiotic evaluation. According to their report, all 10 isolates showed resistance to SGI conditions, with a survival rate of over 50%. Additionally, the isolated yeasts had adhesion abilities exceeding 40% and exhibited strong antioxidant properties. Similarly, Greppi et al. [7] isolated probiotic yeast from traditional African fermented foods and identified *Pichia kudriavzevii* M28, reporting that the yeast had a survival rate of 40.1% in gastric juice and 19.5% in pancreatic juice, with an auto-aggregation rate of 38.9%.

Alkalbani et al. [8] also isolated 12 yeasts, including *Saccharomyces cerevisiae* OK441070 and *P. kudriavzevii* OK441060, from fermented dairy and non-dairy products, and after conducting probiotic tests, reported that the survival rate of the tested strains ranged from 69% to 89%. Moreover, all the isolates exhibited proper antimicrobial properties, hydrophobicity, and auto-aggregation ability. In another study, Lara-Hidalgo et al. [9] evaluated the probiotic properties of *P. kudriavzevii* IPNFG1, *Wickerhamomyces anomalus* IPNFG3, and *Hanseniaspora opuntiae* IPNFG2 isolated from fermented foods and reported that their adhesion to mucin and survival rates in SGI conditions were comparable to commercial strains, with auto-aggregation rates exceeding 90%. The study also confirmed the co-aggregation ability with pathogens, antioxidant capacity, and antimicrobial activity of the yeasts.

Based on the literature review, no reports of probiotic yeast isolation from acorn sourdough have been documented so far. Accordingly, this study aims to evaluate the probiotic, antifungal, and antioxidant properties of the predominant yeast isolated from acorn sourdough.

2-Materials and Methods

Preparation of raw materials

The foodborne microorganisms used in this study (*Escherichia coli* PTCC 1399,

[DOI: 10.22034/FSCT.22.160.162]

Staphylococcus aureus PTCC 1112, *Listeria monocytogenes* PTCC 1298, *Salmonella enterica* PTCC 1709, and *Aspergillus flavus* PTCC 5018) were purchased from the Persian type culture collection (PTCC) of the Iranian research organization for science and technology. The microbial culture media and chemicals were purchased from commercial brands with analytical grade like Merck (Germany) and Chromagar (France). For acorn flour preparation, acorns were procured from a local market, and after removing the outer and inner shells, the kernels were milled and sieved into flour. The properties of the acorn flour were determined based on standardized methods [10]. The acorn flour used in the present study contained 5.7% fat, 6.4% protein, 6.8% reducing sugars, and 8.3% moisture.

Spontaneous fermentation of acorn

A dough yield of 160 (equivalent to 100 g of flour and 60 mL of sterile distilled water) was prepared, and the mixture was fermented for 24 h at 25 °C [11]. To determine pH and total titratable acidity (TTA), 10 g of the sourdough sample was mixed with 90 mL of distilled water and titrated using 0.1 N sodium hydroxide (NaOH) to reach a pH of 8.5. TTA was expressed based on the volume of NaOH used [12], and the pH was measured with a pH meter.

Isolation and identification of the predominant yeast isolate

For isolation, serially ten-fold dilutions of the spontaneously fermented acorn were prepared in ringer solution, and then it was surface-plated on yeast glucose chloramphenicol (YGC) agar medium. After incubation for 24-72 h at 25 °C, single colonies were obtained using the streak plate method [13]. For molecular identification, the predominant yeast's DNA was extracted using a commercial kit (Geneall, South Korea). The DNA was amplified using PCR with internal transcribed spacer (*ITS*) primers *ITS1*: 5′-TCCGTAGGTGAACCTGCGG-3′ and *ITS4*: 5′-TCCTCCGCTTATTGATATGC-3′ [14], and then the PCR products were separated using electrophoresis in 1.5% agarose gel (for 40 min at V= 90 at Tris boric acid EDTA or TBE buffer) and subsequently they were sequenced (Pishgam, Iran). The obtained data was compared with the data available in the National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (BLASTn) algorithm.

Survival of the predominant yeast in simulated gastrointestinal conditions

The yeast isolate was adjusted to a population of 10^8 colony forming units (CFU)/mL and exposed to SGI conditions (pH 2 with 0.1% pepsin for 2 h, followed by pH 8 with 0.3% bile salt and 0.1% pancreatin for 3 h). Survival was determined by surface plating of serially ten-fold diluted samples on YGC agar compared to untreated control sample [15].

Hemolytic activity of the yeast isolate

The yeast isolate was cultured on blood agar containing 5% sheep blood to observe any hemolysis signs or color changes [16].

Auto-aggregation ability of the yeast isolate

The yeast population was adjusted in phosphate-buffered saline (PBS) to 10^8 CFU/mL, and the suspension was incubated at 25 °C for 24 h. The absorbance of the yeast suspension at 600 nm was measured using a spectrophotometer (PGI, UK) and auto-aggregation was calculated using the following equation:

$$\text{Auto-aggregation\%} = [1- (A_f/A_0)] \times 100$$

Where, A_f and A_0 are the absorbance values at the end and beginning of the incubation period, respectively [17].

Hydrophobicity of the predominant yeast

[DOI: 10.22034/FSCT.22.160.162]

The yeast population was adjusted in PBS (10^8 CFU/mL), and 3 mL of the yeast suspension was mixed with 1 mL of xylene or hexane. After vortexing for 30 seconds and incubating at 25 °C for 4 h, the absorbance of the yeast suspension was measured at 600 nm. Hydrophobicity was also calculated using the following formula:

Hydrophobicity% = $[(A_a - A)/A_a] \times 100$

Where, A_a is the absorbance at the beginning and A at the end of the incubation period [18].

Antibacterial activity of the isolate

Fresh 24-hour cultures of foodborne pathogens (*E. coli*, *S. aureus*, *L. monocytogenes*, and *S. enterica*) were prepared and their populations were adjusted. Then, equal populations of the yeast isolate and each bacterium (10^8 CFU/mL) were mixed and incubated for 24 h in brain heart infusion (BHI) broth. Serially ten-fold dilutions of the suspension were surface-plated on specific chromogenic media. After 24 h of incubation at 37 °C, colonies were counted and compared to the control [19].

Co-aggregation ability of the isolate

Equal volumes of yeast and selected foodborne bacterium suspensions (10^8 CFU/mL) were mixed and incubated for 4 h. Absorbance at 600 nm was recorded, and co-aggregation was calculated using the following formula:

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

Where, A_p is the absorbance of the bacterial suspension, A_y is the absorbance of the yeast suspension, and A_{mix} is the absorbance of the yeast-bacteria mixture [9].

Antibiotic and antimycotic susceptibility of the isolate

YGC agar plates with surface cultured yeast were overlaid with antibiotic and antimycotic disks. The inhibition zones diameters were measured and ≤ 14 mm, 15-19 mm and ≥ 20 mm diameters indicating resistance, intermediate sensitivity and sensitivity, respectively [20]

Antifungal activity of the yeast isolate

The overlay method was used against *A. flavus*. After 48 h incubation of yeast cultured on YGC agar, *A. flavus* spores (10^4 spores/mL) were mixed with potato dextrose agar (PDA) and poured onto the yeast cultures. Plates were then incubated at 25 °C until the control plate was fully covered by fungal growth. The inhibition percentage was determined using Image J software [21].

Antioxidant activity

The 24-hour yeast culture was centrifuged (3000 g, 10 min), and the yeast cells were re-suspended in PBS. Then, 800 µL of this suspension was mixed with 1 mL of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) and kept in the dark for 30 min. After centrifugation (3000 g, 10 min), the absorbance of the supernatant was measured at 517 nm, and the DPPH scavenging activity was calculated using the following formula according to Gil-Rodriguez et al. [17].

DPPH scavenging activity% = $[1-(A_{sample}/A_{blank})] \times 100$

Statistical analysis

The results were analyzed using one-way analysis of variance (ANOVA). All tests were performed in triplicate, and data analysis was conducted using SPSS (version 20). Microsoft Office Excel 2019 was used for drawing the charts. Mean comparisons were performed using the least significant difference (LSD) test at $P<0.05$ confidence level.

[DOI: 10.22034/FSCT.22.160.162]

3-Results and Discussion

Identification of the predominant yeast isolate

The predominant yeast isolate was identified through sequencing of the PCR products compared with NCBI database records, revealing *P. kudriavzevii* PA01 with 96% similarity. Specific amplification of the target sequence was verified in the agarose gel electrophoresis as shown in Fig. 1 In some studies, this yeast has been isolated from other sourdoughs. For example, in a study on buckwheat sourdough, *P. kudriavzevii* was isolated, and its probiotic characteristics were verified [5]. In the same vein, *P. kudriavzevii* MK044080.1 was isolated as a potential probiotic yeast from Ethiopian injera sourdough by Muche et al. [22]. The ability of yeasts to maintain in sourdough's acidic environment and their adaptation to specific conditions of stressful sourdough ecosystem are key factors in their dominance during sourdough fermentation [2].

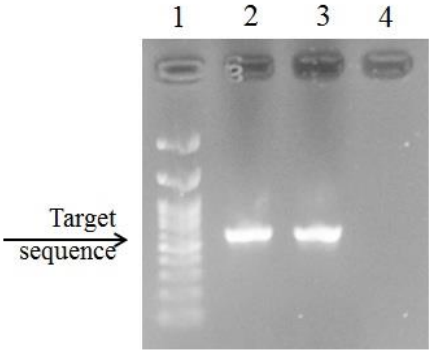

Fig. 1 Gel electrophoresis of the PCR products obtained from amplification of the target sequence (the *ITS* region) of predominant yeast isolated from acorn sourdough (lane 2) compared to the positive and negative control samples (lane 3 and 4, respectively) and 100 bp DNA ladder (lane 1).

Survival and adhesion capabilities of the yeast isolate

The survival rate of *P. kudriavzevii* isolate under SGI conditions was equal to 91.93 ± 0.91%, demonstrating its low tolerance to the environments of the gastrointestinal tract. Additionally, the yeast showed a strong auto-aggregation capability (84.65 ± 1.59%), which is an essential characteristic for its survival and probiotic functionality. Similarly, the survivability of the *P. kudriavzevii* isolate under SGI conditions was equal to 79.26% in the study of Shahryari et al. [5]. These data are in agreement with those recently reported by Rahimi et al. [23] for probiotic yeast isolated from sourdough. Accordingly, probiotic yeasts have the ability to form biofilms, helping them to protect cells from harsh environmental conditions. By utilizing surface cellular compounds, these yeasts enhance their auto-aggregation and survival capabilities, making them more resistant to stomach acidic conditions and bile salts of the intestine.

Antibacterial and co-aggregation abilities of the isolate

P. kudriavzevii isolate showed a significantly (P<0.05) higher inhibitory effect on *L. monocytogenes* compared to the other tested foodborne bacteria studied (Fig. 2) Moreover, there was no significant difference among co-aggregation ability of the isolate with *E. coli*, *S. aureus*, and *S. enterica*.

[DOI: 10.22034/FSCT.22.160.162]

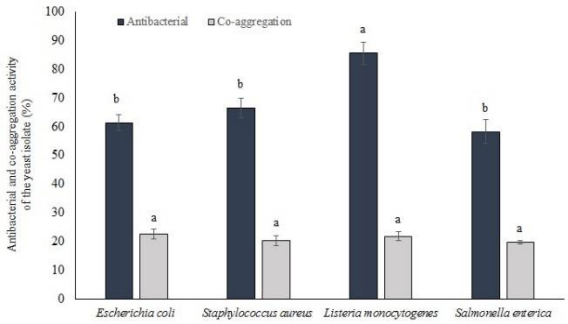

Fig. **2** Inhibitory activity (%) of the *P. kudriavzevii* isolate against *E. coli*, *S. aureus*, *L. monocytogenes*, and *S. enterica* in comparison with the co-aggregation ability of the yeast isolate with the tested foodborne bacteria. Different letters indicate significant differences at $P<0.05$ in terms of each capability.

The antimicrobial activity against foodborne pathogens is considered one of the most important probiotic features. In the present study, the inhibitory effect of the isolate on Gram-positive bacteria was higher than those of Gram-negative bacteria studied. Several studies have confirmed the antimicrobial properties of probiotic yeasts against foodborne pathogens. For example, Kim et al. [24] verified the antibacterial activity of the probiotic yeast *S. cerevisiae* KU200270 against *L. monocytogenes*, while Chen et al. [25] reported that *S. cerevisiae* (isolated from koumiss) exhibited significant antimicrobial activity against *E. coli*, attributing this effect to organic acids such as citric acid and propionic acid. Younis et al. [26] also demonstrated the antimicrobial effects of probiotic yeasts against *S. aureus* and *E. coli*. Similarly, Al-Sahlany et al. [27] identified an antimicrobial peptide in *S. cerevisiae* ATCC 36858 that was resistant to heat (50-90 °C for 30 min) and that was effective against *E. coli* and *Klebsiella aerogenes*. Accordingly, action modes of antibacterial activity in probiotic yeasts include competition for nutrients and epithelial cell binding sites, as well as production of antimicrobial metabolites like organic acids, hydrophobic peptides, and ethanol [3]. In addition, the correlation between co-aggregation ability and antibacterial activity of probiotic yeast has been verified in the study of Rahimi et al. [23], which was in agreement with our findings. The co-aggregation ability as an important mechanism in antibacterial activities of probiotic yeasts is associated with competition between yeast and bacteria for adhesion to epithelial surfaces, and reducing the availability of nutrients for harmful bacteria [2]. In a similar fashion, Menezes et al. [28] reported that probiotic yeasts form a yeast-bacteria complex, leading to the destruction of pathogens. Generally, type I flagella plays a crucial role in the adhesion of pathogenic bacteria to the intestinal epithelium, often using mannose as a receptor. Since yeast cell-walls are rich in mannose, probiotic yeasts can act as alternative receptors, preventing pathogenic bacteria from attaching to gastrointestinal cell receptors [29].

Hemolysis and antibiotic susceptibility of the isolate

According to the results, the *P. kudriavzevii* isolate exhibited no hemolytic activity. Similar studies on the hemolytic activity of probiotic yeasts, such as those by Fadda et al. [18] and Suvarna et al. [30] reported comparable results. The lack of hemolytic activity in yeasts may be due to the absence of hemolytic enterotoxin-producing genes,

[DOI: 10.22034/FSCT.22.160.162]

resulting in no production of blood-destroying toxins [31]. Additionally, the yeast isolate demonstrated resistance to the antibiotics tested. Antibiotic resistance in probiotic yeasts has been reported in numerous studies, including research by Banik et al. [32], Fadda et al. [18], and Perricone et al. [33]. The lack of horizontal gene transfer of this resistance to other yeasts and bacteria makes yeasts, suitable candidates for probiotic applications. In the present study, the predominant yeast isolate was semi-sensitive to natamycin, ketoconazole, and itraconazole, and resistant towards potassium sorbate and calcium propionate (Table 1).

Table 1. Susceptibility of the yeast isolated from acorn sourdough towards antimycotic compounds. Different letters show significant differences at $P<0.05$.

Antimycotic agent (µg of effective component)	Sensitivity	The diameter of inhibition zone (mm)
Itraconazole (10)	semi-sensitive	16.32 ± 1.54^{a}
Ketoconazole (20)	semi-sensitive	17.23 ± 1.59^{a}
Natamycin (30)	semi-sensitive	18.71 ± 0.54^{a}
Potassium sorbate (60)	resistance	0.00^{b}
Calcium propionate (60)	resistance	0.00^{b}

Similar findings regarding the relative sensitivity of probiotic yeasts to antimycotic agents such as ketoconazole and itraconazole have been reported in various studies [32]. Some of yeasts mechanisms for this phenomenon include altering cell membrane permeability, inhibiting mitochondrial protein synthesis, mutation, and reducing ATP hydrolysis activities. Resistance to antifungal compounds may also result from changes in the target site of the antimycotic agents, inhibition of cellular RNA and DNA synthesis, and activation of the proton pump system in the cell membrane [34].

Antifungal and antioxidant activity of the isolate

The inhibitory effect of the predominant yeast isolate on *A. flavus* after 4 days of incubation compared to the control sample is shown in Fig. 3. As can be seen, the yeast isolate inhibited the growth of the target fungus (42.98 ± 0.84%) and prevented its sporulation as color-less growth zone.

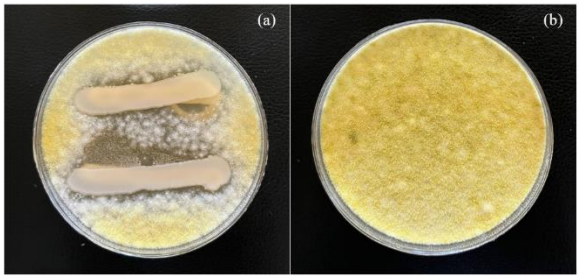

Fig. 3 Antifungal activity of the *P. kudriavzevii* isolate against *A. flavus* (a) compared to the control sample containing the target mold (b) in overlay bioassay.

A similar study found that some yeasts such as *S. cerevisiae* strains exhibited antifungal activities ranging from 2.7% to 100% against *A. flavus*, *Penicillium citrinun*, *Penicillium griseufulvun*, *Aspergillus niger*, and *Aspergillus fumigatus* [21]. Additionally, Alasmar et al. [35] approved the antifungal

[DOI: 10.22034/FSCT.22.160.162]

activity of the yeast *Kluyveromyces marxianus* QKM-4 against 17 fungal species from the genera *Aspergillus*, *Penicillium*, and *Fusarium*, attributing this effect to volatile organic compounds produced by the yeast. Other antifungal mechanisms of probiotic yeasts include the production of metabolites such as carbon dioxide, ethanol, protein compounds, or low molecular weight peptides [21]. The DPPH radical scavenging ability of the yeast isolate in the present study was also equal to 78.67% ± 1.88%. In a study by Romero-Luna et al. [36], this ability was reported to be 63.0%. Probiotic yeasts are capable of producing antioxidant compounds like glutathione, superoxide dismutase, and catalase, which help protect against oxidative damage. Certain compounds produced by yeasts, such as hydrogen peroxide not only possess antioxidant properties but also can damage the cell membrane of fungi, preventing their growth [37].

4-Conclusion

Exploring the properties of probiotic yeasts isolated from substrates that have been less studied opens the possibility of discovering unique characteristics. Based on available data, there has been no prior report on the isolation of potential probiotic yeast from acorn sourdough and the evaluation of its properties. This study demonstrated that *P. kudriavzevii*, the predominant yeast isolated from acorn sourdough, possesses suitable probiotic, antifungal, and antioxidant capabilities. Moreover, there was a direct correlation between co-aggregation and antibacterial activities of the isolate, as well as between its antifungal and antioxidant capabilities. Interestingly, these correlations show potential applications of the isolate for a wide range of purposes after in depth characterization. Given these findings, *P. kudriavzevii* can be proposed as a suitable candidate for usage as a potential probiotic and/or protective functional culture in food and fermentation industries.

Funding

There was no funding.

Data availability

Data will be available based on the request.

Conflict of interest

All authors declare that there is no conflict of interest.

References

[1] Saad, N., Delattre, C., Urdaci, M., Schmitter, J.M., & Bressollier, P. (2013). An overview of the last advances in probiotic and prebiotic field. LWT-Food Science and Technology, *50*(1), 1-16.

[2] Sadeghi, A., Ebrahimi, M., Shahryari, S., Kharazmi, M. S., & Jafari, S. M. (2022). Food applications of probiotic yeasts; focusing on their techno-functional, postbiotic and protective capabilities. Trends in Food Science & Technology, *128*, 278-295.

[3] Hatoum, R., Labrie, S., & Fliss, I. (2012). Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. Frontiers in Microbiology, *3*, 421.

[4] Shruthi, B., Deepa, N., Somashekaraiah, R., Adithi, G., Divyashree, S., & Sreenivasa, M. Y. (2022). Exploring biotechnological and functional characteristics of probiotic yeasts: a review. Biotechnology Reports, e00716.

[5] Shahryari, S., Sadeghi, A., Ebrahimi, M., Sadeghi Mahoonak, A., & Moayedi, A. (2022). Evaluation of probiotic and antifungal properties of the yeast isolated from buckwheat sourdough. Iranian Food Science and Technology Research Journal, *18*(5), 575-588.

[6] Shruthi, B., Adithi, G., Deepa, N., Divyashree, S., & Sreenivasa, M. Y. (2024). Probiotic and functional attributes of yeasts isolated from different traditional fermented foods and products. Probiotics and Antimicrobial Proteins, 1-19.

[7] Greppi, A., Saubade, F., Botta, C., Humblot, C., Guyot, J.P., Cocolin, L. (2017). Potential probiotic *Pichia kudriavzevii* strains and their ability to enhance folate content of traditional cereal-based African fermented food. Food Microbiology, *62*, 169-177.

[8] Alkalbani, N. S., Osaili, T. M., Al-Nabulsi, A. A., Obaid, R. S., Olaimat, A. N., Liu, S. Q., & Ayyash, M. M. (2022). *In vitro* characterization and identification of potential probiotic yeasts isolated from fermented dairy and non-dairy food products. Journal of Fungi, *8*(5), 544.

[DOI: 10.22034/FSCT.22.160.162]

[9] Lara-Hidalgo, C. E., Dorantes-Álvarez, L., Hernández-Sánchez, H., Santoyo-Tepole, F., Martínez-Torres, A., Villa-Tanaca, L., & Hernández-Rodríguez, C. (2019). Isolation of yeasts from guajillo pepper (*Capsicum annuum* L.) fermentation and study of some probiotic characteristics. Probiotics and Antimicrobial Proteins, *11*, 748-764.
[10] AACC, International. (2010). Approved methods of the American association of cereal chemists. 11th Ed. The St. Paul.
[11] Purabdolah, H., Sadeghi, A., Ebrahimi, M., Kashaninejad, M., & Mohamadzadeh, J. (2022). Evaluation of probiotic and antifungal properties of the predominant LAB isolated from fermented acorn (*Quercus persica*). Journal of Food Science and Technology (Iran), *19*(124), 171-183.
[12] Katina, K., Heiniö, R. L., Autio, K., & Poutanen, K. (2006). Optimization of sourdough process for improved sensory profile and texture of wheat bread. LWT-Food Science and Technology, *39*(10), 1189-1202.
[13] Palla, M., Agnolucci, M., Calzone, A., Giovannetti, M., Di Cagno, R., Gobbetti, M., *et al.* (2019). Exploitation of autochthonous Tuscan sourdough yeasts as potential starters. International Journal of Food Microbiology, *302*, 59-68.
[14] White, T.J., Bruns, T., Lee, S.J.W.T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications, *18*(1), 315-322.
[15] Hsiung, R. T., Fang, W. T., LePage, B. A., Hsu, S. A., Hsu, C. H., & Chou, J. Y. (2021). *In vitro* properties of potential probiotic indigenous yeasts originating from fermented food and beverages in Taiwan. Probiotics and Antimicrobial Proteins, *13*, 113-124.
[16] Bonatsou, S., Karamouza, M., Zoumpopoulou, G., Mavrogonatou, E., Kletsas, D., & Papadimitriou, K. (2018). Evaluating the probiotic potential and technological characteristics of yeasts implicated in cv. Kalamata natural black olive fermentation. International Journal of Food Microbiology, *271*, 48-59.
[17] Gil-Rodriguez, A.M., Carrascosa, A.V., & Requena, T. (2015). Yeasts in foods and beverages: *In vitro* characterisation of probiotic traits. LWT- Food Science and Technology, *64*, 1156-1162.
[18] Fadda, M.E., Mossa, V., Deplano, M., Pisano, M.B., & Cosentino, S. (2017). *In vitro* screening of *Kluyveromyces* strains isolated from Fiore Sardo cheese for potential use as probiotics. LWT- Food Science and Technology, *75*, 100-106.
[19] Zarali, M., Sadeghi, A., Jafari, S. M., Ebrahimi, M., & Mahoonak, A. S. (2023). Enhanced viability and improved *in situ* antibacterial activity of the probiotic LAB microencapsulated layer-by-layer in alginate beads coated with nisin. Food Bioscience, *53*, 102593.
[20] Amorim, J. C., Piccoli, R. H., & Duarte, W. F. (2018). Probiotic potential of yeasts isolated from pineapple and their use in the elaboration of potentially functional fermented beverages. Food Research International, *107*, 518-527.
[21] Ruggirello, M., Nucera, D., Cannoni, M., Peraino, A., Rosso, F., Fontana, M., *et al.* (2019). Antifungal activity of yeasts and lactic acid bacteria isolated from cocoa bean fermentations. Food Research International, *115*, 519-525.
[22] Muche, N., Geremew, T., & Jiru, T. M. (2023). Isolation and characterization of potential probiotic yeasts from Ethiopian injera sourdough. 3 Biotech, *13*(9), 300.
[23] Rahimi, D., Sadeghi, A., Kashaninejad, M., & Ebrahimi, M. (2024). Postbiotic characterization of a potential probiotic yeast isolate, and its microencapsulation in alginate beads coated layer-by-layer with chitosan. Heliyon, *10*(7).
[24] Kim, Y. J., Yu, H. H., Song, Y. J., Park, Y. J., Lee, N. K., & Paik, H. D. (2021). Anti-biofilm effect of the cell-free supernatant of probiotic *Saccharomyces cerevisiae* against *Listeria monocytogenes*. Food Control, *121*, 107667.
[25] Chen, Y. J., Wang, C. J., Hou, W. Q., Wang, X. S., Gali, B. G., YANG, S. Q., ... & WU, Y. G. (2017). Effects of antibacterial compounds produced by *Saccharomyces cerevisiae* in Koumiss on pathogenic *Escherichia coli* Os and its cell surface characteristics. Journal of Integrative Agriculture, *16*(3), 742-748.
[26] Younis, G., Awad, A., Dawod, R. E., & Yousef, N. E. (2017). Antimicrobial activity of yeasts against some pathogenic bacteria. Veterinary World, *10*(8), 979.
[27] Al-Sahlany, S. T. G., Altemimi, A. B., Abd Al-Manhel, A. J., Niamah, A. K., Lakhssassi, N., & Ibrahim, S. A. (2020). Purification of bioactive peptide with antimicrobial properties produced by *Saccharomyces cerevisiae*. Foods, *9*(3).
[28] Menezes, A. G. T., Ramos, C. L., Cenzi, G., Melo, D. S., Dias, D. R., & Schwan, R. F. (2020). Probiotic potential, antioxidant activity, and phytase production of indigenous yeasts isolated from indigenous fermented foods. Probiotics and Antimicrobial Proteins, *12*, 280-288.
[29] Kline, K. A., Fälker, S., Dahlberg, S., Normark, S., & Henriques-Normark, B. (2009). Bacterial adhesins in host-microbe interactions. Cell Host & Microbe, *5*(6), 580-592.
[30] Suvarna, S., Dsouza, J., Ragavan, M. L., & Das, N. (2018). Potential probiotic characterization and

[DOI: 10.22034/FSCT.22.160.162]

effect of encapsulation of probiotic yeast strains on survival in simulated gastrointestinal tract condition. Food Science and Biotechnology, *27*(3), 745-753.
[31] Fernández-Pacheco, P., Ramos Monge, I. M., Fernández-González, M., Poveda Colado, J. M., & Arévalo-Villena, M. (2021). Safety evaluation of yeasts with probiotic potential. Frontiers in Nutrition, *8*, 659328.
[32] Banik, A., Mondal, J., Rakshit, S., Ghosh, K., Sha, S. P., Kumar, Halder, S., Ghosh, C., & Mondal, K. C. (2019). Amelioration of cold-induced gastric injury by a yeast probiotic isolated from traditional fermented foods. Journal of Functional Foods, *59*, 164–173.
[33] Perricone, M., Bevilacqua, A., Corbo, M., & Sinigaglia, M. (2014). Technological characterization and probiotic traits of yeasts isolated from Altamura sourdough to select promising microorganisms as functional starter cultures for cereal-based products. Food Microbiology, *38*, 26-35.
[34] Kanafani, Z. A., & Perfect, j. R. (2014). Resistance to antifungal agents: mechanisms and clinical impact. Clinical Infectious Diseases, *46*(1), 120-128.
[35] Alasmar, R., Ul-Hassan, Z., Zeidan, R., Al-Thani, R., Al-Shamary, N., Alnaimi, H., ... & Jaoua, S. (2020). Isolation of a novel *Kluyveromyces marxianus* strain QKM-4 and evidence of its volatilome production and binding potentialities in the biocontrol of toxigenic fungi and their mycotoxins. ACS Omega, *5*(28), 17637-17645.
[36] Romero-Luna, H. E., Hernández-Sánchez, H., Ribas-Aparicio, R. M., Cauich-Sánchez, P. I., & Dávila-Ortiz, G. (2019). Evaluation of the probiotic potential of *Saccharomyces cerevisiae* Strain (C41) isolated from Tibicos by in vitro studies. Probiotics and Antimicrobial Proteins, *11*, 794-800.
[37] Khan, A., Ahmad, A., Akhtar, F., Yousuf, S., Xess, I., Khan, L.A., & Manzoor, N. (2011) Induction of oxidative stress as a possible mechanism of the antifungal action of three phenylpropanoids. FEMS Yeast Research, 11(1):114-122.

[DOI: 10.22034/FSCT.22.160.162]

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

ارزیابی ویژگی‌های پروبیوتیکی، ضد قارچی و آنتی‌اکسیدانی مخمر جدا شده از خمیرترش بلوط

حسین پورعبدالله[۱]، علیرضا صادقی*[۱]، سید مهدی جعفری[۱،۲]، مریم ابراهیمی[۳]، هدی شهیری طبرستانی[۱]

۱- گروه علوم و مهندسی صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان، گرگان، ایران

۲- مرکز تحقیقات حلال، سازمان غذا و دارو، وزارت بهداشت، درمان و آموزش پزشکی، تهران، ایران

۳- مرکز تحقیقات سلامت غذا، دارو و فراورده‌های طبیعی، دانشگاه علوم پزشکی گلستان، گرگان، ایران

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۳/۸/۲۲

تاریخ پذیرش: ۱۴۰۴/۰۲/۲۱

کلمات کلیدی:

خمیرترش بلوط،

جدایه مخمری،

ویژگی‌های پروبیوتیکی،

اثر ضد قارچی،

اثر آنتی‌اکسیدانی

DOI: 10.22034/FSCT.22.160.162.

* مسئول مکاتبات:

sadeghi.gau@gmail.com;
asadeghi@gau.ac.ir

چکیده

احتمال مواجهه با مخمرهای پروبیوتیک که از قابلیت‌های عملکردی مناسبی برخوردار باشند در بستره‌های طبیعی که کمتر مورد مطالعه قرار گرفته‌اند وجود دارد. در این پژوهش، مخمر غالب از خمیرترش بلوط، جداسازی و با استفاده از PCR شناسایی شد. سپس ویژگی‌های پروبیوتیکی شامل زنده‌مانی در شرایط شبیه‌سازی شده دستگاه گوارش، قابلیت خود اتصالی و دگر اتصالی، آبگریزی، اثر ضد باکتریایی، مقاومت آنتی‌بیوتیکی، آنتی‌مایکوتیکی و قابلیت همولیز خون و همچنین قابلیت آنتی‌اکسیدانی و اثر ضدقارچی این جدایه مخمری بر روی *Aspergillus flavus* مورد مطالعه قرارگرفت. توالی‌یابی محصولات PCR منجر به شناسایی مخمر *Pichia kudriavzevii* به عنوان جدایه مخمری غالب خمیرترش بلوط شد. میزان زنده‌مانی جدایه مذکور در شرایط شبیه‌سازی شده دستگاه گوارش ۹۱/۹۳ درصد بود. قابلیت خود اتصالی این جدایه برابر با ۸۴/۶۵ درصد و میزان آبگریزی آن در برابر هگزان و زایلن به ترتیب ۳۵/۱۵ و ۲۱/۷۰ درصد بود. همچنین اثر ضد باکتریایی مخمر *P. kudriavzevii* مورد مطالعه در این پژوهش در برابر *Listeria monocytogenes* معادل ۸۵/۵۸ درصد و به شکل معنی‌داری ($P<0/05$) از سایر عوامل بیماری‌زای مورد آزمون، بیشتر بود. کمترین اثر ضد باکتریایی نیز در برابر *Salmonella enterica* مشاهده شد. میزان قابلیت دگر اتصالی جدایه مخمری غالب در برابر عوامل بیماری‌زای مورد آزمون، تفاوت معنی‌داری نداشت. علاوه بر این، جدایه مذکور فاقد فعالیت همولیتیکی بود و نسبت به تمامی آنتی‌بیوتیک‌های مورد بررسی، مقاوم بود اما در مقابل ترکیبات آنتی‌مایکوتیک ایتراکونازول، کتوکونازول و ناتامایسین، حساسیت نسبی نشان داد و همچنین نسبت به سوربات پتاسیم مقاوم بود. اثر ضد قارچی این جدایه در برابر *A. flavus* تایید شد و میزان قابلیت آنتی‌اکسیدانی آن ۷۸/۶۷ درصد بود. بر این اساس، جدایه *P. kudriavzevii* از قابلیت مناسبی برای استفاده به عنوان کشت پروبیوتیک جهت تولید محصولات غذایی تخمیری برخوردار است.

[DOI: 10.22034/FSCT.22.160.162]