



Original research

Probiotic characteristics of the predominant yeast isolated from rice bran sourdough and application of its para-probiotics as food preservatives

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ABSTRACT

In the present study, probiotic characteristics of the predominant yeast isolated from rice bran sourdough were evaluated. Then, dead cells of the isolate were used as bio-preservatives in the formulation of the home-made chicken sausage. The yeast isolate was molecularly identified as *Wickerhamomyces anomalus*, which exhibited a high survival rate (78.92%) under simulated gastrointestinal conditions. The isolate showed no hemolytic activity. The yeast's auto-aggregation and hydrophobicity were also equal to 70.63% and 44.25%, respectively. This yeast demonstrated significant antibacterial effects against Gram-positive foodborne bacteria tested. Moreover, it showed relative sensitivity to antifungal compounds such as fluconazole and resistance to natamycin. Additionally, the yeast significantly inhibited the growth of *Aspergillus flavus*, reducing it by approximately 49.77%. Interestingly, dead cells of the yeast isolate significantly ($p < 0.05$) reduced *Staphylococcus aureus* compared to the control sample during a weak storage period of the home-made chicken sausage as a potent clean-label strategy. Accordingly, the yeast isolate and its dead cells can be considered as bio-additives with promising antibacterial effects in the supplemented sausage.

Keywords: Rice bran sourdough; Probiotic yeast; Antibacterial; Dead cells; Clean-label.

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1. Introduction

Probiotics (PROs) are defined as viable and active microorganisms that, when consumed in adequate amounts, confer health benefits to the host body. For this purpose, PRO microorganisms must possess the ability to survive the conditions of the gastrointestinal tract (GIT), including low pH and pepsin enzyme in the stomach, as well as higher pH, bile salts, and pancreatic enzyme in the intestine. Additionally, they must be able to multiply at human body temperature (Hsu and Chou, 2021). Fermented foods like sourdough (a mixture of water and flour) are rich sources of PROs (Sadeghi et al., 2022; Much et al., 2023). Yeasts isolated from sourdough can generally tolerate stress conditions such as those found in the GIT due to the acidic environment and also have the

ability to attach to the intestinal cells (Hsiung et al., 2021; Sadeghi et al., 2025a). Yeasts play a significant role as beneficial PROs due to their characteristics such as diversity in cellular structure and morphology, ability to utilize a wide range of nitrogen, carbon and phosphorus sources, resistance to stressful conditions (including survival in low pH and gastrointestinal conditions), survival at low water activity (a_w) and oxygen levels, as well as antioxidant and antimicrobial activities. Moreover, the intrinsic resistance of yeasts to common antibiotics and their inability to transfer genes to pathogenic microorganisms make their safe use as PROs in food products feasible (Wang et al., 2024; Hsiung et al., 2021). In general, viability under gastrointestinal stress, adhesion properties, and antimicrobial activities are essential elements for the selection of a proper PRO candidate. Survival under simulated gastrointestinal (SGI) conditions reflects the ability of a strain to remain active

Abbreviations: a_w : water activity, BHI: brain heart infusion, GIT: gastrointestinal tract, ITS: internal transcribed spacer, PBS: phosphate buffered saline, PDA: potato dextrose agar, PRO: probiotic, PTCC: Persian type culture collection, SGI: simulated gastrointestinal, YCW: yeast cell-wall, YGC: yeast extract glucose chloramphenicol

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during gastrointestinal transit, which is a prerequisite for exerting health-promoting effects. Adhesion properties indicate the potential for colonization and competitive exclusion of pathogens, thereby improving persistence within the intestine. Furthermore, antimicrobial effects are associated with modulation of the gut microbiota. Accordingly, these *in vitro* analyses provide mechanistic insight into strain-specific potentials and serve as screening approaches to identify effective PROs prior to further validations (Sadeghi et al., 2022).

There are some studies focused on isolating yeasts with PRO potential from various types of sourdough. For example, Shams Shargh et al. (2025a) evaluated the PRO capability of a yeast strain isolated from fermented sprouted soybeans. The *Pichia kudriavzevii* ShS01 demonstrated 82.70% survival under SGI conditions and exhibited antibacterial activity against *Staphylococcus aureus* and other Gram-positive pathogenic bacteria, along with higher co-aggregation ability. Taheri et al. (2025a) isolated the *Saccharomyces cerevisiae* THY2 with PRO characteristics from honey. This isolate showed good hydrophobicity (88.22%) and auto-aggregation (95.27%), and had antifungal activity against *Aspergillus flavus*, while also showing resistance to all antifungal agents tested except natamycin. Another study reported the *P. kudriavzevii* FBS02 as a selected yeast strain that tolerated high heat, exhibited 95.45% and 39.24% auto-aggregation and hydrophobicity, respectively. The yeast isolate demonstrated significantly greater antibacterial effects against *Salmonella enterica* compared to other foodborne bacteria studied (Shahryari et al., 2025). Rahimi et al. (2024) isolated 11 yeast strains from wheat germ sourdough, selecting *S. cerevisiae* RWGS07 with 95.74% survival in SGI conditions. This selected yeast reduced *Aspergillus niger* growth by 77.35% and showed the highest inhibitory effect (75.84%) against *Listeria monocytogenes* compared to other foodborne bacteria tested.

Purabdollah et al. (2025) evaluated the PRO characteristics of the *P. kudriavzevii* isolated from acorn sourdough. They found that this yeast lacked hemolytic activity, showed relative sensitivity to itraconazole, ketoconazole, and natamycin, but was resistant to potassium sorbate. Additionally, this strain exhibited significant antifungal activity against *A. flavus*. In another study, the predominant yeast isolated from honey was identified as *S. cerevisiae*, which showed 18.32% antifungal effect against *A. flavus* and the highest co-aggregation ability with *S. aureus* (Taheri et al., 2025b). Shahryari et al. (2022) isolated *P. kudriavzevii* from buckwheat sourdough, reporting a viability of 62.79% against sequential acid and bile treatment compared to the control sample. This isolate showed no hemolytic ability and good antifungal effects against *A. niger* and *A. flavus*. Shams Shargh et al. (2025b) isolated *R. mucilaginosa* from sprouted soybean sourdough; it showed greater inhibitory effects against Gram-negative foodborne bacteria than Gram-positive ones, with the highest antibacterial and co-aggregation activity against *Escherichia coli*. Its hydrophobicity was 69.93% and 32.56% against xylene and hexane, respectively.

Para-probiotics have been defined as non-viable bioactive food components that confer health benefits upon the host. These non-viable cells are gaining more and more importance in the antimicrobial process of food production. Their importance primarily rests on their ability to offer greater food safety and product quality; all this, while responding to growing consumer demands for clean labels and more natural modes of preservation. The stability of para-probiotics, the predictability of their effects, and straight forward of their incorporation into processing environments are features that give them an advantage since these properties are not shared by live PROs, which need careful maintenance of cell

viability. Given this stability, para-probiotics can exhibit consistent antimicrobial effects with considerable ease of application at an industrial level (Sadeghi et al., 2025b; Sionek and Gantner, 2025). Given the limited research on PRO yeast isolation from rice bran sourdough, the present study explored the PRO and antifungal features of the predominant yeast isolated from rice bran sourdough. Rice (*Oryza sativa*) bran, as a largely underutilized agro-industrial by-product, has remarkable potential applications. It is rich in fermentable carbohydrates, proteins, and bioactive phytochemicals as excellent carbon and nitrogen sources. This composition makes rice bran a cost-effective medium for microbial fermentation. Moreover, rice bran creates a selective niche for metabolically versatile and stress-tolerant microorganisms, thereby facilitating the isolation of novel strains with a wide range of potential applications. Consequently, the valorization of rice bran through fermentation contributes to waste reduction and offers a promising platform for developing innovative functional foods (Sadeghi et al., 2019). The novelty of the present study was to the select of a potential PRO yeast isolated from rice bran sourdough and the evaluation of its non-viable cells (para-probiotics) in a food model to propose a safer and proper alternative for functional food development.

2. Materials and Methods

2.1. Microbial cultures and reagents

The foodborne microorganisms used in this study (*A. flavus* PTCC 5018, *E. coli* PTCC 1399, *S. aureus* PTCC 1112, *L. monocytogenes* PTCC 1298, and *S. enterica* PTCC 1709) were obtained from the Persian type culture collection (PTCC). All microbiological culture media and analytical-grade chemical reagents were also purchased.

2.2. Rice bran fermentation

To prepare the rice bran sourdough, a dough yield (dough-to-flour ratio) of 400 was used. Fermentation was carried out at 30 °C for 24 h. To obtain a stable predominant microbial flora, back-slopping was applied (20% of the previous day's sourdough was inoculated into the new sourdough formulation) until a constant total titratable acidity was achieved (Sadeghi et al., 2019).

2.3. Isolation of predominant yeasts

For this purpose, serial tenfold dilutions of the rice bran sourdough were prepared and spread-plated onto yeast extract glucose chloramphenicol (YGC) agar, followed by incubation at 28 °C for 48–96 h. Microscopic morphology of the isolated yeasts, along with selected biochemical characteristics, was subsequently examined.

2.4. Screening of the isolates

A suspension of each isolate was first centrifuged at 10,000 g for 5 min at 4 °C (Sigma, Germany). After removal of the supernatant, the pellet was re-suspended in phosphate buffered saline (PBS) containing pepsin and adjusted to pH 2 with 1N hydrochloric acid, corresponding to a turbidity of 0.5 McFarland. The obtained suspension was incubated at 37 °C for 3 h. Next, the pH of the

suspension was adjusted to 6.5 using 1 N NaOH, followed by exposure to pancreatin and 0.3% bile salts for 4 h. The number of viable yeasts after these treatments was compared with that of the untreated control (Zhang et al., 2011).

2.5. Molecular identification of the selected isolate

For molecular identification of the selected yeast (the isolate showed the highest viability under SGI conditions), PCR using internal transcribed spacer (ITS) primers and sequencing of the PCR products were performed. For yeast, a 650-bp fragment of the ITS region was amplified and sequenced (White et al., 1990).

2.6. Evaluation of the antibacterial activity of the selected isolate

The antibacterial activity of the selected isolate was assessed using the well-diffusion method. For this purpose, the optical densities of freshly prepared cultures of the selected isolate and the foodborne bacteria (*L. monocytogenes*, *S. aureus*, *E. coli*, and *S. enterica*) were adjusted to match a turbidity of 0.5 McFarland. Then, 50 µL of each bacterial suspension was spread plated on brain heart infusion (BHI) agar plates. After approximately 30 minutes, wells were made at the center of the plates, and 50 µL of the selected isolate culture was added to each well. The cultured plates were incubated at 30 °C, and after 24 h, the antibacterial effect was evaluated by measuring the inhibition zones (Rahimi et al., 2024; Fernandez-Pacheco et al., 2018).

2.7. Auto-aggregation ability of the selected isolate

Following adjustment of the selected isolate's cell concentration to 10⁸ CFU/mL using a 24-h activated culture, the optical density of the suspension was measured at 600 nm after 3 h of incubation and compared with the initial reading using a spectrophotometer (PGI, UK). The auto-aggregation percentage was calculated using the equation (Eq. (1)) (Fernandez-Pacheco et al., 2018).

$$\text{Auto-aggregation (\%)} = \left[1 - \left(\frac{A_3}{A_0} \right) \right] \times 100 \quad (1)$$

where: A_0 = absorbance at the beginning of the incubation and A_3 = absorbance after 3 h of incubation.

2.8. Co-aggregation ability of the selected isolate

To evaluate the co-aggregation ability of the selected isolate with the studied foodborne bacteria, cells from a 24-h culture of *E. coli* were harvested by cold centrifugation and washed twice with PBS. A mixture containing equal volumes of the selected isolate and suspension of each foodborne bacterium (adjusted to the same cell concentration) was then prepared. The absorbance of the individual suspensions and their mixture was measured at 600 nm after 4 h of incubation at 37 °C. Co-aggregation was also calculated according to the equation (Eq. (2)) (Collado et al., 2007):

$$\text{Co-aggregation (\%)} = \left[\left(AY + \frac{AP}{2} \right) - A_{\text{mix}} / \frac{(AY+AP)}{2} \right] \times 100 \quad (2)$$

where: A_Y = absorbance of the yeast suspension, A_P = absorbance of the pathogenic bacterial suspension, and A_{mix} = absorbance of the mixed yeast–pathogen suspension.

2.9. Cell-surface hydrophobicity of the selected isolate

First, an active culture of the yeast isolate was centrifuged at 5,000 g for 5 min at 4 °C. The pellet was washed twice with PBS, and then 3 mL of the resulting suspension was mixed with 1 mL of xylene. After incubation at 28 °C for 3 h, the absorbance of the aqueous (lower) phase was measured at 600 nm and used to calculate hydrophobicity according to the equation (Eq. (3)) (Alkalbani et al., 2022):

$$\text{Hydrophobicity (\%)} = \left[1 - \left(\frac{A_{\text{final}}}{A_{\text{initial}}} \right) \right] \times 100 \quad (3)$$

where: A_{initial} = absorbance of the suspension before mixing, and A_{final} = absorbance of the suspension after incubation.

2.10. Sensitivity of the yeast isolate to antimycotic agents

For this assay, the 24-h culture of the selected isolate was surface-plated on YGC agar plates. Antimycotic discs were then placed on the surface of each plate. After incubation at 30 °C for 24 h, the diameter of the inhibition zones around the discs was measured. Sensitivity was classified as resistant, intermediate, or sensitive when inhibition zones were <10 mm, 10–20 mm, and >20 mm, respectively (Rahimi et al., 2024).

2.11. Antifungal activity of the selected isolate

For this purpose, the selected isolate was first inoculated onto YGC agar. Then, a spore suspension of *A. flavus* (10⁵ spores/mL), an aflatoxin-producing fungus, was mixed with potato dextrose agar (PDA) and poured as a second layer over the yeast culture. After solidification of the upper layer, plates were incubated at 30 °C for 48 h. Finally, the diameter of the fungal growth inhibition zone around the selected isolate was measured (Magnusson et al., 2003).

2.12. In situ antibacterial activity of the yeast dead cells

In situ inhibitory activity of the heat-killed (autoclaved at 121 °C for 15 min) cells of the yeast isolate against *S. aureus* was determined in home-made chicken sausage (containing 80% chicken meat, 6% ice powder, 6% vegetable oil, 1% NaCl, 1% yeast dead cells, 6% mixture of fresh sweet pepper, soy protein isolate and whole wheat flour without preservative) through a challenge test (inoculation of 10⁶ CFU/g of *S. aureus* into the product) during a week storage period of the product at 4 °C. The presence of the targeted bacterium was also checked in the produced sausage before inoculation with the bacterium using spread plating of the serially ten-fold diluted samples on the specific Chromagar medium (Chromagar, France).

2.13. Statistical analysis

The obtained data were studied using a completely randomized design and one-way analysis of variance. All tests were conducted in triplicate. Data analysis was performed using SPSS software, and graphs were generated using Microsoft Office Excel. Mean

comparisons were done using the least significant difference test at a $p < 0.05$. The t -test statistical analysis was also used to compare two independent samples.

3. Results and Discussion

3.1. Screening of the isolates

Among the predominant yeast isolates obtained from rice bran sourdough, isolate Y1 exhibited the highest viability ($78.92 \pm 1.45\%$) under SGI, which was significantly ($p < 0.05$) higher than that of the other isolate ($60.03 \pm 5.11\%$). Accordingly, this isolate was selected (due to its higher viability under SGI conditions) for further characterization.

Muche et al. (2023) reported that among five yeast strains isolated from an Ethiopian injera, all isolates were able to tolerate and grow under acidic and alkaline conditions. Among them, *S. cerevisiae* KY073577.1 showed the highest viability, whereas *P. kudriavzevii* MK044080.1 exhibited the lowest tolerance when exposed to SGI conditions. Likewise, Shams Shargh et al. (2025b) demonstrated that *R. mucilaginosa* isolated from sprouted soybean sourdough exhibited 49.61% viability under SGI conditions. Microorganisms isolated from fermented substrates generally possess the ability to survive and grow at low pH due to the inherently acidic environment of these products. In general, acid tolerance enables PROs to pass through the GIT and reach the colon, where they exert their functional effects. Upon exposure to bile salts, cellular homeostasis is typically disrupted, leading to detachment of lipid layers and membrane proteins, ultimately causing leakage of intracellular contents and cell death. However, cell-wall integrity and adaptive mechanisms protect PROs against such damage (Alkabani et al., 2022; Sadeghi et al., 2022). The survivability of PRO yeasts under SGI conditions is largely dependent to their adaptive stress-response potentials compared to the non-PRO strains. The YCW also forms a multilayered structure that provides mechanical resistance against gastrointestinal stress. Additionally, the presence of membrane sterols like ergosterol in yeasts further enhances their membrane stability in these conditions (Sadeghi et al., 2022). Accordingly, adaptation potentials enable PRO yeasts to maintain viability during gastrointestinal transit and reach the colon with sufficient population to exert beneficial effects.

3.2. Hemolytic activity

The selected yeast isolate exhibited no hemolytic activity on blood agar (γ -hemolysis) and was therefore considered safe.

Menezes et al. (2020) reported that none of the 83 yeasts isolated from various fermented substrates exhibited the ability to lyse blood cells. In another study, among 16 isolated yeasts, only *Candida metapsilosis* showed hemolytic activity (Hsiung et al., 2021). In general, three types of hemolytic activities (alpha, beta, and gamma) are known. Hemolytic activity is one of the most important preliminary safety assessments for potential PRO strains because the ability to lyse blood cells can lead to intestinal epithelial tissue damage, ultimately resulting in anemia or edema in the host (Vesterlund et al., 2007). Hemolytic activity assessment is a critical safety criterion in the selection of PRO candidates, as it serves as an indicator of potential cytotoxicity and pathogenicity. PRO strains intended for food or clinical applications must exhibit non-hemolytic (γ -hemolysis) behavior to ensure they do not damage host

erythrocytes or produce virulence-associated factors. The presence of such mechanisms is commonly associated with opportunistic or pathogenic microorganisms rather than safe commensals (Sadeghi et al., 2022). Therefore, the absence of hemolytic activity provides essential evidence of *in vitro* biosafety and supports the suitability of a strain for PRO or para-probiotic applications in food systems.

3.3. Molecular identification of the selected isolate

Based on gel electrophoresis of the PCR products as shown in Fig. 1, amplification of the target sequence in the selected yeast was confirmed. Basic local alignment search tool alignment of the obtained sequence with the National Center for Biotechnology Information database revealed *Wickerhamomyces anomalus* as the predominant yeast isolate from rice bran sourdough.

He et al. (2022) reported the isolation of yeasts such as *S. cerevisiae* and *Candida humilis* with PRO potential from a type of fermented rice substrate. According to Vrancken et al. (2010), among 21 traditional sourdough samples, *W. anomalus* was identified as one of the predominant yeast species. The authors stated that after *S. cerevisiae*, *W. anomalus* represented the second most abundant species in the fermented substrates studied. Factors such as substrate type and fermentation conditions like fermentation temperature, fermentation time and number of back-slopping processes play key roles in shaping the microbial biodiversity of predominant sourdough yeasts. Substrate composition determines the availability of fermentable micronutrients, thereby selectively promoting the growth of specific microbial groups. The optimal temperature for isolating and obtaining these yeasts ranges between 25–27 °C as another crucial element. The back-slopping process also affects microbial population dynamics to obtain predominant strains with better compatibility and adaptability potentials (Sadeghi et al., 2022).

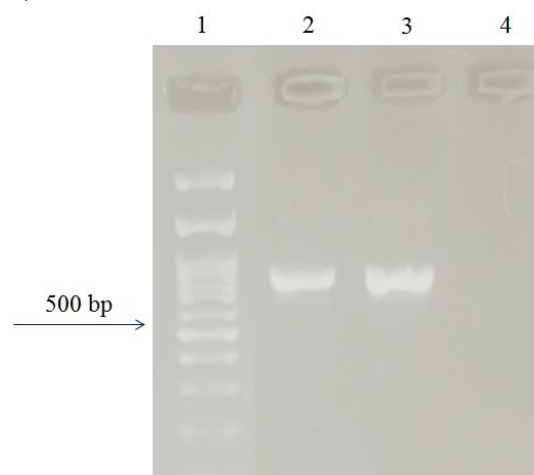


Fig. 1. Gel electrophoresis of PCR products of the selected yeast isolate (lane 2) compared with the positive control (lane 3), negative control (lane 4), and the 100-bp DNA ladder (lane 1).

3.4. Adhesion-related properties of the selected isolate

The auto-aggregation ability of the selected yeast isolate was $70.63 \pm 2.18\%$. The hydrophobicity of the yeast isolate toward xylene was also equal to $44.25 \pm 1.70\%$.

Huang et al. (2024) reported that all 13 yeast isolates exhibited auto-aggregation values above 70%, with *Pichia kluyveri*

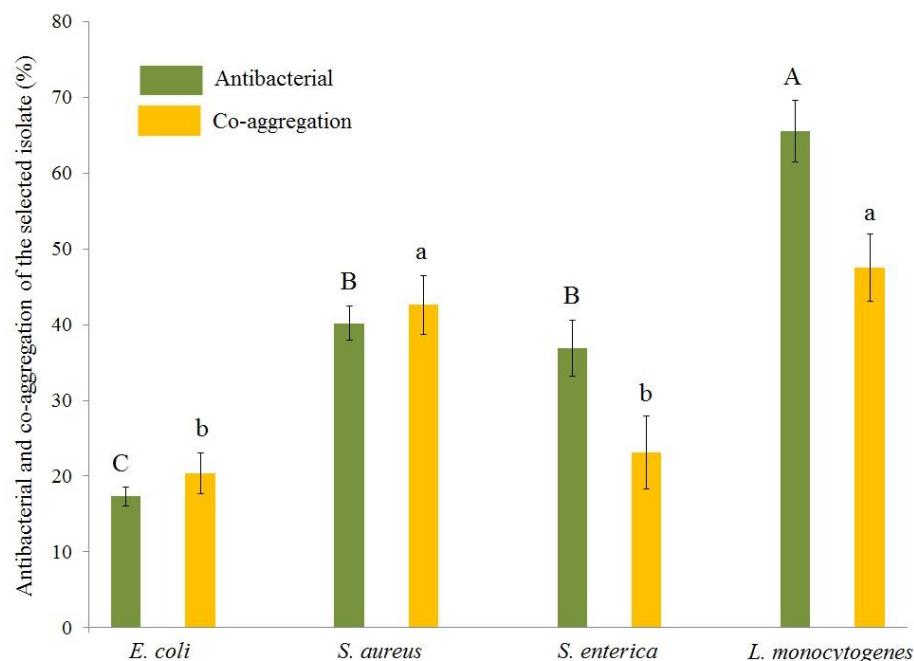


Fig. 2. Antibacterial and co-aggregation activities of the selected yeast isolate obtained from rice bran sourdough. Different lowercase and uppercase letters indicate significant differences ($p < 0.05$) among co-aggregation and antibacterial values, respectively.

KM982973.1 showing the highest level (91.16%). These isolates also demonstrated hydrophobicity values ranging from 35.33% to 89.73%. Auto-aggregation is considered a key PRO characteristic because the ability of cells to adhere to one another enhances their attachment to intestinal epithelial cells, thereby improving colonization and biofilm formation. It also prevents adherence of undesirable microorganisms to the GIT, ultimately supporting host gut health (Kragh et al., 2016). In a study by Kanyakam and Prakitchaiwattana (2025), among 23 yeasts isolated from different fermented substrates, 12 isolates with desirable viability under SGI conditions were selected for hydrophobicity assessment. All 12 isolates showed hydrophobicity levels above 50%. Shahryari et al. (2025) reported that the PRO *P. kudriavzevii* FBS02 exhibited 39.24% hydrophobicity and 95.45% auto-aggregation. Similar to the present findings, no correlation was observed between hydrophobicity and auto-aggregation in the aforementioned study. Hydrophobicity, like auto-aggregation, contributes to health-promoting effects by improving microbial persistence in the GIT (Wang et al., 2024). The adhesion capacity of PRO yeasts is related to complex physicochemical interactions primarily associated with cell-wall structure and surface-expressed adhesins. Cell surface hydrophobicity and charge distribution further influence the strength and specificity of attachment (Sadeghi et al., 2022).

3.5. Antibacterial and co-aggregation potentials of the selected isolate

The results of the antibacterial and co-aggregation evaluations of *W. anomalus* in this study are shown in Fig. 2. As observed, the highest antibacterial activity and co-aggregation ability of the selected yeast isolate were recorded against *L. monocytogenes*, with values of 65.50% and 47.52%, respectively, which were

significantly ($p < 0.05$) higher than those observed for the other foodborne pathogens (Fig. 2). Moreover, the antibacterial and co-aggregation effects of the yeast isolate were stronger against Gram-positive bacteria than Gram-negative ones.

Shams Shargh et al. (2025a) evaluated the inhibitory activity of *P. kudriavzevii* SHS01 against four foodborne bacteria and found that its antimicrobial effect was greater against *S. aureus* and *L. monocytogenes* compared to Gram-negative bacteria. Coutinho et al. (2021) reported that *S. cerevisiae* UFMGC11120 exhibited moderate co-aggregation with *Salmonella Typhi*, while *R. mucilaginosa* UFMGCB183 showed low co-aggregation with *E. coli* and *L. monocytogenes*. A PRO yeast may exhibit stronger antibacterial activity against Gram-positive bacteria in comparison with Gram-negative species, primarily due to the permeability of the cell envelope. Gram-positive bacteria possess a simple cell-wall composed of peptidoglycan without an outer membrane, which makes them more susceptible to antimicrobial metabolites of yeasts. Furthermore, some Gram-negative bacteria often express detoxification mechanisms that further reduce susceptibility (Rahimi et al., 2024). Co-aggregation is one of the antimicrobial mechanisms of PROs, creating a competitive environment that prevents pathogens from colonizing the gut. Yeasts inhibit the attachment and translocation of pathogenic bacteria to the intestinal villi, thereby reducing pathogen activity. They can also prevent pathogen growth through several mechanisms, including competition for nutrients, lowering environmental pH via organic acid production, and secretion of antimicrobial metabolites like ethanol (Liu et al., 2021). Considering that no significant difference was observed in the co-aggregation capacity of the yeast with *S. aureus* and *L. monocytogenes*, and given that adhesion/co-aggregation represents one of the principal antimicrobial mechanisms associated with yeast cell-wall components, this property is highly relevant to the

Table 1. Antimycotic resistance of the isolated yeast from rice bran sourdough. Different letters indicate significant differences at $p < 0.05$. Inhibition zone diameters < 10 mm, $10\text{--}20$ mm, and > 20 mm correspond to resistant (R), intermediate (I), and sensitive classifications, respectively.

Sensitivity	Inhibition diameter zone (mm)	Antimycotic agent (concentration)
I	10.47 ± 1.90^a	Fluconazole (150 mg)
R	8.33 ± 0.81^b	Itraconazole (100 mg)
R	4.50 ± 1.06^c	Ketoconazole (200 mg)
R	0.00 ± 0.00^d	Natamycin (50 mg)
R	0.00 ± 0.00^d	Calcium propionate (60 mg)
R	0.00 ± 0.00^d	Potassium sorbate (60 mg)

investigated application. Notably, autoclaved (non-viable) yeast cells may even exhibit enhanced binding potential due to increased surface exposure and availability of cell-wall functional groups, thereby improving physical interactions with pathogenic bacteria (Taheri et al., 2025a; Sadeghi et al., 2025b). Consequently, the retention or potential enhancement of adhesion capacity after thermal inactivation supports the effectiveness of dead yeast cells as antimicrobial or para-probiotic agents within the studied food system.

3.6. Resistance of the selected isolate to antimycotic agents

The antimycotic resistance results of the yeast isolate are presented in Table 1. According to the findings, the yeast isolate was intermediately sensitive to fluconazole, while it was resistant to ketoconazole, itraconazole, natamycin, potassium sorbate, and calcium propionate.

Coutinho et al. (2021) reported that all *S. cerevisiae* isolates were sensitive to the antifungal agents amphotericin B and fluconazole, while *R. mucilaginosa* strains were sensitive to amphotericin B but resistant to fluconazole. Maione et al. (2024) showed that *P. kudriavzevii* MH5459281, the selected yeast isolate, exhibited intermediate resistance to fluconazole and ketoconazole, whereas it was resistant to itraconazole. Yeasts exhibit antifungal resistance and tolerance through several mechanisms, including alterations in membrane permeability, changes in gene expression, and inhibition of DNA and RNA synthesis (Sadeghi et al., 2023). One of the important characteristics of PRO yeasts is their resistance to antifungal agents. This feature is critical for the practical application of PRO yeasts in the food industry, where they are exposed to preservatives, as well as during treatment with antifungal drugs. Resistance of PRO yeasts to antimycotic agents is mediated by multifactorial cellular defense mechanisms. Alterations in membrane lipid composition and ergosterol biosynthesis reduce the binding affinity of azole and polyene antifungals, thereby limiting membrane disruption. In addition, enhanced cell-wall thickness by increased β -glucan and chitin deposition can reduce drug permeability (Sadeghi et al., 2022; Sadeghi et al., 2025b).

3.7. Antifungal activity of the selected isolate

The inhibitory effect of the yeast isolate on *A. flavus* compared to the control is shown in Fig. 3. As observed, the selected yeast reduced the growth of *A. flavus* by $49.77 \pm 3.64\%$.

Shams Shargh et al. (2025a) reported that *P. kudriavzevii* exhibited notable antifungal activity against *A. flavus*, effectively inhibiting its growth. In another study, the antifungal effect of *R. mucilaginosa* isolated from a fermented substrate was evaluated against *A. niger* and *A. flavus*. The yeast significantly reduced both mycelial growth and sporulation of *A. flavus* (Shams Shargh et al.,

2025b). The antifungal activity of yeasts can be attributed to the production of various protein metabolites, such as mycocins, and the secretion of enzymes like kinases (Ruggirello et al., 2019). The antifungal activity of PRO yeasts is mediated through multiple complementary mechanisms. One principal mechanism is competitive exclusion, whereby yeasts rapidly colonize ecological niches. Additionally, PRO yeasts secrete a range of antifungal metabolites. Certain yeast strains also produce hydrolytic enzymes that degrade structural components of fungal cell-walls. (Sadeghi et al., 2022; Sadeghi et al., 2025a).

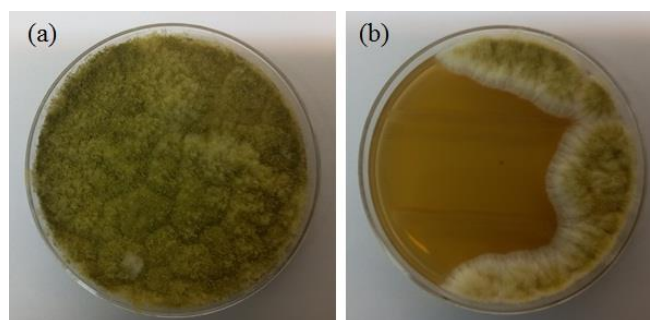


Fig. 3. Antifungal activity of the selected yeast isolate (b) compared to the control sample (a) in overlay bioassay.

3.8. In situ antibacterial effect of the yeast dead cells

It should be noted that although the inhibitory effect of the yeast isolate against *L. monocytogenes* was greater than that observed for *S. aureus*, according to established food safety standards, *S. aureus* is considered one of the most critical hazard-associated microorganisms in heat-treated meat products, whereas *L. monocytogenes* is regarded as comparatively less critical in this specific context (INSO, 2021). Accordingly, *S. aureus* was selected for *in situ* analysis. *In situ* inhibitory activity of the yeast dead cells against *S. aureus* in the produced sausage in a challenge test during a week storage period of the product is illustrated in Fig. 4. Accordingly, there was a significant difference ($p < 0.05$) between control (sausage infected with *S. aureus*) and treated samples every day during the storage period. Moreover, *S. aureus* decreased significantly after 5 days of the storage period compared to the first day (about 0.5 Log CFU/g) in the treated samples containing yeast dead cells + *S. aureus*.

Application of dead cells of PROs as efficient antimicrobial agents in the food supply chain is an emerging way to develop clean-label products. There are some promising reports about the antibacterial activity of structural components derived from yeast

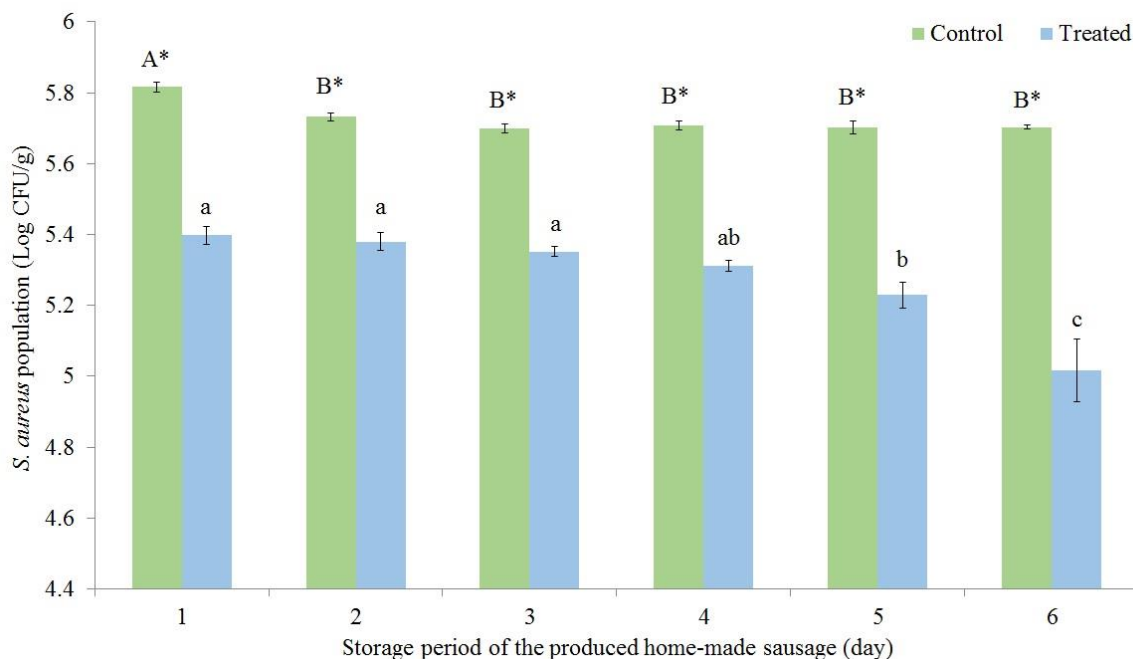


Fig. 4. *S. aureus* population in a home-made chicken sausage supplemented with yeast dead cells (treated) and without yeast dead cells (control) in a challenge test during a week storage period at 4 °C. The different letters indicate a significant difference ($p < 0.05$) among the *S. aureus* population in the same sample (uppercase letters for control and lowercase letters for treated samples) during the storage period. Star symbol also shows a significant difference between control and treated samples on the same day, which was determined using t-test statistical analysis.

dead cells. For example, it is reported that the yeast β -d-glucans induced antimicrobial peptide expressions in broilers challenged with *Salmonella enteritidis* (Shao et al., 2016). Application of yeast cell-wall (YCW) extract not only increased the permeability of *E. coli* outer membrane, but also disrupted its membrane integrity in the study of Liu et al. (2023). It is hypothesized that the co-aggregation ability of the yeast isolate with the targeted bacterium, which was verified in the previous sections of the present study, is responsible for this inhibitory effect. Moreover, mannan and β -glucan of the YCW are involved in formation of a yeast-bacterium complex that limits the accessibility of the bacterium to the available nutrients. Water-binding activity of the YCW components may also affect a_w of the product and subsequent viability/activity of the targeted bacterium. The antibacterial activity of PRO yeasts is mediated through a combination of metabolite-induced inhibition and competitive exclusion. Furthermore, YCW components may contribute to contact-dependent inhibition and pathogen co-aggregation (Sadeghi et al., 2022). It should be noted that there were some limitations in the present study, such as PRO tests being only *in vitro* and para-probiotic effects being evaluated in a single food model against one pathogen.

4. Conclusion

A predominant yeast isolated from rice bran sourdough showed proper PRO characteristics, including high viability under SGI conditions, adhesion properties, and antimicrobial activities. Moreover, heat-killed cells of the yeast isolate exhibited potent antibacterial activity in the supplemented home-made chicken

sausage. Evaluation of PRO characteristics remains essential even when the intended application involves para-probiotic (non-viable or autoclaved) cells of the same strain in a food matrix. Although para-probiotics do not rely on metabolic activity or *in vivo* colonization, many of their functional effects are mediated by structural components and bioactive molecules originally associated with PRO functionality. Properties such as adhesion capacity, co-aggregation, antimicrobial activity, and resistance to gastrointestinal stress provide mechanistic insight into the stability and bioactive potential of the strain. Considering the limitations associated with synthetic preservatives in food-supply chain, application of para-probiotic ingredients of PROs as clean-label strategies is a promising alternative. In meat systems, para-probiotics can inhibit a wide range of spoilage and pathogenic microorganisms, thereby extending shelf-life and lessening the use of synthetic preservatives, which is crucial aspect in the competition for meat product advancement. Besides, looking ahead, the para-probiotics in meat production are expected to undergo a significant increase in the coming years, the increase will be fuelled by research on more bioactive molecules and their optimized carriers. Autoclaved cells of PRO yeasts represent a promising functional ingredient for future applications in processed meat products such as sausages and deli meats. As para-probiotics, these inactivated cells retain structurally intact bioactive components that may exert antimicrobial effects without the safety concerns associated with live microbial incorporation. In meat systems, autoclaved yeast cells could function as natural bioprotective agents by inhibiting pathogenic and spoilage microorganisms through adsorption mechanisms and competitive binding. Additionally, non-viable yeast preparations offer greater processing compatibility with heat-treated meat products and eliminate risks related to microbial

overgrowth during storage. Consequently, autoclaved PRO yeast cells hold substantial potential as multifunctional clean-label additives for enhancing safety, stability, and functional value in processed meat formulations. As regulatory conditions become more conducive and consumer awareness increases, para-probiotics are set to form the backbone of next-generation preservation strategies of the meat industry.

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Conflict of interest

The authors declare that there is no conflict of interest.

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