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Evaluation of probiotic and antifungal properties of predominant yeast isolated from honey

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ABSTRACT

Evaluation of probiotic properties of microorganisms isolated from stressful substrates has received considerable attention. Among probiotic microorganisms, yeasts are distinguished from lactic acid bacteria due to their bigger size, better adhesion ability, and resistance to antibiotics without the possibility of transferring resistance genes. In the present study, probiotic and antifungal properties of the predominant yeast isolated from natural honey were investigated. Sequencing results of the PCR products led to the identification of *Saccharomyces cerevisiae* as the predominant yeast isolated from honey. Moreover, the isolate had no hemolytic activity and showed the highest sensitivity towards natamycin among the studied antimycotic agents. In addition, although the yeast isolate had no proper survival under simulated gastrointestinal conditions, it had relatively high auto-aggregation (93.86%) and cell-surface hydrophobicity (76.36%). The highest co-aggregation ability of the isolate was also observed with Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus*, and the inhibition activity of the isolate against *B. cereus* was significantly (p<0.05) higher than those of the other studied food-borne bacteria. The yeast isolate also showed 32.18% antifungal effect on *Aspergillus flavus*. Accordingly, the predominant yeast isolated from honey has suitable capabilities for application as a protective culture in fermentation industries.

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1- Introduction

The probiotic potentials of microbial flora isolated from stress-induced substrates have been investigated and confirmed. Thus, stress-induced substrates can be suitable sources for isolating probiotic microorganisms [1, 2]. Among the key features of probiotic microorganisms are antimicrobial effects, nutritional benefits, and the ability to maintain the balance of the body's microbial flora to prevent gastrointestinal and immune system disorders [3]. Viability in adverse gastrointestinal conditions (presence of enzymes, bile salts, stomach and intestinal pH), safety, ability to adhere to intestinal epithelial cells, resistance to inhibitory factors such as antibiotics, and lack of antibiotic resistance gene transfer are other significant features of probiotic microorganisms [4, 5].

Most probiotic studies have focused on lactic acid bacteria, with less attention given to other microorganisms with functional characteristics, such as certain yeasts [6]. A notable feature of yeasts is their larger size compared to bacteria, which may enhance their adhesion to the gastrointestinal tract and restrict the growth of pathogenic microorganisms. Other attributes of yeasts include inherent resistance to antibiotics, tolerance to unfavorable gastrointestinal conditions, and positive health effects [7].

Studying microorganisms isolated from special substrates like honey can lead to encountering strains with interesting potentials. To date, studies have been conducted on isolating and evaluating the probiotic properties of yeasts isolated from honey or similar habitats. For instance, research on 104 yeast strains isolated from various honey samples showed that 58 isolates could grow in acidic pH, at 37 °C, and in the presence of bile salts. Moreover, all isolates exhibited high auto-aggregation capability (70-100%) [5, 8]. Additionally, Tauber et al. (2019) isolated *Wickerhamomyces anomalus* CBS 262 from the honey bee gut and observed that this yeast could acidify the environment, favoring *Lactobacillus* bacteria and reducing the growth of the fungus *Nosema ceranae* [9]. According to Khalafalla et al. (2019), the yeasts *Zygosaccharomyces mellis* MK005880, *Lachancea thermotolerans* MK000703, and *W. anomalus* MH997572 isolated from the honey bee gut exhibited tolerance to acidic pH and 3% bile salt concentration, were non-hemolytic, and showed high antibiotic resistance [10]. Based on the 2023 study by Chelucci et al., the yeasts *Starmerella bombicola* CBS 9710, *Candida magnolia* CBS 2677, *Pichia guilliermondii* W1171, *Aureobasidium* sp., *Rhodotorula* sp., *Curvibasidium* sp., and *Metschnikowia* sp. isolated from bee pollen demonstrated significant antioxidant and anti-inflammatory activities, suggesting potential for use as probiotics [11].

Given the limited studies on the probiotic properties of yeasts isolated from natural honey in the country, the present study aimed to evaluate the probiotic and antimicrobial capabilities of the predominant yeast isolated from natural honey.

2-Materials and Methods

1.1 Isolation of Predominant Yeasts from Honey

Natural honey was obtained from the Hyrcanian forests. For initial enrichment, 10 grams of honey were dissolved in 90 mL of brain heart infusion (BHI) broth (Merck, Germany) and incubated at 25 °C for 24 hours. Subsequent serial dilutions from 10^{-1} to 10^{-6} were prepared in sterile ringer's solution (Merck, Germany). All dilutions were surface-plated on yeast glucose chloramphenicol (YGC) agar (Merck, Germany) and incubated at 25 °C for 48 hours. Colonies were then spread, stained, and observed under a light microscope (Zeiss, Germany), and a predominant isolate was selected for further study [12].

2.2 Identification of the Predominant Yeast Isolate

The DNA of the predominant yeast isolate (with the largest population among the yeast

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isolates) was extracted using a kit (GeneAll, South Korea) and amplified via PCR using internal transcribed spacer (ITS)1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers in a thermocycler (Corbett, Australia) over 35 cycles with an annealing temperature of 55 °C. Initial confirmation of amplification was achieved by transferring PCR products to 1.5% agarose gel containing SYBR Safe stain (Invitrogen, USA) and running electrophoresis in Tris-borate-EDTA (TBE) buffer at 90 V for 40 minutes. For final identification, PCR products were sequenced (Pishgam, Iran) and compared with national center for biotechnology information (NCBI) database entries using the basic local alignment search tool (BLAST) procedure [13].

2.3 Viability Assessment of the Selected Isolate in Simulated Gastrointestinal Conditions

An inoculum of 10^8 colony forming units (CFU)/mL of the selected isolate in BHI broth was prepared, and the pH was adjusted to approximately 2 with 1N HCl (Merck, Germany). The suspension was then incubated with 2 mg/mL pepsin (Sigma, USA) at 37 °C for 1.5 hours. After adjusting the pH to around 7.5 with 1N NaOH (Merck, Germany), the suspension was incubated with 0.3% w/v bile salt (Sigma, USA) and 0.5 mg/mL pancreatin (Sigma, USA) at 37 °C for two hours. Finally, the number of viable yeasts was determined by surface plating on YGC agar compared to a control sample [14].

2.4 Evaluation of Co-Aggregation Ability of the Selected Yeast Isolate

Equal volumes and concentrations of active cultures of the selected yeast isolate (with an optical density of around 0.6) and foodborne bacteria *Bacillus cereus* PTCC 1015, *Staphylococcus aureus* PTCC 1112, *Listeria monocytogenes* PTCC 1298, *Salmonella enterica* PTCC 1709, and *Escherichia coli* PTCC 1399 (with an optical density of around 0.05) were prepared. The optical density of these suspensions was measured separately and in mixed form at 600 nm using a spectrophotometer (PG Instruments, UK) after 24 hours of incubation at 37 °C. The percentage of co-aggregation was calculated using the following formula [15], where A_{yeast}, $A_{bacteria}$, and A_{mix} are the optical densities of the yeast, foodborne bacteria, and their mixture, respectively.

$[(A_{yeast}+A_{bacteria})/2-(A_{mix})]/[(A_{yeast}+A_{bacteria})/2]\times 100$

2.5 Evaluation of Auto-aggregation and Hydrophobicity of the Selected Yeast Isolate

For auto-aggregation assessment, an active yeast culture in BHI medium with an optical density of 0.6 (A0) was incubated at 25 °C for 24 hours, and the optical density was measured again (A1). The auto-aggregation percentage was calculated as follows [16]: $(1-(A1/A0))\times 100$

To assess hydrophobicity, a yeast suspension with an optical density of 0.6 (A0) in BHI medium was mixed with three mL of xylene (Merck, Germany). The mixture was incubated at 25 °C for two hours, and the absorbance of the lower phase was measured at 600 nm (AF). The hydrophobicity percentage was calculated as follows [17]: $(1-(AF/A0))\times 100$

2-6- Examination of Antibacterial Activity of the Selected Isolate

To assess the antibacterial effects of the selected yeast isolate, active cultures of the yeast and various foodborne bacteria, including *E. coli*, *L. monocytogenes*, *S. aureus*, *S. enterica*, and *B. cereus*, were prepared. Each bacterial culture was separately mixed with the yeast isolate. The mixtures were incubated for 24 hours at 25 °C. After incubation, serial dilutions of these mixtures were prepared and cultured on chromogenic agar medium. The cultures were incubated for 24 hours at 37 °C, and the number of colonies was then counted and compared to the control samples (bacteria without added yeast).

2-7- Examination of the Yeast Isolate's Resistance to Antimycotic Compounds

To determine the resistance of the selected yeast isolate to antimycotic compounds, a 24-

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hour active culture of the yeast was prepared and cultured on YGC agar medium. Sterile paper discs impregnated with different antimycotic compounds, including ketoconazole, itraconazole, fluconazole, potassium sorbate, natamycin, and calcium propionate, were placed on the medium. After incubation for 24 hours at 25 °C, the diameter of the inhibition zones around the discs was measured using Image J software (version 1.42e).

2-8- Examination of Hemolytic Activity of the Selected Yeast Isolate

To evaluate the hemolytic activity of the yeast isolate, it was cultured on blood agar medium (Merck, Germany) containing 5% fresh sheep blood. The type and color of the halo formed (green halo: alpha hemolysis, white or clear halo: beta hemolysis, no halo formation: gamma hemolysis) were then examined.

2-9- Examination of Antifungal Activity of the Selected Isolate

To assess the antifungal effect of the selected yeast isolate, the overlay culture method was used against the fungi *Aspergillus flavus* PTCC 5006 and *Aspergillus niger* PTTC 5012. Initially, an active culture of the yeast was streaked on YGC medium in parallel lines with a specified distance between them. Then, a suspension containing 10^5 spores/mL of each fungus in potato dextrose agar (PDA) agar medium was prepared and poured over the first layer. The plates were incubated at 25 °C until the entire surface of the control plate (without yeast) was covered by the fungi. Finally, the diameter of the inhibition zones around the yeast was determined using Image J software.

2-10- Statistical Analysis of Results

All tests were conducted in a completely randomized design with three replications. The data were analyzed using SPSS software (version 27) and one-way analysis of variance (ANOVA). The means were compared using the least significant difference at a $p<0.05$ significance level. The graphs were drawn using Microsoft Office Excel 2019.

3-Results and Discussion

3.1. Isolation of Major Yeasts from Honey

After isolating the predominant yeasts from honey, three isolates with codes THY1, THY2, and THY3 were pre-screened based on their colony morphology and microscopic appearance, and were tested for viability under simulated gastrointestinal conditions. Observations showed that isolate THY3 had a significantly higher viability rate of 99.14 ± 2.16% under these conditions ($p<0.05$), while the other two isolates showed no viability.

According to a study conducted by Binetti and colleagues (2013) on yeasts isolated from food products, the viability of some yeast strains under simulated gastrointestinal conditions decreased by approximately two logarithmic cycles, with this ability being completely strain-dependent [23]. A study by Suvarna and colleagues in 2017 on the viability of four yeasts (*Pichia barkeri* VIT-SJSN01, *Yarrowia lipolytica* VIT-ASN04, *W. anomalus* VIT-ASN01, and *Saccharomyces cerevisiae* VIT-ASN03) under simulated gastrointestinal conditions showed viability rates ranging from 48-59% [24]. Another study by Hébrard et al. (2010) examined the viability of *Saccharomyces boulardii* CNCM I-745® under simulated gastrointestinal conditions over time, reporting about 10% viability [25]. A report by Alkalbani and colleagues (2022) also found that yeast strains isolated from food products exhibited varying levels of viability under adverse gastrointestinal conditions, ranging from 7.6% to 96.4% [26]. Moreover, a study by Khalafalla and colleagues (2019) indicated that all yeast strains isolated from the honey bee digestive tract were capable of tolerating similar intestinal conditions [10]. Viability under adverse gastrointestinal conditions is one of the most important characteristics of a probiotic microorganism, as it directly correlates with its effectiveness. Probiotic yeasts use various mechanisms, such as neutralizing pH, altering gene expression, and adjusting membrane lipid composition, to survive under these conditions. Additionally, the resistance of microorganisms to adverse intestinal conditions can be attributed to the

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hydrolysis and consumption of bile [27, 28]. In cases where a microorganism does not have sufficient viability in the upper gastrointestinal tract but possesses other suitable probiotic properties, methods such as microencapsulation are recommended as an efficient strategy to improve its viability in the gastrointestinal system [24].

3.2. Identification of the Selected Yeast Isolate

Based on the electrophoresis results of PCR products, as shown in Figure 1, the amplification of target sequence in the selected yeast isolate was confirmed, and sequencing results identified the yeast as *S. cerevisiae* with 96% similarity. In 2012, Saksinchai and colleagues isolated 186 yeast strains from 37 honey samples collected from 12 different bee species. After morphological and physiological studies, they identified an ascomycete yeast named *Zygosaccharomyces siamensis* [29]. Furthermore, a study by Silva and colleagues in 2020 on microbial isolates from natural honey revealed that 55 isolates belonged to yeasts such as *Papiliotrema flavescents* DMKU-CE139, *Rhodotorula mucilaginosa* SM6-1, *Starmerella meliponinorum* CBS 9117, and *S. cerevisiae*. Among these isolates, some strains of *S. cerevisiae* were capable of producing ethanol and glycerol [30]. Another study in 2021 by Echeverrigaray and colleagues on honey from 17 different bee species found that yeast populations in honey samples with higher water activity had the greatest diversity, with *Zygosaccharomyces* and *Starmerella* being the dominant genera. Additionally, the presence of *Wickerhamomyces sydowiorum* in honey was reported for the first time [31]. Moreover, a 2023 study by Ziuzia and colleagues on yeasts isolated from lemon honey samples in Poland identified 15 selected isolates belonging to species such as *Y. lipolytica*, *C. magnolia*, and *Starmerella magnoliae*. Screening revealed that the best producers of beneficial compounds were strains of *Y. lipolytica*, known for producing large amounts of erythritol and citric acid [32]. Honey, due to its unique properties such as high osmotic pressure, low water content, pH around 4, and the presence of inhibitory compounds, inhibits the growth of many microorganisms [33, 34]. Therefore, most microorganisms found in honey are part of the natural flora and originate from the bee's gut, flower pollen, or the surrounding environment, indicating a high likelihood of encountering microorganisms with probiotic capabilities among them [8].

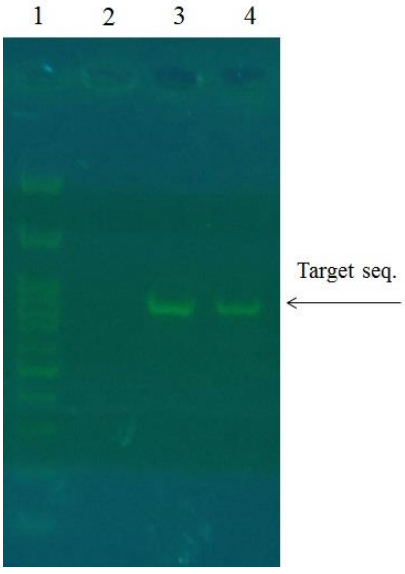

Fig. 1. Gel electrophoresis of the PCR products obtained from amplification of DNA extracted from selected yeast isolated from natural honey. Lane1: 100 bp DNA ladder, lane 2: negative control, lane 3:

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positive control (amplified DNA extracted from baker's yeast) and lane 4: amplified DNA extracted from selected yeast isolate.

3-3. Examination of Co-Aggregation and Antibacterial Activity of the Selected Yeast Isolate

The results of the co-aggregation capability and antibacterial effect of the selected yeast isolate are shown in Figure 2. The findings revealed that the *S. cerevisiae* isolate had a significantly higher co-aggregation ($p<0.05$) with Gram-positive bacteria *B. cereus* and *S. aureus*, and the least co-aggregation with Gram-negative *E. coli*. Additionally, the inhibition effect of the isolate against the Gram-positive bacterium *B. cereus* was significantly higher than against the other studied bacteria, with the lowest inhibitory effect observed against the Gram-negative *S. enterica*. There was also a direct relationship between the co-aggregation capability of this isolate and the inhibition of *B. cereus* growth.

According to a 2020 study by Kanpiengjai et al. on the co-aggregation ability of the yeast *Sporidiobolus ruineniae* A45.2 with foodborne bacteria, the highest percentage of co-aggregation was observed with the bacteria *Salmonella Typhimurium*, *S. aureus*, *L. monocytogenes*, and *B. cereus* with co-aggregation rates of 45.8%, 44%, 37.8%, and 36.2%, respectively, which is consistent with the results of the present study [35]. In a study conducted by Ragavan et al. (2019) on the probiotic yeast *Lipomyces starkeyi* VIT-MN03, it was found that, contrary to the present study, the highest percentage of co-aggregation was observed with Gram-negative bacteria [16]. Co-aggregation refers to the adherence of different microorganisms to each other, where in yeasts, the cell-wall plays a crucial role. Co-aggregation can occur through the binding of bacterial pili to the mannans in the yeast cell-wall as well as through non-specific hydrophobic and electrostatic interactions [36, 37]. In 2021, two yeasts, *Kluyveromyces lactis* B10 and *Torulaspora delbrueckii* B14, isolated from food products, exhibited high auto-aggregation against *S. enterica*. When fed to mice infected with salmonellosis, these yeasts reduced mortality by 90% [38]. Menezes et al. in 2020 examined strains of *S. cerevisiae* and *Pichia kluyveri* isolated from food for their adhesive and antimicrobial capabilities. They found that these yeasts had suitable co-aggregation abilities with foodborne bacteria *E. coli*, *L. monocytogenes*, and *Salmonella enteritidis*. However, these yeasts did not produce any antimicrobial metabolites; thus, their antimicrobial effect was attributed to competition for nutrients and adhesion sites [39]. In another study on the yeasts *S. cerevisiae* UFMGCB 11120 and *R. mucilaginosa* UFMGCB 18377, both showed high co-aggregation capabilities with the foodborne bacterium *S. Typhimurium*. Furthermore, after feeding these yeasts to mice infected with salmonella, *R. mucilaginosa* UFMGCB 18377 protected the mice against salmonella infection and prevented weight loss [40]. In another study, 18 yeast strains isolated from food were evaluated for their probiotic properties, including co-aggregation and antimicrobial activity. It was found that the co-aggregation levels against the foodborne pathogen *S. enteritidis* ranged from 19% to 25%. These isolates also exhibited suitable antimicrobial effects on this pathogen and limited its adhesion to intestinal epithelial cells [41]. The primary mechanisms through which probiotic yeasts exert antimicrobial effects include adherence to pathogens, modification of intestinal epithelial cell structure and function, and the production of certain inhibitory metabolites. Some of these metabolites produced by different yeast strains include phenolic compounds, ethanol, organic acids, carbon dioxide, hydrogen peroxide, and mycosins [42, 43]. The adherence of probiotic microorganisms to pathogens can prevent these pathogens from accessing nutrients, hinder their adhesion to intestinal epithelial cells, and facilitate their expulsion from the gastrointestinal tract through intestinal motility [44].

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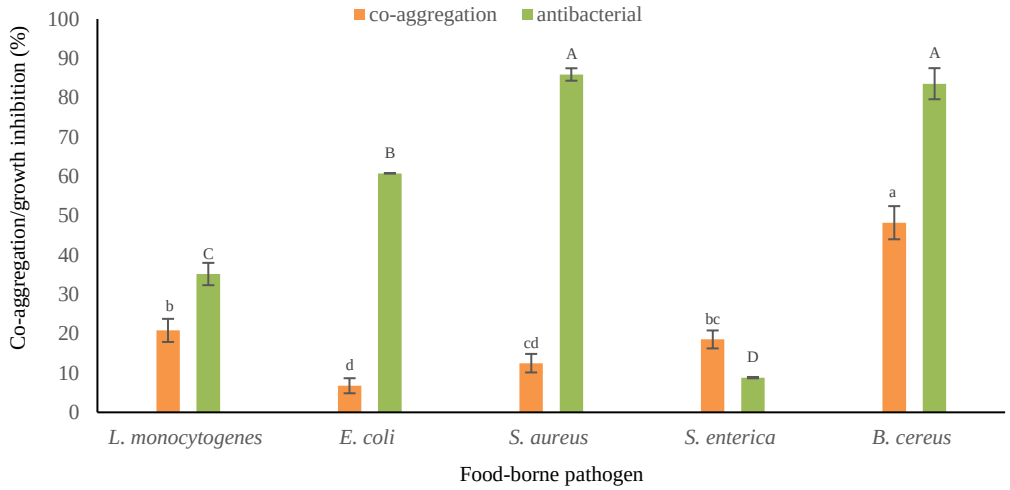

Fig. 2. Co-aggregation and antibacterial capabilities of predominant yeast isolated from natural honey against studied food-borne bacteria. The different lowercase and uppercase letters indicate significant differences ($p<0.05$) among co-aggregation and antibacterial activities of the selected yeast isolate, respectively.

3-4- Investigation of Auto-aggregation and Hydrophobicity of Selected Yeast Isolate

In the present study, the auto-aggregation and hydrophobicity values of *S. cerevisiae* were found to be 93.86 ± 0.06% and 76.36 ± 0.16%, respectively. Auto-aggregation is defined as the ability of a microorganism to adhere to its conspecific strains, which is an important characteristic of probiotic microorganisms [45]. According to a study by Binetti et al. in 2013 on strains of the yeasts *Kluyveromyces marxianus*, *S. cerevisiae*, *Clavispora lusitaniae*, *K. lactis*, and *Galactomyces geotrichum*, it was observed that the auto-aggregation capacity of these isolates ranged from 16% to 70% [23]. Another study by Díaz-Vergara et al. in 2017 reported that strains of *K. marxianus* isolated from food showed auto-aggregation capacities of approximately 33.02% to 41.65%, which were lower than the auto-aggregation capabilities of the isolates in the present study [46]. Auto-aggregation in yeasts is a complex phenomenon occurring during the exponential or stationary phases and is influenced by differences in cell-wall composition and surface proteins. This property can enhance the adhesion of microorganisms to intestinal epithelial cells, improve colonization, form biofilms, protect the gastrointestinal tract, and prevent the attachment of undesirable microorganisms to the gastrointestinal tract [45, 47].

According to a study conducted by Suvarna et al. in 2018 on four yeast strains, the highest hydrophobicity values were observed in *Y. lipolytica* VIT-ASN04 and *P. barkeri* VIT-SJSN01 against diethyl ether, with values of 96% and 74%, respectively [24]. Another study by Diguță et al. in 2022 found that yeast strains isolated from food exhibited different levels of hydrophobicity against hexane and xylene, ranging from 5.93% to 55.43% [48]. The hydrophobicity of microorganisms is associated with their ability to adhere to epithelial cells and colonize the gastrointestinal tract, potentially enhancing their beneficial health effects, prolonging their impact on gastrointestinal immunity, and providing a barrier against the colonization of pathogenic microorganisms. Additionally, the hydrophobicity of microorganisms has a direct relationship with other adhesion abilities, such as auto-aggregation, and can serve as a basis for determining the adhesive capacity of probiotic microorganisms [49, 50].

3-5- Investigation of Resistance to Antimycotic Compounds and Hemolytic Activity

As shown in Table 1, the study of antimycotic resistance revealed that *S. cerevisiae* showed significant sensitivity ($p<0.05$) to natamycin.

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Although this isolate also formed an inhibition zone against ketoconazole, the diameter of the inhibition zone was less than 14 mm, indicating that the isolate was resistant to this compound and other antimycotic agents studied, including calcium propionate, potassium sorbate, itraconazole, and fluconazole. Furthermore, the isolate did not exhibit any hemolytic activity.

Table 1. Antimycotic susceptibility of the predominant yeast isolated from natural honey. Diameter of inhibition zone equal to 14, 15-17 and higher than 20 mm, respectively show resistance, intermediate and sensitive. The different letters indicate significant difference at $p<0.05$.

Antimycotic compounds (concentration)	Inhibition zone diameter (mm)	Sensitivity
Ketoconazole (200 mg)	9.95 ± 0.21^{b}	Resistant
Natamycin (50 mg)	20.10 ± 0.42^{a}	Sensitive
Calcium propionate (60 mg)	0.00^{c}	Resistant
Potassium sorbate (60 mg)	0.00^{c}	Resistant
Itraconazole (100 mg)	0.00^{c}	Resistant
Fluconazole (150 mg)	0.00^{c}	Resistant

An important feature of probiotic yeasts is their resistance to antimycotic agents. This ability is crucial for the practical application of probiotic yeast in the food industry, where preservatives are used, and during treatments with antifungal drugs. Fernández-Pacheco and colleagues in 2021 examined probiotic yeasts for their sensitivity to antimycotic agents. They found that fluconazole and nystatin had the least and most impact on the yeasts studied, respectively [51]. Similarly, Qasim in 2022 investigated the sensitivity of *Rhodotorula* species to natamycin, noting that the inhibition zone created by natamycin was approximately 30 mm, indicating high sensitivity of *Rhodotorula* to this compound [52]. Common mechanisms by which yeasts resist antimycotic agents include modifying or altering membrane permeability, inhibiting DNA and RNA synthesis, and changing gene expression [53-55].

Oliveira Coelho and colleagues in 2019 isolated *S. cerevisiae* LPBF3 from a honey-based kefir beverage and found that this yeast lacked hemolytic activity [56]. Another study on yeasts isolated from food revealed that among the strains studied, only *Candida metapsilosis* exhibited the ability to hemolyze blood cells [57]. Hemolytic activity is an important pathogenicity factor in microorganisms, facilitating access to iron and potentially causing anemia in the host. Therefore, assessing this characteristic in probiotic microorganisms is essential and is one of the initial tests for confirming their safety [58, 59].

3.6. Antifungal Effect of the Selected Yeast Isolate

Figure 3 shows the inhibitory effect of *S. cerevisiae* on the fungi *A. flavus* and *A. niger* compared to the control samples. As depicted, the yeast isolate demonstrated significant inhibitory capability against the growth of *A. flavus*, forming a clear inhibition zone and reducing the growth of this fungus by 32.18 ± 1.32%. Although the isolate did not create an inhibition zone against *A. niger*, it prevented the complete black discoloration of the fungus.

Ruggirello et al. in 2019 isolated strains of *Saccharomyces* and *Candida* from food and investigated their antifungal effects on *Aspergillus* and *Penicillium*. They observed that these yeasts exhibited significant antifungal properties, reducing the growth of

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these fungi by an average of 56.1%. They attributed the antifungal activity of these yeasts to the production of proteinaceous metabolites like mycocins [60]. Similarly, da Cunha et al. in 2018 used strains of *Candida stellimalicola* to inhibit the growth of *Penicillium italicum* in citrus fruits, linking the antifungal capabilities to the production of chitinase enzymes and inhibition of fungal conidial germination [61]. Kunyeit and colleagues (2019) confirmed the potential of two probiotic yeasts, *S. cerevisiae* KTP and *Issatchenkia occidentalis* ApC, as strategies against common fungal infections. Their study found that these yeast strains could inhibit the adhesion, biofilm formation, and hyphal development of four *Candida* species (*Candida tropicalis* MYA 3404, *Candida krusei*, *Candida glabrata*, and *Candida parapsilosis* CDC317), thereby suppressing their pathogenicity [62]. Dikmetas et al. in 2023 investigated the antifungal effects of species from the genera *Moesziomyces*, *Meyerozyma*, and *Metschnikowia* on *A. flavus*. They reported that volatile organic compounds produced by some of these yeasts could reduce fungal mycelium growth and sporulation by up to 91%. Additionally, volatile compounds from *Metschnikowia fructicola* 1-UDM significantly reduced aflatoxin production by the fungus [63]. Fungi are major producers of lethal toxins in food, which can cause cancer, liver failure, and genetic disorders in humans. Therefore, their control in food products is of great importance. Given the adverse effects of industrial fungicides, there is a growing interest in biological control of fungi. Yeasts, due to their non-toxic nature and practical properties, have garnered significant attention as biological control agents. They can control fungi through mechanisms such as producing antifungal metabolites such as mycocins, enzymes like chitinases, and competition for nutrients [64-66].

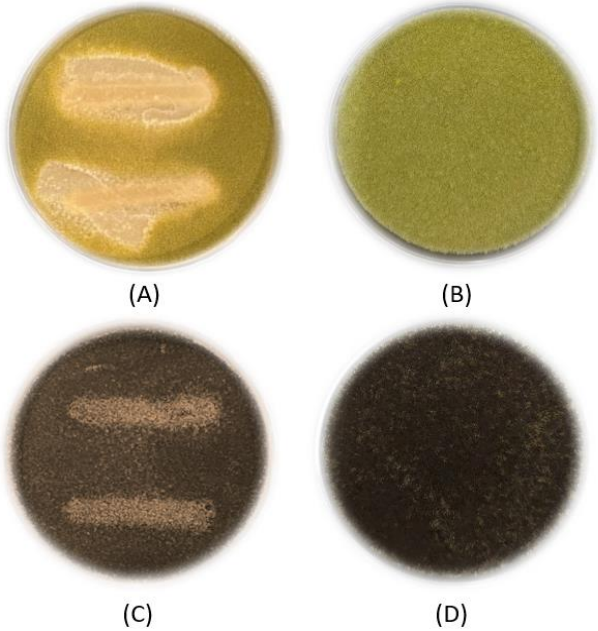

Fig. 3. Antifungal effect of predominant yeast isolated from natural honey on *A. flavus* (A) and *A. niger* (C) compared to their corresponding controls (B and D) in overlay bioassay.

4-Conclusion

Given the significance of antimicrobial and probiotic capabilities of yeasts isolated from food sources, evaluating the potential characteristics of these microorganisms for application in the food industry has become increasingly important. On the other hand, considering the appropriate adhesion properties and inherent resistance of probiotic yeasts to antibiotics, these beneficial microorganisms have gained a special position in food

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biotechnology. With the risks and limitations associated with synthetic preservatives in the food industry, the introduction of suitable biological alternatives such as probiotic yeasts is also noteworthy. In this study, the probiotic and antimicrobial characteristics of a predominant yeast isolate from honey were investigated. The results confirmed the yeast's good auto-aggregation, surface hydrophobicity, co-aggregation, and antibacterial properties. Additionally, the isolate exhibited effective antifungal activity. Therefore, this yeast isolate can be used as a protective culture or a starter culture in fermentation industries.

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ارزیابی ویژگی‌های پروبیوتیکی و ضد قارچی مخمر غالب جدا شده از عسل

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چکیده

همواره ارزیابی قابلیت‌های پروبیوتیکی میکروارگانیسم‌های جدا شده از بسترهای تحت تنش حائز اهمیت بوده است. از بین میکروارگانیسم‌های پروبیوتیک، مخمرها نسبت به باکتری‌های اسید لاکتیک به واسطه اندازه بزرگ‌تر، قابلیت‌های اتصال مناسب‌تر و همچنین مقاومت به آنتی‌بیوتیک‌ها بدون امکان انتقال ژن‌های مقاومت متمایز می‌شوند. در پژوهش حاضر، ویژگی‌های پروبیوتیکی و ضد قارچی مخمر غالب جدا شده از عسل طبیعی مورد ارزیابی قرار گرفت. توالی‌یابی محصولات PCR منجر به شناسایی *Saccharomyces cerevisiae* به عنوان مخمر غالب جدا شده از عسل گردید. همچنین جدایه مذکور، فاقد فعالیت همولیزی بود و از بین ترکیبات ضد قارچ مورد مطالعه، بیشترین حساسیت را نسبت به ناتامایسن نشان داد. علاوه بر این، اگر چه جدایه مخمری مذکور دارای زنده‌مانی مناسبی در شرایط شبیه‌سازی شده دستگاه گوارش نبود اما از قابلیت‌های خود اتصالی (۹۳/۸۶٪) و آبگریزی سطحی (۷۶/۳۶٪) نسبتا بالایی برخوردار بود. بیشترین قابلیت دگر اتصالی جدایه مذکور نیز با باکتری‌های گرم مثبت *Bacillus cereus* و *Staphylococcus aureus* مشاهده شد و بازدارندگی جدایه مذکور در برابر *B. cereus* به شکل معنی‌داری (p <۰/۰۵) از سایر باکتری‌های غذازاد مورد مطالعه بیشتر بود. جدایه مذکور همچنین ۳۲/۱۸٪ اثر ضد قارچی بر علیه *Aspergillus flavus* از خود نشان داد. بر این اساس، مخمر غالب جدا شده از عسل، از قابلیت‌های مناسبی جهت استفاده به عنوان کشت محافظت کننده در صنایع تخمیری برخوردار است.

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