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Original article

Isolation and characterization of lactic acid bacteria from wheat and fenugreek and evaluation of their antifungal and antibacterial activities

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Abstract

Introduction: Lactic acid bacteria (LAB) constitute a major group of probiotic microorganisms recognized for their ability to inhibit pathogenic microbes, modulate the microbiota, and enhance host protection through the production of organic acids, antimicrobial compounds, and other bioactive metabolites.

Methods: The aim of this study was to isolate and characterize lactic acid bacteria from wheat and fenugreek seeds and to evaluate their antibacterial and antifungal activities for potential probiotic and biocontrol applications. Eight LAB strains isolated from wheat and fenugreek seeds were characterized morphologically, biochemically, and functionally, and subsequently assessed for their antibacterial and antifungal potential.

Results: Macroscopic and microscopic observations showed that all strains were Gram-positive, primarily cocci and rods, devoid of DNase activity, and capable of producing biofilm, suggesting profiles compatible with probiotic use. Antibacterial activity varied depending on both the LAB strain and the target pathogen. Inhibition zones ranged from 0 to 15 mm against Staphylococcus aureus (0–10 mm), Escherichia coli (6–11 mm), Listeria monocytogenes (0–15 mm), Bacillus cereus (0–11 mm), Micrococcus luteus (0–11 mm), and Salmonella typhimurium (0–7 mm). Activity was attributed to medium acidification and the production of antimicrobial metabolites, including organic acids and bacteriocins. Selective antifungal activity was also observed, with inhibition zones ranging from 0 to 27 mm against Candida albicans (0–20 mm), Candida krusei (0–22 mm), Candida neoformans (0–26 mm), Aspergillus brasiliensis (0–25 mm), and Aspergillus fumigatus (0–27 mm). These results reflect strain–target-specific interactions and confirm the potential of the LAB strains to inhibit opportunistic yeasts and molds of sanitary relevance.

Conclusion: Overall, these findings quantitatively demonstrate the biotechnological potential of LAB strains derived from wheat and fenugreek seeds as biocontrol agents and probiotic candidates for the prevention of foodborne infections and reduction of fungal contamination.

Keywords: probiotics, wheat, fenugreek seeds, antimicrobial metabolites, biocontrol, pathogenic microorganisms.

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1. Introduction

Lactic acid bacteria (LAB), recognized as Generally Recognized as Safe (GRAS), are promising biocontrol agents because they produce organic acids, bacteriocins, and antifungal metabolites capable of inhibiting fungal and bacterial pathogens, reducing mycotoxins such as zearalenone (ZEN) produced by Fusarium spp., and limiting biofilm formation in food-processing and agricultural settings [1]. Several strains, particularly *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, and *Lactococcus lactis*, have demonstrated antagonistic activity against Fusarium spp., Botrytis cinerea, and Listeria monocytogenes through acidification of the medium, nutritional competition, and partial degradation of toxins [2].

Beyond their intrinsic antimicrobial properties, the ecological origin of LAB plays a critical role in shaping their metabolic diversity and antagonistic potential. Plant-based matrices are especially suitable reservoirs of functional LAB due to their richness in fermentable carbohydrates, fibers, and bioactive compounds that favor LAB colonization and metabolic activity.

Wheat grains and cereal-based substrates naturally harbor diverse LAB communities associated with spontaneous or traditional fermentations, where selective pressures favor strains capable of producing organic acids, bacteriocins, and antifungal metabolites [3]. Strains isolated from silages and fermented cereal products have demonstrated inhibitory capacities reaching 80–90% under agri-food conditions [2]. Similarly, fenugreek seeds constitute a nutrient-rich matrix characterized by a high

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content of polysaccharides, saponins, and phenolic compounds, which not only support LAB growth but may also stimulate the production of bioactive antimicrobial metabolites. These characteristics collectively justify the selection of wheat and fenugreek as promising sources of antagonistic LAB for biocontrol and probiotic applications [4].

Despite the availability of such naturally protective microorganisms, fungal and bacterial contamination of cereals remains a major concern, leading to significant post-harvest losses and serious health risks associated with mycotoxin exposure [5]. Growing concerns regarding the environmental impact and declining efficacy of chemical fungicides have further emphasized the need for sustainable alternatives. In this context, biocontrol strategies based on beneficial microorganisms, including fermentative and endophytic LAB, have emerged as viable approaches capable of limiting pathogen growth and activating natural biological defense mechanisms [6].

For the effective and safe application of LAB as biocontrol agents, rigorous identification and functional characterization are essential. LAB are therefore phenotypically evaluated using morphological, biochemical, and functional assays to confirm their GRAS status, technological suitability, and antimicrobial potential [7]. Standardized in vitro methods, such as agar diffusion assays, allow quantification of inhibitory effects mediated by acidification, competitive exclusion, and membrane-disrupting antimicrobial metabolites, supporting their use in long-term biocontrol strategies.

Based on this scientific rationale, the main objective of the present study was to isolate and characterize lactic acid bacteria from local plant matrices—specifically ensiled wheat and fermented fenugreek seeds—and to evaluate their antifungal and antibacterial activities. Antifungal potential was assessed against Candida albicans ATCC 90028, Candida krusei ATCC 6258, Candida neoformans ATCC 14116, Aspergillus brasiliensis ATCC 16404, and Aspergillus fumigatus ATCC 204305, while antibacterial activity was evaluated against common foodborne pathogens: Staphylococcus aureus ATCC 25923, Micrococcus luteus NCIMB 8166, Escherichia coli ATCC 35218, Listeria monocytogenes ATCC 19115, Salmonella typhimurium ATCC 1408, and Bacillus cereus ATCC 11778.

2. Materials and Methods

Material

The plant material used in this study consisted of wheat and fenugreek seeds collected from the Béja and Sahel regions. A total of 10 wheat samples and 5 fenugreek samples were obtained. Wheat samples were harvested between May and June 2022, whereas fenugreek seeds were collected between March and April 2022. All samples were stored at 4 °C in sealed containers prior to analysis to prevent degradation. Before analysis, the wheat and fenugreek samples were air-dried at room temperature for 7 days and then ground into a fine powder to ensure experimental uniformity.

MRS agar and MRS broth were used to promote the growth of lactic acid bacteria (LAB). Congo Red agar was employed to evaluate the biofilm-forming capacity of the isolates, while DNase agar was used to assess DNase enzyme production. Mueller–Hinton agar was used for antimicrobial susceptibility testing. Tryptic Soy Agar (TSA) supplemented with EL supported LAB growth, and Tryptic Soy Broth (TSB) was used for enrichment of reference strains. All culture media were purchased from Biolife Italiana (Italy).

Isolation and Purification of Lactic Acid Bacteria from Wheat and Fenugreek Seeds

Isolation was performed on MRS agar under facultative aerobic conditions using the decimal dilution method (10^{-1} to 10^{-8}). Plates were incubated at 25 °C, 30 °C, or 37 °C for 24–72 h to evaluate bacterial growth under different environmental, food-related, and physiological conditions. Purification was achieved by repeated subculturing of well-isolated colonies on solid media. Only Gram-positive and catalase-negative strains were selected [8].

Physiological and Biochemical Identification of the Isolates

The selection and purification of isolates were based on the following criteria:

Macroscopic Observation

Colonies grown on different culture media were examined for macroscopic characteristics, including size, color, shape, margin, surface texture, and overall appearance.

Catalase Production

Catalase activity was assessed to distinguish catalase-positive from catalase-negative bacteria. Catalase presence was indicated by the formation of oxygen bubbles when bacterial cells were brought into contact with hydrogen peroxide (H_2O_2), according to the reaction: Inoculum + $H_2O_2 \rightarrow H_2O + ½\ O_2$.

A few drops of H_2O_2 were placed on a microscope slide, and bacterial cells from isolated colonies were added using a sterile loop. Immediate bubble formation indicated catalase-positive activity, whereas the absence of bubbles indicated catalase-negative bacteria [9].

Oxidase Test

The oxidase test was performed by placing an Ox disc in a hemolysis tube and moistening it with a drop of distilled water. A portion of the colony was spread onto the disc. After approximately 10 min, the appearance of a dark purple color that later turned black indicated a positive oxidase reaction [10].

Microscopic Observation

Morphological identification relied on microscopic observation of cell shape and arrangement. Bacterial smears were prepared, stained, and examined under a light microscope. Proper smear preparation was essential to ensure homogeneous cell distribution and accurate visualization of bacterial structures.

Smear Preparation

A drop of sterile water was placed on a clean glass slide. A small amount of culture was mixed into the drop using a platinum loop to form a homogeneous suspension. The smear was spread in circular motions to form a thin layer over two-thirds of the slide. After air-drying, the smear was heat-fixed by passing the slide over a Bunsen burner flame three to four times for approximately 0.5 s each. Slides were allowed to cool before staining.

Gram Staining

Gram staining was performed as a preliminary step for bacterial characterization. The fixed smear was stained with crystal violet for 1 min and rinsed with water. Lugol's iodine was applied for 1 min, followed by decolorization with alcohol for 5–10 s until runoff was clear. The slide was immediately rinsed and counterstained with diluted fuchsin for 1 min. After rinsing and drying, slides were examined microscopically. Gram-positive bacteria appeared violet, whereas Gram-negative bacteria appeared pink [11].

Biochemical Tests

Biofilm-forming capacity was evaluated using Congo Red Agar (CRA; Sigma), as described by Binda et al. (2020) [12]. CRA was prepared by mixing 0.8 g Congo red and 36 g sucrose in 1 L Brain Heart Infusion agar. Plates were incubated at 37 °C for 24 h under aerobic conditions, followed by overnight incubation at room temperature. Biofilm-producing strains formed irregular black colonies, whereas non-producers appeared pink-red [7]. LAB isolates were streaked onto DNase agar and incubated at 37 °C for 48 h. DNase activity was indicated by the presence of a clear pinkish zone around colonies [13].

Antimicrobial Activity

The reference strains used were: Staphylococcus aureus ATCC 25923 (Gram+), Micrococcus luteus NCIMB 8166 (Gram+), Escherichia coli ATCC 35218 (Gram−), Listeria monocytogenes ATCC 19115 (Gram+), Salmonella typhimurium ATCC 1408 (Gram−), and Bacillus cereus ATCC 11778 (Gram+).

Antibacterial activity was evaluated using a modified agar well diffusion method described by Jacobsen et al. (1999) and Schellenberg et al. (2007). LAB pre-cultures were grown in MRS broth at 37 °C for 48 h, and reference strains were grown in TSB at 37 °C for 24 h [14].

After incubation, LAB cultures were centrifuged, and both pellets (for direct activity) and supernatants (for bacteriocin activity) were tested. MRS agar plates were prepared, and four wells were punched in each plate. Plates were incubated at 37 °C for 24 h, and inhibition zones were measured. Results are expressed as mean ± SD from three independent experiments (n = 3) [10].

Three yeasts (Candida albicans ATCC 90028, Candida krusei ATCC 6258, Candida neoformans ATCC 14116) and two filamentous fungi (Aspergillus brasiliensis ATCC 16404 and Aspergillus fumigatus ATCC 204305) were used. Filamentous fungi were incubated for at least 48 h, and yeasts were cultured on YPD medium for 24 h at 37 °C.

Yeast suspensions were prepared from five colonies grown on Sabouraud agar and adjusted to 0.5 McFarland (~10^5–10^6 cells/mL). Aspergillus spores were harvested with sterile water, centrifuged, adjusted to OD_{630} = 0.6–1.0, and diluted 1:10.

Antifungal activity was assessed by the agar well diffusion method on Sabouraud agar supplemented with 2% glucose. Plates were incubated at 30 °C for 48 h, and inhibition zones were measured.

3. Results

Identification of lactic acid bacteria

After 24 h of incubation at 37 °C, 30 °C, and 25 °C, the 20 bacterial strains grown on MRS agar formed colonies exhibiting distinct macroscopic features. The colonies were circular, smooth, convex, and opaque, with variations in size and opacity among the different strains. Selected colonies were subcultured to obtain pure isolates for further analyses, including Gram staining and biochemical tests (Figure 1).

Fig. 1: Macroscopic appearance of lactic acid bacteria colonies isolated on MRS agar after 24 hours of incubation. Colonies are circular, smooth, and well-defined; demonstrating typical morphology of lactic acid bacteria used for further isolation and identification procedures.

Macroscopic examination of the eight lactic acid bacterial strains cultured on MRS agar at 37 °C showed homogeneous colony morphology (Table 1). All strains produced small to very small, circular colonies with a whitish appearance. No pigmentation, irregular margins, or mucoid texture was observed among the isolates.

Microscopic examination combined with basic biochemical tests revealed consistent characteristics among the eight LAB isolates (Table 2). All strains were Gram-positive, catalase-negative, and oxidase-negative. Regarding cell morphology, six isolates (S1, S2, S3, S4, S5, and S7) exhibited a coccoid shape, whereas two isolates (S6 and S8) displayed a bacillary (rod-shaped) morphology. Biofilm production was positive for all eight strains (Table 3).

Table 1. Macroscopic morphology of lactic acid bacterial strains cultured on MRS agar at 37°C.

Strain	Medium / Conditions	Macroscopic characteristics		
		Shape	Size	Color
S1 : BAA (C3) S2 : BB (C1) S3 : BBA1 (C'1) S4 : BB (C2) S5 : BBA1 (C1) S6 : BBA 1(C2) S7 : BAA1 (C1) S8 : G. F	MRS 37°C	Spherical	Very small	Whitish

Table 2. Microscopic observation of the isolates

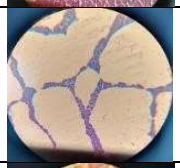
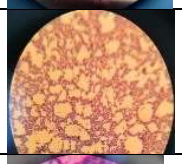
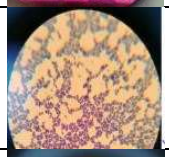
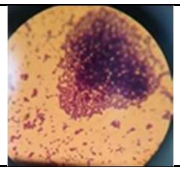
Strain	Catalase	Oxidase	Gram reaction	Observation	Observation / Photo
S1 : BAA (C3)	Negative	Negative	Positive	Cocci	
S2 : BB (C1)	Negative	Negative	Positive	Cocci	
S3 : BBA1 (C'1)	Negative	Negative	Positive	Cocci	
S4 : BB (C2)	Negative	Negative	Positive	Cocci	
S5 : BBA1 (C1)	Negative	Negative	Positive	Cocci	
S6 : BBA1 (C2)	Negative	Negative	Positive	Bacilli	
S7 : BAA1 (C1)	Negative	Negative	Positive	Cocci	
S8 : G. F	Negative	Negative	Positive	Bacilli	

Table 3. Results of the Congo red assay

Strain	Medium	Observation / Photo
S1 : BAA (c3) S2 : BB (C1) S3 : BBA1 (C'1) S4 : BB (C2) S5 : BBA1 (C1) S6 : BBA1 (C2) S7 : BAA1 (C1) S8 : G. F	Congo Red Agar	

DNase activity

DNase activity of the eight LAB isolates was evaluated on DNase agar. After incubation, hydrochloric acid was applied to the agar surface to visualize DNA hydrolysis. No clear zones or halos were observed around any of the colonies (Figure 2). All tested isolates were negative for DNase activity.

Fig 2. Macroscopic appearance of Lactobacillus colonies on DNase agar. Colonies are visible as opaque, circular spots with no surrounding clear zones, indicating the absence of DNase activity. The lack of DNA hydrolysis confirms that all eight isolates are non-pathogenic and unable to degrade extracellular DNA

Antibacterial activity

The antibacterial activity of the eight LAB strains was evaluated using the agar diffusion method. Inhibition zone diameters were measured and expressed as mean ± standard deviation (SD) in millimeters (mm) based on three independent experiments (n = 3) (Table 4).

Against Staphylococcus aureus, inhibition zones ranged from 7 to 10 mm. Strains S3, S4, S5, and S6 produced inhibition zones of 9–10 mm, whereas strains S1 and S8 showed no inhibition. For Micrococcus luteus, inhibition was observed for strains S1 (11 mm), S2 (7 mm), and S6 (7 mm), while the remaining strains showed no inhibition. All tested strains inhibited Escherichia coli, with inhibition zone diameters ranging from 6 to 11 mm; the largest zones were recorded for strains S2 (10 mm) and S5 (11 mm).

Regarding Listeria monocytogenes, inhibition zones were observed for strains S4 (15 mm), S1 (10 mm), and S2 (11 mm), whereas strains S3, S5, S6, S7, and S8 showed no activity. For Salmonella typhimurium, inhibition was detected only for strains S1, S7, and S8, with diameters ranging from 5 to 7 mm. The other strains showed no inhibition. Against Bacillus cereus, inhibition zones of 11 mm were observed for strains S1, S5, S6, and S8, while the remaining strains were inactive. Statistical analysis indicated significant differences ($p < 0.05$) in inhibition zone diameters among the tested LAB strains for several indicator bacteria.

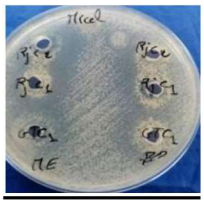
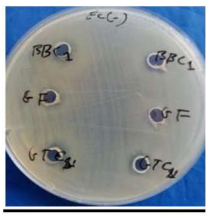
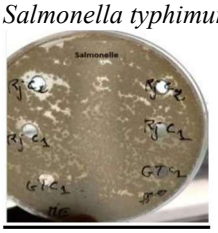
Antifungal activity

The antifungal activity of the eight LAB isolates against yeast and filamentous fungi is presented in Table 5 as mean inhibition zone diameters ± SD (mm) from three independent experiments (n = 3).

Against Candida albicans, all LAB isolates showed inhibition, with diameters ranging from 7 to 20 mm. The largest inhibition zones (20 mm) were recorded for strains S3, S5, and S6, followed by S4 (17 mm), S2 (16 mm), S7 (15 mm), and S1 (12 mm); S8 showed the smallest inhibition zone (7 mm).

For Candida krusei, inhibition zones ranged from 0 to 22 mm. Strains S1 and S8 exhibited the largest zones (22 mm), followed by S2 (17 mm). Smaller zones were observed for strains S3 and S4 (11 mm) and S6 (7 mm), while strains S5 and S7 showed no inhibition.

Table 4. Results of the antibacterial activity assay (mean inhibition zone ± SD, n = 3).

Reference strains	S1 BAA (c3)	S2 BB (C1)	S3 S3 : BBA1 (C`1)	S4 BB (C2)	S5 BBA1 (C1)	S6 BBA1 (C2)	S7 BAA1 (C1)	S8 G.F
Staphilococcus auerus 	0 ± 0	7 ± 0	9 ± 0	10 ± 0	9 ± 0	9 ± 0	7 ± 0	0 ± 0
Micrococcus luteus 	11 ± 0	7 ± 0	0 ± 0	0 ± 0	0 ± 0	7 ± 0	0 ± 0	0 ± 0
Escherichia coli 	7 ± 0	10 ± 0	9 ± 0	7 ± 0	11 ± 0	6 ± 0	7 ± 0	6 ± 0
Listeria monocytogenes 	10 ± 0	11 ± 0	0 ± 0	15 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Salmonella typhimurium 	7 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	6 ± 0	5 ± 0
Bacillus cereus 	11 ± 0	0 ± 0	0 ± 0	0 ± 0	11 ± 0	11 ± 0	0 ± 0	11 ± 0

(All values are mean inhibition zone ± SD, n = 3 ; significant differences assessed by ANOVA, $p < 0.05$).

Regarding Candida neoformans, inhibition zones ranged from 7 to 26 mm. The largest inhibition was observed for strain S8 (26 mm), followed by S5 (22 mm), S3 (19 mm), S4 (17 mm), S6 (16 mm), and S2 (15 mm). Strains S1 and S7 showed smaller zones of 8 mm and 7 mm, respectively.

Against Aspergillus brasiliensis, inhibition zones ranged from 0 to 25 mm. Strains S8 (25 mm), S4 (23 mm), S2 (22 mm), and S1 (20 mm) showed measurable inhibition, while S3 had a smaller zone (7 mm). No inhibition was observed for strains S5, S6, and S7.

For Aspergillus fumigatus, inhibition zones ranged from 0 to 27 mm. The largest zone was recorded for strain S1 (27 mm), followed by S2 (23 mm) and S8 (15 mm). Smaller zones were observed for strains S3 (11 mm), S4 (7 mm), and S7 (6 mm), whereas S5 and S6 showed no inhibition.

Statistical analysis indicated significant differences ($p < 0.05$) in inhibition zone diameters among the LAB strains for all tested fungal species.

4. Discussion

The isolation and characterization of LAB led to the selection of eight strains exhibiting homogeneous, whitish, round colonies, some surrounded by a transparent zone, confirming their purity. These morphological characteristics are consistent with those reported for LAB isolated from raw fermented milk, which typically form circular, white to gray-white colonies with diameters ranging from 0.1 to 0.5 mm [9]. Microscopic examination following Gram staining showed that six isolates were cocci arranged in chains, pairs, or aggregates, whereas two isolates exhibited a rod-shaped morphology. All strains were Gram-positive, in agreement with previous reports describing a predominance of cocci (59%) and rods (41%) among LAB isolated from Moroccan and Nigerian raw milk [15]. Functional characterization revealed that all strains were capable of biofilm production, a trait commonly associated with enhanced resistance to environmental stress and antimicrobial agents. In addition, none of the isolates exhibited DNase activity, suggesting a low pathogenic potential. These characteristics align with those reported for LAB considered safe for probiotic applications, which are typically catalase-negative and lack major virulence factors [16].

The antibacterial activity assays revealed marked variability depending on both the LAB strain and the target bacterium, with inhibition zone diameters ranging from 7 to 15 mm against Gram-positive and Gram-negative pathogens (Table 4). Several strains (S2, S3, S4, S5, S6, and S7) inhibited the growth of Staphylococcus aureus, likely through medium acidification resulting from lactic acid production. This observation is consistent with studies reporting that organic acids and pH reduction (around 3.8) represent the main inhibitory mechanisms against S. aureus [17]. Activity against Micrococcus luteus was observed for strains S1, S2, and S6, with S1 showing the highest inhibition (11 mm), in agreement with reports describing inhibition mediated by organic acids and bacteriocins produced by LAB [18]. All tested strains inhibited Escherichia coli, with S5 exhibiting the highest activity (11 mm), supporting previous findings on the antagonistic effects of LAB mediated by organic acids, bacteriocins, and hydrogen peroxide [19,20]. Regarding Listeria monocytogenes, only strains S1, S2, and S4 showed inhibitory activity, with S4 being the most effective. This selective inhibition may be associated with acidification ($pH < 4.5$) and the production of specific bacteriocins, such as nisin, known for their antilisterial activity [20]. Against Salmonella, inhibition was observed only for strains S1, S7, and S8, with S1 showing the largest inhibition zone, consistent with previous studies reporting inhibition mediated by lactic acid production and bacteriocins [18]. In addition, strains S1, S5, S6, and S8 inhibited Bacillus cereus, suggesting the involvement of multiple mechanisms including acidification, nutrient competition, and bacteriocin production [21]. Collectively, these results highlight the antimicrobial potential of the selected LAB strains and support their relevance as probiotic candidates capable of modulating intestinal microbiota and reducing the risk of foodborne infections [22].

The antifungal activity results (Table 5) further demonstrate the selective inhibitory capacity of the LAB strains against yeast and filamentous fungi. Moderate to strong inhibition was observed against Candida albicans, with inhibition zones reaching 20 mm for strains S3, S5, and S6, in agreement with studies reporting susceptibility of this opportunistic yeast to probiotics and bioactive metabolites [23]. In contrast, Candida krusei exhibited a heterogeneous response, with strong inhibition by strains S1 and S8 and complete resistance in others, reflecting its intrinsic resistance mechanisms, including efflux pumps and ERG11 mutations [24]. For Candida neoformans, large inhibition zones observed for strains S8 and S5 indicate notable susceptibility, consistent with previous reports describing the antifungal activity of microbial or phenolic compounds targeting its capsular structures [25].

Among filamentous fungi, strong inhibition of Aspergillus brasiliensis by strains S2, S4, and S8 contrasted with the absence of activity in other isolates, highlighting strain-dependent variability. Similarly, Aspergillus fumigatus showed high sensitivity to strains S1 and S2, suggesting potential relevance for controlling opportunistic fungal pathogens [26].

The selective nature of these antifungal effects underscores the need for further investigations, including minimum inhibitory concentration (MIC) assays and synergy studies with conventional antifungal agents, particularly in contexts where fungal contamination and mycotoxin exposure, such as zearalenone, coexist.

This study has some limitations. The identification of LAB was based exclusively on phenotypic, biochemical, and morphological characteristics, without molecular confirmation such as 16S rRNA gene sequencing. Consequently, taxonomic assignment remains tentative and should be confirmed in future studies.

In addition, antimicrobial assays were conducted using non-neutralized culture supernatants that were not treated with proteolytic enzymes. Therefore, the relative contributions of organic acids, hydrogen peroxide, and bacteriocins to the observed antimicrobial activity could not be determined. Future work will focus on mechanistic studies using neutralized and enzyme-treated supernatants to clarify the nature of the inhibitory compounds.

Table 5. Results of the antifungal activity assay (mean inhibition zone ± SD, n = 3).

Reference strains	The 8 potential biocontrol LAB isolates	Inhibition zone diameter (mm)
Candida albicans	S1 : BAA (c3)	12 mm ± 0
	S2 : BB (C1)	16 mm ± 0
	S3 : BBA1 (C'1)	20 mm ± 0
	S4 : BB (C2)	17 mm ± 0
	S5 : BBA1 (C1)	20 mm ± 0
	S6 : BBA1 (C2)	20 mm ± 0
	S7 : BAA1 (C1)	15 mm ± 0
	S8 : G.F	7 mm ± 0
Candida krusei	S1 : BAA (c3)	22 mm ± 0
	S2 : BB (C1)	17 mm ± 0
	S3 : BBA1 (C'1)	11 mm ± 0
	S4 : BB (C2)	11 mm ± 0
	S5 : BBA1 (C1)	0 mm ± 0
	S6 : BBA (C2)	7 mm ± 0
	S7 : BAA (C2)	0 mm ± 0
	S8 : G.F	22 mm ± 0
Candida neoformans	S1 : BAA (c3)	8 mm ± 0
	S2 : BB (C1)	15 mm ± 0
	S3 : BBA1 (C'1)	19 mm ± 0
	S4 : BB (C2)	17 mm ± 0
	S5 : BBA1 (C1)	22 mm ± 0
	S6 : BBA1 (C2)	16 mm ± 0
	S7 : BAA 1(C1)	7 mm ± 0
	S8 : G.F	26 mm ± 0
Aspergillus brasiliensis	S1 : BAA (c3)	20 mm ± 0
	S2 : BB (C1)	22 mm ± 0
	S3 : BBA1 (C'1)	7 mm ± 0
	S4 : BB (C2)	23 mm ± 0
	S5 : BBA1 (C1)	0 mm ± 0
	S6 : BBA 1(C2)	0 mm ± 0
	S7 : BAA 1(C1)	0 mm ± 0
	S8 : G.F	25 mm ± 0
Aspergillus fumigatus	S1 : BAA (c3)	27 mm ± 0
	S2 : BB (C1)	23 mm ± 0
	S3 : BBA1 (C'1)	11 mm ± 0
	S4 : BB (C2)	7 mm ± 0
	S5 : BBA1 (C1)	0 mm ± 0
	S6 : BBA1(C2)	0 mm ± 0
	S7 : BAA1 (C1)	6 mm ± 0
	S8 : G.F	15 mm ± 0

Conclusion

The results demonstrate that the isolated lactic acid bacterial strains exhibit morphological, biochemical, and functional characteristics compatible with potential applications in food and probiotic contexts. Their ability to inhibit a wide range of pathogenic bacteria and fungi highlights the important roles of organic acids, bacteriocins, and other antimicrobial metabolites in their mechanisms of action.

The selectivity observed between different strains and target microorganisms underscores the need for further investigations, including determination of minimum inhibitory concentrations, studies on synergistic interactions with conventional antifungals, and in vivo testing. In this perspective, the studied strains represent promising candidates for the development of natural biocontrol alternatives, contributing to improved food safety and the valorization of fermentative microorganisms.

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Authors' contributions

Haifa Dhif: Formal analysis, investigation, writing—original draft preparation, writing—review & editing, visualization. Jalila Ben Salah-Abbès: Writing—review & editing. Kamel Chaeib: Conceptualization, writing—review & editing, validation. Samir Abbès: Writing—review & editing, supervision.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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