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Probiotic and Antifungal Attributes of Lactic Acid Bacteria Isolates from Naturally Fermented Brazilian Table Olives

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Abstract: Research with fermented olives as a source of wild Lactic Acid Bacteria (LAB) strains with probiotic and biotechnological characteristics constitutes a promising field of work. The present study evaluated in vitro probiotic, antifungal, and antimycotoxigenic potential of LAB isolates from naturally fermented Brazilian table olives. Among fourteen LAB isolates, the *Levilactobacillus brevis* CCMA 1762, *Lactiplantibacillus pentosus* CCMA 1768, and *Lacticaseibacillus paracasei* subsp. *paracasei* CCMA 1770 showed potential probiotic and antifungal properties. The isolates showed resistance to pH 2.0 (survival ≥ 84.55), bile salts (survival ≥ 99.44), and gastrointestinal tract conditions (survival ≥ 57.84%); hydrophobic cell surface (≥27%); auto-aggregation (≥81.38%); coaggregation with *Escherichia coli* INCQS 00181 (≥33.97%) and *Salmonella* Enteritidis ATCC 564 (≥53.84%); adhesion to the epithelial cell line Caco-2 (≥5.04%); antimicrobial activity against the bacteria *S.* Enteritidis ATCC 564 (≥6 mm), *Listeria monocytogenes* ATCC 19117 (≥6 mm), *Staphylococcus aureus* ATCC 8702 (≥3 mm), and the fungi *Penicillium nordicum* MUM 08.16 (inhibition ≥ 64.8%). In addition, the strains showed the ability to adsorb the mycotoxins aflatoxin B1 (≥40%) and ochratoxin A (≥34%). These results indicate that LAB strains from naturally fermented Brazilian table olives are potentially probiotic and antifungal candidates that can be used for food biopreservation.

Keywords: adhesion ability; antifungal activity; fermented olives; in vitro digestive simulation; mycotoxins; probiotic selection

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1. Introduction

In addition to benefiting human health, table olives are greatly appreciated worldwide for their nutritional value and sensory characteristics due to phenols and antioxidant compounds in their composition [1]. However, olive drupes cannot be eaten immediately after harvest due to oleuropein (which is responsible for the bitterness). Therefore, this fruit needs to go through several processing methods to become edible, including methods involving chemical treatment of fruits (Sevillian and Californian methods) and the natural method of fermentation (olives processed in brine without pretreatment with chemicals) [2].

The consumption of table olives is widespread throughout the world. In the 2020–2021 season, the production of table olives exceeded 2.6 million tons, with Spain and Greece

being the main contributors in the Mediterranean [3,4]. Although olives are widely consumed in Brazil, their production is low, and most of them are imported (approximately 10,367.9 tons of table olives until August 2021) [3]. Therefore, studies on Brazilian olives are important for the characterization and valorization of this product. Table olives can contain microorganisms with biotechnological properties to be explored as a fermented food item.

The natural fermentation process of olives occurs in extreme environments (olive and brine), characterized by low nutrients and high levels of polyphenols and sodium chloride. The microbiota surviving the fermentation process mainly comprises lactic acid bacteria (LAB) and yeasts. The dominant microorganisms in this environment have important physiological properties that allow their use as probiotics [5].

Therefore, research with fermented olives as a source of wild LAB strains with probiotic and technological characteristics constitutes a promising field of work [6]. Many recent works have demonstrated several health benefits of probiotic LAB found in fermented olives. The most studied activities were mainly antibacterial, antifungal, anti-adherent, antiproliferative, anticancer, metal detox, and immunostimulant [7–9]. However, there are no data regarding LAB isolated from fermented Brazilian table olives about probiotic potential or antifungal activity.

The criteria for selecting probiotic LAB strains are safety, including the absence of the secretion of harmful metabolites, survival in gastrointestinal conditions, and the ability to inhibit pathogens [10,11]. The most studied probiotic properties include resistance to low pH and bile salts or tolerance toward gastric juice and pancreatic digestion—in some cases, all of them—with the main objective of achieving the survival of probiotics in the gastrointestinal tract (GIT) [12]. Recommended safety criteria in selecting probiotics include isolation environment, taxonomic identification, and absence of virulence and transferable antibiotic resistance genes [13].

The high acidification capacity of LAB is necessary to guarantee the biotransformation of the olives and the inhibiting pathogenic microorganisms responsible for deterioration [14]. Due to this important capacity, LAB has been intensively studied as a bioprotective agent and can be used to prevent fungal contamination and mycotoxin production [15]. So, using lactic acid bacteria with probiotic potential and biopreservation capacity confirms that LAB can be biological agents with high and promising applicability in food safety [16].

Although many probiotic LAB strains are characterized, the search for new probiotic strains continues to be of interest due to the vast possibilities of using these microorganisms, especially their incorporation into food matrices [17]. However, given the lack of knowledge in this sector of olives in Brazil, this study evaluated the in vitro probiotic potential, antifungal activities, and adsorption of mycotoxins of LAB isolates from naturally fermented Brazilian table olives.

2. Materials and Methods

2.1. Microorganisms and Growth Conditions

A total of fourteen LABs were studied to assess the probiotic potential of each. The species *Levilactobacillus brevis* (*L. brevis*), *Lacticaseibacillus paracasei* subsp. *paracasei* (*L. paracasei*), and *Lactiplantibacillus pentosus* (*L. pentosus*) (Table 1) were evaluated. The strains were isolated from naturally fermented Brazilian table olives (Greek-style) of two cultivars, Grappolo 541 and Ascolano, harvested at the green stage from the Experimental Farm of EPAMIG (Minas Gerais Agricultural Research Company) in the city of Maria da Fé-MG, Brazil (22°18′ south latitude and 45°23′ west longitude).

Table 1. Bacteria strains from table olives used in this study (n = 14).

Bacteria Strain	N° of Isolates	Code	Cultivar and Fermentation Time
Levilactobacillus brevis	3	**CCMA1762**	Ascolano (time 120 days)
		CCMA1765	Ascolano (fruit)
		CCMA1766	Ascolano (time 60 days)
Lacticaseibacillus paracasei subsp. *paracasei*	10	CCMA1763	Ascolano (time 120 days)
		CCMA1764	Ascolano (time 60 days)
		CCMA1767	Ascolano (time 30 days)
		CCMA1769	Ascolano (time 120 days)
		CCMA1770	Grappolo (time 60 days)
		CCMA1771	Grappolo (fruit)
		CCMA1772	Ascolano (time 60 days)
		CCMA1773	Ascolano (fruit)
		CCMA1774	Grappolo (time 120 days)
		CCMA1775	Grappolo (time 30 days)
Lactiplantibacillus pentosus	1	**CCMA1768**	Ascolano (time 120 days)
Lacticaseibacillus paracasei subsp. *paracasei*	1	LBC-81	(Danisco A/S, Copenhagen, Denmark)

Strains showing potential properties for use as probiotics during the simulated digestion are bolded.

The identification of strains was performed by MALDI-TOF, using the Maldi-tof Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany), and the cluster was identified through sequence analysis of the 16S rRNA gene using primers 27-F (5′-AGAGTT TGATCCTGGCTCAG-3′) and 1512-R (5′-GGCTACCTTGTTACGACT-3′) (Simões et al., 2021). All isolates were deposited in the Culture Collection of Agricultural Microbiology (CCMA) of the Federal University of Lavras. CCMA is registered with the World Data Centre for Microorganisms (WDCM) as CCMA-UFLA under WDCM 1083. For their use, each LAB strain was cultivated in Man Rogosa and Sharpe (MRS) (Oxoid, Basingstoke, UK) broth and incubated at 37 °C for 48 h.

The bacteria pathogenic strains employed in the coaggregation and antibacterial activity assays were *Escherichia coli* (EPEC) INCQS 00181 CDC 055, *Salmonella* Enteritidis ATCC 564, *Listeria monocytogenes* ATCC 19117, and *Staphylococcus aureus* ATCC 8702. The pathogens were grown at 37 °C for 24 h in BHI (Brain heart infusion, Himedia, Einhausen, Germany). The reference strain selected for all assays was the lyophilized *Lacticaseibacillus paracasei* subsp. *paracasei* LBC-81 (Danisco A/S, Copenhagen, Denmark) bacterium (Fonseca, et al. [18]. This microorganism was reactivated in MRS broth at 37 °C for 48 h. A growth curve was elaborated to standardize LAB, pathogens, and the reference strain inoculum; absorbance was measured at 600 nm, and colony counting was performed using an MRS agar for LAB and a BHI agar for pathogens. The plates were incubated at 37 °C for 48 h, and the inoculum was standardized at 7 to 8 log CFU mL^{-1}.

The fungal strains used in antifungal activity were *Aspergillus flavus* MUM 08.201 and *Penicillium nordicum* MUM 08.16, obtained from the MUM Culture Collection (Micoteca da Universidade do Minho, Braga, Portugal). The fungi were reactivated on MEA agar (Malt Extract Agar, Himedia, Einhausen, Germany) at 25 °C in the dark. After 7 days, the fungal spores were suspended in a solution of 0.1% peptone and 0.05% Tween 80 (Fisher Scientific, Waltham, MA, USA). The concentration was standardized at 10^6 spores/mL using a Neubauer chamber.

2.2. Tolerance to Low pH and Bile Salts

The fourteen LAB isolates were tested for tolerance to acid pH and bile salt concentration, according to Ramos et al. [19], with certain modifications. The isolates were grown and centrifuged (3000× g for 5 min at 4 °C), after which the strains were resuspended in MRS broth with pH adjusted to 2.0 using an acid solution (1 N HCl) or supplemented with

bile salts (Oxgall, Neogen, Lansing, MI, USA) at 0.3 % (*v*/*v*). The cells were incubated for 3 h at 37 °C. Samples were obtained at the beginning (time 0 h) and end of incubation (time 3 h) to determine the total viable count. Dilutions were made (up to 10^7) and spread plated onto MRS agar incubated at 37 °C for 48 h; viable cells were counted (log CFU mL^{-1}). The percentage of survival at pH 2.0 or bile salt was calculated using the equation:

$$\text{Survival at pH 2 (\%) or bile salt (\%)} = \text{Final viable count/Initial viable count} \times 100$$

2.3. Cell Surface Hydrophobicity

For twelve strains selected in the previous assay, the cell surface hydrophobicity assay was performed according to dos Santos, et al. [20], with some modifications. The LAB was cultivated, and the pellet cells were obtained by centrifugation (7000× *g* for 5 min at 4 °C). After the pellets were washed twice and resuspended in PBS solution (50 mM K_2HPO_4/KH_2PO_4, pH 6.5, Sigma-Aldrich, Saint Louis, MO, USA) to achieve an optical density of 0.6–0.7 (PBS containing 8 log CFU mL^{-1}) (A_0) at 600 nm, the organic solvent n-hexadecane (Sigma-Aldrich, Saint Louis, MO, USA) was mixed (1:5) with the cell suspension and vortexed for 2 min. After 1 h of incubation at 37 °C, the absorbance of the aqueous layer formed was measured at 600 nm (A). The following equation calculated cell surface hydrophobicity:

$$\text{Hydrophobicity (H\%)} = [(A_0 - A)/A_0] \times 100$$

The values for A_0: absorbance values measured before the extraction and A: absorbance values measured after the extraction with n-hexadecane.

2.4. Auto-Aggregation and Coaggregation Assays

The isolates were grown in an MRS broth medium at 37 °C for 48 h to determine specific cell–cell interactions (auto-aggregation) and with pathogenic strains (coaggregation), according to Del Re, et al. [21], with minor modifications. After incubation, the cultures were centrifuged (7000× *g* for 5 min), washed twice, and resuspended in phosphate-buffered saline (50 mM K_2HPO_4/KH_2PO_4, pH 6.5). In addition, 4 mL of the cell suspensions were mixed and incubated at room temperature for 24 h. After the incubation, absorbance at 600 nm was measured using a UV spectrophotometer (Thermo Scientific, Waltham, MA, USA) (A_0). The auto-aggregation percentage was expressed as:

$$\text{Auto-aggregation (\%)} = 1 - (A_0/A_t) \times 100,$$

where A_0 represents the optical density at the beginning of the experiment and A_t represents the data after 24 h.

For coaggregation, LAB cultures and pathogenic microorganisms, *Escherichia coli* (EPEC) INCQS 00181 CDC 055 or *Salmonella* Enteritidis ATCC 564, were mixed in equal parts (2 mL) in pairs (LAB + selected pathogen). Control groups of individual microorganisms were tested as references. The suspensions' absorbance at 600 nm was measured right after mixing 4 h of incubation at 37 °C. Samples were retrieved following the procedure from the auto-aggregation assay. All experiments were performed in triplicate.

The percentage of coaggregation was calculated using the equation by Handley, et al. [22]:

$$\text{Coaggregation (\%)} = ((Ax + Ay)/2) - A(x + y) \times 100,$$

$$Ax + Ay/2$$

where x and y represent each of the two strains in the control tubes, and $(x + y)$ represents the mixture. Six strains with higher values of cell surface properties were selected for the next set of assays.

2.5. In Vitro Assessment of Safety Attributes

2.5.1. Gelatinase Activity

Selected LAB isolates were tested for gelatinase production using a tryptone-neopeptone-dextrose (TND) agar (g/L: tryptone 17.0, neopeptone 3.0, dextrose 2.5, NaCl 5.0, K_2HPO_4 2.5 and agar 20.0, Sigma-Aldrich, Saint Louis, MO, USA) containing 0.4% gelatin. The LABs were cultivated, and 10 µL of the cell cultures were plated in plates containing the TND medium (incubated at 37 °C for 48 h). After the incubation, a saturated ammonium sulfate solution was added to flood the Petri plates. The positive reaction was defined by developing clear zones around the spots [23].

2.5.2. DNase Production

The LAB isolates were subjected to the analysis of DNase enzyme production. The strains were grown, and 10 µL of the cell cultures were plated in a DNase agar medium (HiMedia, Einhausen, Germany). The plates were incubated at 37 °C for 48 h. Then, an HCl (2 mM) solution was added enough to flood the Petri plates, and the confirmation of a DNase enzyme production was determined by a clear zone around the colonies [23].

2.5.3. Hemolytic Activity

The strains were also tested for hemolytic activity; 10 µL of the LAB cell cultures was inoculated on a culture medium of tryptone soy agar 10% (TSA, bacto™ Bd, Franklin Lakes, NJ, USA), supplemented with 5% (*v*/*v*) of defibrated ram's blood. The plates were incubated for 48 h at 37 °C. The positive result consisted of a clear zone of hydrolysis around the colonies. Therefore, isolates with this result cannot be selected for potential probiotic use [24].

2.6. Antibacterial Activity

Antibacterial activity was evaluated following the methodology described by Prado, et al. [25] with modifications. The LAB cells were centrifuged (7000× *g* for 5 min at 4 °C), and the supernatant (pH adjusted to 7.0) was used for the diffusion technique in wells. An amount of 50 µL was added to the wells on a BHI medium containing 1 mL of the pathogen. The plates were incubated at 37 °C for 48 h, and the positive control was a culture medium without microorganisms. Growth-free inhibition zones around the well confirmed the antibacterial activity.

2.7. Adhesion of LAB Strains to Caco-2 Cell Lines

2.7.1. Growth and Maintenance of Caco-2 Cells

The Caco-2 cells supplied by the Rio de Janeiro Cell Bank (BCRJ, Rio de Janeiro, Brazil) were subcultured in 24-well tissue culture plates (Sarstedt, Germany) to a 2×10^5 cells/mL. The medium used was a modified minimal essential (MEM) by Eagle, supplemented with 10% (*v*/*v*) heat-inactivated fetal bovine serum, 1 × non-essential amino acids, and 0.1 mg/mL gentamicin (Invitrogen, Gibco, Naerum, Denmark). Cultivation took 21 days (at 37 °C, in a humidified atmosphere of 5% CO_2). The cell medium was changed on alternate days until cells obtained subconfluence (80–90%) and were then sub-passed [19].

2.7.2. Adhesion to Caco-2 Cell Line

The human colon adenocarcinoma cell line (Caco-2) adhesion test was performed according to the methodology used by Ramos, Thorsen, Schwan, and Jespersen [19], with certain modifications. Selected LAB isolates were cultivated (24 h, 37 °C) in MRS broth and washed twice with phosphate-buffered solution (10 mM K_2HPO_4/KH_2PO_4 and 150 mM NaCl, pH 7.4). Then, they were resuspended in Eagle's MEM at an approximate concentration of 8 log CFU mL^{-1}. For the assay, 1 mL of the suspension of each bacterium was incubated in each well together with the cell line culture (90 min, 37 °C, 5% CO_2 atmosphere). Next, washed with 1 mL of PBS three times to remove non-adherent bacteria cells, and the cell line cultures were lysed with 1 mL of Triton-X solution (0.1% *v*/*v* in

PBS, 10 min, 37 °C). Then, serial dilutions of the solution with released bacterial cells were performed and enumerated on MRS agar (37 °C, 48 h). The percentage of initial bacteria and the counts after washings (log CFU mL^{-1}) were used to calculate the adhesion capacity. The assays were repeated twice and performed in triplicate.

2.8. Survival of LAB during In Vitro Digestion

The LAB that showed higher adhesion to the Caco-2 cell line (*L. brevis* CCMA1762, *L. pentosus* CCMA1768, and *L. paracasei* CCMA1770) were selected and exposed to simulated gastrointestinal conditions according to de Madureira, et al. [26] to evaluate their probiotic potential. The test was carried out in an incubator at 37 °C with mechanical agitation (TE-424 TECNAL, Orbital Shaker Incubator, São Paulo, Brazil) to simulate peristaltic movements with rotation adjustment in each tested phase (esophagus–stomach, duodenum, and ileum). The stages of digestion were simulated—oral phase: α-amylase solution (100 U/mL 0.1 M $CaCl_2$) pH 6.9; esophagus–stomach: pepsin (25 mg/mL diluted in 0.1 M HCl), at a rate of 0.05 mL/mL, for 90 min at 130 rpm, and in this step, the pH was gradually reduced using 1 M HCl solution (Mainville et al., 2005); duodenum: pancreatin (1 g/L diluted in 0.1 M $NaHCO_3$) and bile salts (6 g/L diluted in 0.1 M $NaHCO_3$), with a proportion of 0.25 mL/mL in the system, pH 5.0, for 30 min, at 45 rpm (Laurent et al., 2007); and ileum: 6.5 pH, using 0.1 mM/L $NaHCO_3$, for 60 min, at 45 rpm. All enzymes and bovine bile salts were supplied by Sigma Aldrich (St. Louis, MO, USA). After each simulation phase, serial dilutions were performed in a sterile saline solution (0.9% NaCl) and inoculated MRS medium to enumerate viable cells (log CFU mL^{-1}).

2.9. Antifungal Activity

The strains *L. brevis* CCMA1762, *L. pentosus* CCMA1768, and *L. paracasei* CCMA1770 were evaluated against the fungi *Aspergillus flavus* MUM 08.201 and *Penicillium nordicum* MUM 08.16. The LAB cells were centrifuged (7000× *g* for 5 min at 4 °C) followed by filtration (0.2 µm, polypropylene), and the supernatant was stored at 4 °C and used in the assays. All experiments were performed in duplicate. For antifungal analysis, the "poisoned food technique" was used according to Guimarães, et al. [27] with some modifications. The LAB supernatant was added to the MEA medium (adjusted final composition) in proportions of 25% and poured into plates. After the medium was solidified, 10 µL of the fungal spore suspension (10^6 spores/mL) was inoculated in the center of the plates. Controls were performed without the addition of supernatant. The plates were incubated for 7 days in the dark at 25 °C, and every day the diameters of the fungal colonies were measured, and the percentage of inhibition determined was determined (% Microbial inhibition = [1 − (MEA with LAB supernatant diameter/MEA control diameter)] × 100).

2.10. Aflatoxin B1 and Ochratoxin A Removal by Selected Lactic Acid Bacteria

The strains *L. brevis* CCMA1762, *L. pentosus* CCMA1768, and *L. paracasei* CCMA1770 LAB to eliminate AFB1 and OTA mycotoxins were evaluated by Taheur, et al. [28] with some modifications. Standard stock solutions of Aflatoxin B1 and Ochratoxin A (Sigma-Aldrich, Saint Louis, MO, USA) were prepared in methanol at 2 mg/mL. For each bacterium, 5 mL of MRS broth was contaminated with mycotoxins (2 µg/mL) and inoculated with 0.1 mL of the inoculum of already grown bacteria. Negative controls were prepared without inoculation of bacteria. After incubation (37 °C, 7 days), the adsorption of mycotoxins AFB1 and OTA were tested, the tubes were centrifuged (7000× *g* for 5 min), and 2 mL of the supernatant was added and vortexed into a solution of acetonitrile/methanol/acetic acid (78/20/2, *v*/*v*/*v*) (Fisher Scientific, Waltham, MA, USA), then left overnight at room temperature in the dark. After this period, all samples were filtered (0.2 µm, polypropylene) and preserved at −20 °C until analyzed by HPLC.

2.11. Mycotoxins Analysis

High-performance liquid chromatography (HPLC) with fluorescence detection was used to quantify AFB1 and OTA mycotoxins (Varian Prostar 210 pump system, Varian Prostar 410 autosampler, Jasco FP-920 fluorescence detector, and Jones Chromatography 7971 column heater—30 °C). The system was operated by Varian 850-MIB data and Galaxie system chromatography data. The YMC-Pack ODS-AQ analytical C18 column (250 × 4.6 mm I.D., 5 µm) and respective pre-column were used.

The mobile phase deionized water/acetonitrile/methanol (3/1/1, *v*/*v*/*v*, 1.0 mL/min) was used for the detection of AFB1, after post-photochemical column derivatization (PHRED unit-Aura Industries, San Diego, CA, USA, ex: 365 nm, in: 435 nm) [29]. The mobile phase acetonitrile/water/acetic acid (99/99/2, *v*/*v*/*v*, 1.0 mL/min) was used for the detection of OTA, with ex: 333 nm and at: 460 nm Abrunhosa, et al. [30]. For the detection, the injection volume was 50 µL for both mycotoxins. Standard curves were prepared for the quantification of mycotoxins, with concentrations ranging from 0.005 to 1.0 µg/mL. AFB1: LOD = 0.004 µg/mL and LOQ = 0.013 µg/mL, mean recovery 93 ± 7.0%, OTA: LOD = 0.005 µg/mL and LOQ = 0.017 µg/mL, mean recovery f 113 ± 6.7%.

2.12. Statistical Analyses

The SISVAR 5.1 software [31] was used to analyze variance and the Scott–Knott test. Differences in results were considered statistically significant when $p < 0.05$.

3. Results

3.1. Screening of LAB Strains

The pre-selection of LAB cultures was first based on their ability to survive at low pH levels and in the presence of bile salts. Twelve LAB strains showing the highest survival rate in this assay were selected and evaluated for cell surface characteristics (hydrophobicity, self-aggregation, and coaggregation with pathogens) and safety attributes. Six strains with higher values concerning cell surface properties and safety were selected as probiotic candidates for further characterization by antimicrobial activity and adhesion capacity to Caco-2 cells. Finally, three bacteria were selected for survival during in vitro digestion.

3.2. Acid and Bile Salt Tolerance

Fourteen strains obtained from naturally fermented table olive were exposed to acidic conditions and the presence of bile salts at 37 °C (Table 2). After 3 h of incubation, twelve strains showed tolerance under these conditions, maintaining survival at pH 2 and at a concentration of 0.3% bile salts (>6 log CFU mL^{-1}), which is comparable or statistically similar ($p > 0.05$) to the reference strain *L. paracasei* LBC-81 (>7.79 log CFU mL^{-1} after 3 h of incubation) (Table 2). The strains *L. paracasei* CCMA1775 and *L. paracasei* CCMA1764 showed reduced viability ($p < 0.05$) at pH 2 and a 0.3% concentration of bile salts after 3 h of incubation (<4.5 log CFU mL^{-1}) and were not considered in the following tests. The other isolates had a high survival rate ranging from 84.38 to 100% in both exposure conditions.

Table 2. Viability of LAB isolates after exposure to acid and bile salt conditions.

LAB Strains	Acid Condition Time of Exposure (h)			Bile Salt Condition Time of Exposure (h)		
	T0	T3	Survival (%)	T0	T3	Survival (%)
L. brevis CCMA1766	7.92 ± 0.01^{aB}	9.15 ± 0.34^{aA}	100	7.67 ± 0.16^{aA}	8.35 ± 0.07^{aA}	100
L. paracasei CCMA1763	7.52 ± 0.02^{aA}	7.97 ± 0.04^{bA}	100	7.42 ± 0.02^{aB}	8.81 ± 0.16^{aA}	100
L. paracasei CCMA1764	7.86 ± 0.02^{aA}	4.25 ± 0.49^{dB}	54.07	7.80 ± 0.06^{aA}	4.09 ± 0.16^{cB}	52.43
***L. pentosus* CCMA1768**	7.65 ± 0.04^{aA}	7.88 ± 0.14^{bA}	100	7.42 ± 0.06^{aA}	7.38 ± 0.45^{bA}	99.44
L. paracasei CCMA1769	7.63 ± 0.04^{aA}	7.73 ± 0.04^{bA}	100	7.73 ± 0.04^{aA}	7.70 ± 0.05^{aA}	99.65
***L. paracasei* CCMA1770**	7.88 ± 0.04^{aA}	7.41 ± 0.34^{bA}	94.13	7.59 ± 0.08^{aA}	8.23 ± 0.11^{aA}	100
***L. brevis* CCMA1762**	7.96 ± 0.03^{aA}	6.73 ± 0.05^{cB}	84.55	7.56 ± 0.09^{aA}	8.36 ± 0.48^{aA}	100
L. paracasei CCMA1772	7.65 ± 0.06^{aA}	6.72 ± 0.03^{cB}	87.88	7.75 ± 0.05^{aA}	7.74 ± 0.19^{aA}	99.86
L. paracasei CCMA1771	7.85 ± 0.13^{aA}	6.64 ± 0.06^{cB}	84.56	7.84 ± 0.01^{aA}	8.44 ± 0.41^{aA}	100
L. brevis CCMA1765	7.53 ± 0.08^{aA}	6.73 ± 0.05^{cB}	89.39	7.86 ± 0.02^{aA}	7.85 ± 0.16^{aA}	99.87
L. paracasei CCMA1767	7.89 ± 0.02^{aA}	6.76 ± 0.08^{cB}	85.64	7.79 ± 0.12^{aA}	7.55 ± 0.27^{aA}	97.02
L. paracasei CCMA1773	7.87 ± 0.04^{aA}	6.64 ± 0.06^{cB}	84.38	7.81 ± 0.03^{aA}	7.91 ± 0.10^{aA}	100
L. paracasei CCMA1774	7.77 ± 0.05^{aA}	6.80 ± 0.16^{cB}	87.49	7.80 ± 0.06^{aB}	8.64 ± 0.29^{aA}	100
L. paracasei CCMA1775	7.65 ± 0.06^{aA}	4.04 ± 0.06^{dB}	52.81	7.62 ± 0.06^{aA}	4.02 ± 0.05^{cB}	52.76
L. paracasei LBC-81	7.91 ± 0.02^{aA}	7.79 ± 0.12^{bA}	98.48	7.63 ± 0.06^{aA}	8.60 ± 0.18^{aA}	100

Results Log CFU mL^{-1} are expressed as mean ± SD, determined in duplicate. Mean values of different letters, lower case in columns and uppercase in rows differ significantly ($p < 0.05$) by the Scott–Knott test. Time of exposure: **T0**: Initial mean count; **T3**: Mean count after 3 h. Survival at pH 2 (%) or bile salt (%) = Final viable count (Log CFU mL^{-1})/Initial viable count (log CFU mL^{-1})) × 100. Strains showing potential properties as probiotics used during the simulated digestion are bolded.

3.3. Cell Surface Hydrophobicity

Microbial adhesion to non-polar solvents (n-hexadecane) reflects cell surface hydrophobicity. In this study, *L. brevis* CCMA1762 (31.23 ± 1.7%), *L. paracasei* CCMA1768 (28.13 ± 2.1%), and *L. paracasei* CCMA1770 (27 ± 0.7%) showed higher ($p < 0.05$) adhesion to the solvent n-hexadecane and are, therefore, able to interact with more cell bodies compared to other strains, similar ($p > 0.05$) to the hydrophobicity of reference strain *L. paracasei* LBC-81 (29.59 ± 0.9%). For the other isolates, the percentage of hydrophobicity ranged from 15.4 ± 1.4 to 24.03 ± 1.2 % (Figure 1).

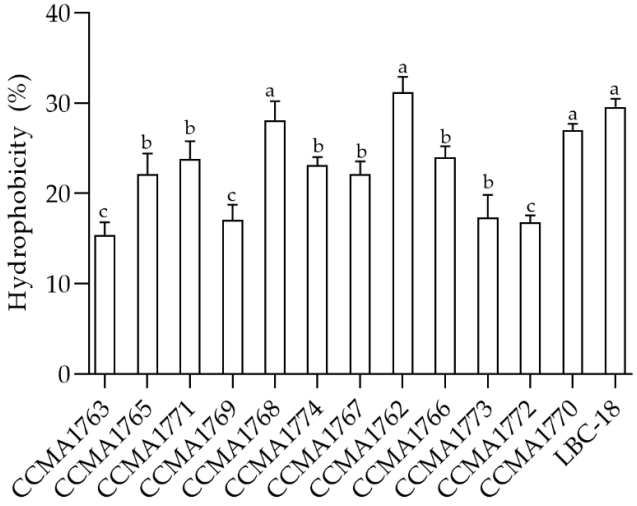

Figure 1. Percentage (%) of Hydrophobicity obtained for the different LAB isolates. Bar indicates SD. Bars followed by different letters differ significantly ($p < 0.05$) by the Scott–Knott test.

3.4. Auto-Aggregation and Coaggregation of the Pathogens' Ability

Auto-aggregation is closely associated with adhesion [32]. Both are responsible for colonization in the GIT; however, coaggregation is associated with interaction with pathogenic bacteria. All strains showed a high percentage of auto-aggregation (>60%) after 24 h of incubation (Figure 2). Among the potential probiotic strains, *L. pentosus*

CCMA1768 (88.83 ± 1.7%), *L. paracasei* CCMA1771 (84.75 ± 0.7%), *L. paracasei* CCMA1766 (84.77 ± 2.1%), *L. paracasei* CCMA1770 (81.85 ± 1.6%), *L. brevis* CCMA1762 (81.38 ± 2.1%), and *L. paracasei* CCMA1774 (80.34 ± 0.7%) provided auto-aggregation percentages similar ($p > 0.05$) to that of the reference strain (81.86 ± 2.1%). The other isolates, despite showing a lower percentage of auto-aggregation ($p < 0.05$) compared to the reference strain, still showed relatively high values of auto-aggregation, ranging from 74.07 ± 1.6 to 79.08 ± 2.3%.

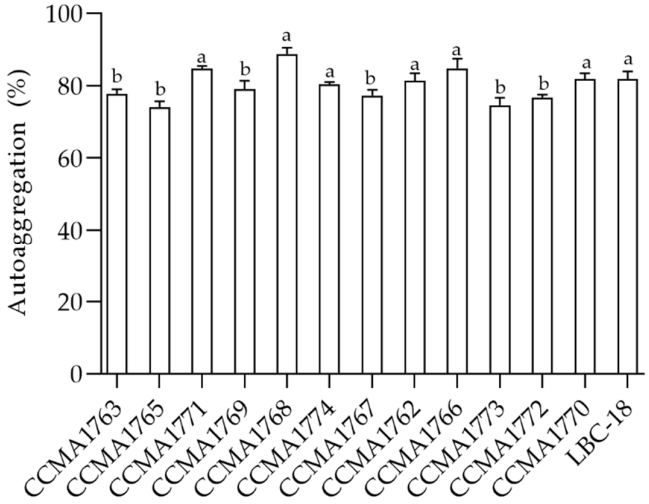

Figure 2. Percentage of auto-aggregation of LAB isolates Bar indicates SD. Bars followed by different letters differ significantly ($p < 0.05$) by the Scott–Knott test.

The coaggregation percentage of the LAB against *S.* Enteriditis and *E. coli* was evaluated for the selected bacteria (Figure 3a,b). The following strains showed a higher ($p < 0.05$) coaggregation percentage with the pathogen *S.* Enteriditis: *L. paracasei* CCMA1771 (63.6 ± 3.4%), *L. pentosus* CCMA1768 (64.79 ± 2%), and *L. brevis* CCMA1762 (63.02 ± 1%). The coaggregation rates of the remaining strains with the pathogen *S.* Enteriditis ranged from 28.43 ± 0.5 to 56.09 ± 0.6%, whereas the reference strain *L. paracasei* LBC-81 was 54 ± 0.9%.

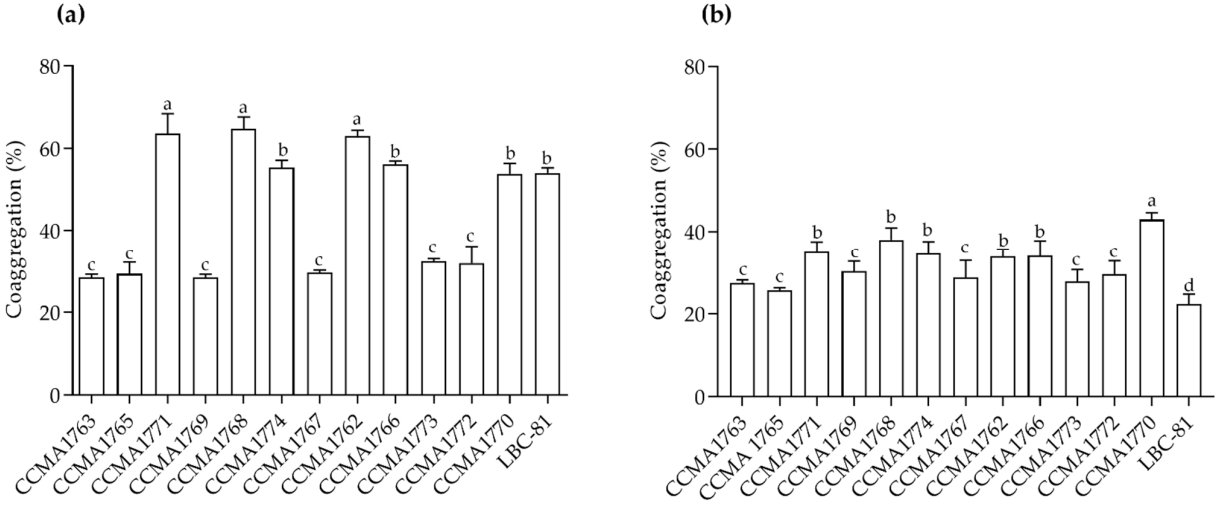

Figure 3. Percentage (%) coaggregation of LAB isolates with pathogenic microorganisms: (**a**): coaggregation with *Salmonella* Enteritidis ATCC 564; (**b**): coaggregation with *Escherichia coli* (EPEC) CDC 055. Bar indicates SD. Bars followed by different superscript letters differ significantly ($p < 0.05$) by the Scott–Knott test.

All selected strains were able to co-aggregate with the pathogen *E. coli*, presenting coaggregation rates higher ($p < 0.05$) than the reference strain *L. paracasei* LBC-81 (22.37 ± 1.7%). Values ranged from 25.6 ± 0.5% for *L. brevis* CCMA1765 to 42.95 ± 1.2% for *L. paracasei* CCMA1770.

3.5. In Vitro Assessment of Safety Attributes

The isolates tested in this study did not appear positive for gelatinase, DNase, and hemolytic activity (data not shown), validating their relative safety as probiotic candidates.

3.6. Antibacterial Activity

The inhibitory activity of strains against the pathogenic bacteria was recorded for six bacteria that showed the best results for cell surface tests and were safe to be used as probiotics. The inhibition halos were in the range of 4.3 (+)–9 mm (+++) (Table 3). All the strains showed antagonistic activity against all tested pathogens, with *S. aureus* ATCC 8702 being the most sensitive to the effects of LAB, in which a clearly defined zone of inhibition ≥ 8 mm was observed in the cell-free supernatant wells of *L. paracasei* CCMA1774, CCMA 1770, and *L. brevis* CCMA 1766 bacteria. Such high inhibition rates were also observed for other pathogenic microorganisms—the supernatant of the *L. paracasei* CCMA71 bacterium showed greater inhibition halos for the pathogen *S*. Enteritidis ATCC 564, and the supernatant of *L. brevis* CCMA1762 and *L. pentosus* CCMA1768 bacteria showed greater efficiency against the pathogen *L. monocytogenes* ATCC 19117.

Table 3. Antibacterial activity of LAB isolates.

LAB Strains Code	*S. aureus* ATCC 8702	*S.* Enteritidis ATCC 564	*L. monocytogenes* ATCC 19117
***L. pentosus* CCMA1768**	+	++	+++
L. paracasei CCMA1771	++	+++	++
L. paracasei CCMA1774	+++	++	++
***L. paracasei* CCMA1770**	+++	++	++
L. brevis CCMA1766	+++	++	++
***L. brevis* CCMA1762**	++	++	+++
L. paracasei LBC-81	++	++	++

Activity: + = the presence of a clear zone of growth inhibition around well ≤ 6 mm; ++ = presence of a clear zone of growth inhibition around well ≥ 6 mm; +++ = presence of a clearly defined inhibition zone ≥ 8 mm surrounding the wells containing cell-free supernatant. Strains showing potential properties as probiotics used during the simulated digestion are bolded.

3.7. Caco-2 Cell Line Adhesion Assay

The ability to directly adhere to mammalian epithelial cells, such as Caco-2, is an important requirement for potential probiotic use and is another way to assess microbial adhesion properties. Thus, six bacteria evaluated LAB's adhesion to the human colon tumor cell lines Caco-2 in vitro (Figure 4). The isolates obtained a percentage of adhesion between 1.84 ± 1.2% (*L. paracasei* CCMA1771) and 8.47 ± 4.26% (*L. brevis* CCMA1762). All the tested isolates showed a percentage ($p < 0.05$) of adhesion to Caco-2 cells that was similar to or higher than the reference strain *L. paracasei* LBC-81 (2.9 ± 1.22). The *L. brevis* CCMA 1762 strain presented the highest ($p < 0.05$) adhesion rate, following the isolate *L. pentosus* CCMA1768 (5.58 ± 3.47%) and the strain *L. paracasei* CCMA1770 (5.04 ± 1.87%).

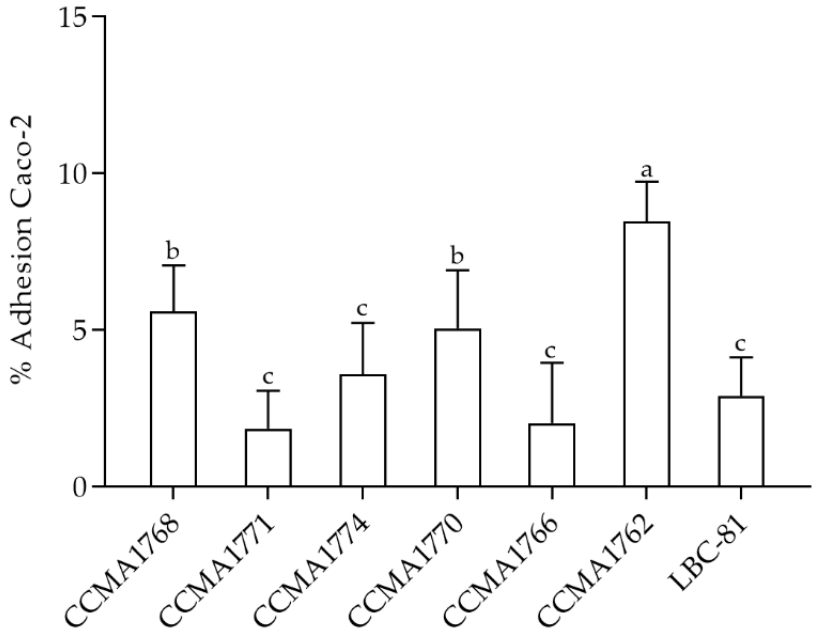

Figure 4. Adhesion capacity of LAB strains to Caco-2 cells. Bar indicates SD. Bars followed by different letters differ significantly ($p < 0.05$) by the Scott–Knott test.

3.8. Survival of LAB during In Vitro Digestion

Resistance to the conditions of the GIT is an important aspect when considering possible probiotic strains. In the present study, three selected strains were submitted to simulated gastrointestinal digestion (SGID) (Table 4). Before the simulation, the concentration of bacteria was between 7.03 ± 0.08 and 7.21 ± 0.01 CFU mL^{-1}. In the initial step (mouth conditions), the strains did not decrease significantly ($p > 0.05$) and maintained their viability ≥ 7.02 ± 0.03 CFU mL^{-1}. During their exposure to esophagus/stomach conditions, there was a reduction of 1 log CFU mL^{-1}. The strains *L. pentosus* CCMA1768 and *L. brevis* CCMA1762 significantly decreased ($p < 0.05$) their viable cell numbers after 50 min of contact with gastric juice (pH 2.8). The concentration of viable cells of the strain *L. paracasei* CCMA1770 remained significantly unchanged ($p > 0.05$) until 70 min of contact with gastric juice at pH 2.3. Among the three potential probiotic LAB studied, *L. brevis* CCMA1762 appeared to be more resistant to the transit throughout the GIT, presenting with a higher ($p < 0.05$) viable cell count at the end of the digestion simulation than the other tested LAB strains, and a survival rate of 80.23%. The survival rate for bacterium *L. pentosus* CCMA1768 was 61.22%, and that for bacterium *L. paracasei* CCMA1770 was 57.84%.

3.9. Antifungal Activity and Elimination of Mycotoxins

The effect of the supernatant from LAB added to culture media was tested in plates against the fungi *A. flavus* and *P. nordicum* (Table 5). For *A. flavus*, the presence of LAB supernatant was insufficient to inhibit the fungus growth at the end of 7 days of incubation, and the hyphal radial growth was similar to controls (without adding bacteria). The inhibition rate ranged from 3.5 ± 0.8 to 6.5 ± 0.5%. The bacterium *L. paracasei* CCMA1770 showed the highest inhibition ($p < 0.05$) to the fungus *A. flavus* until the sixth day of incubation, but after 7 days, the percentage of inhibition was equal ($p > 0.05$) for all supernatants used.

Table 4. Conditions used during the simulated digestion and the resultant viable cell counts of different LAB strains.

Organ	Condition	Stirring (rpm)	pH	Time (min)	Viable Cell Counts (Log CFU mL^{-1})		
					LAB Strains Code		
					L. pentosus CCMA1768	*L. brevis* CCMA1762	*L. paracasei* CCMA1770
Before simulation	-	-	-	-	7.04 ± 0.01 ^{aA}	7.03 ± 0.08 ^{aA}	7.21 ± 0.01 ^{aA}
Mouth	Amylase solution	200	6.9	2	7.02 ± 0.03 ^{aA}	7.04 ± 0.04 ^{aA}	7.21 ± 0.01 ^{aA}
Esophagus–Stomach	Pepsin	130	5.5	10	6.95 ± 0.05 ^{aA}	6.98 ± 0.01 ^{aA}	7.09 ± 0.06 ^{aA}
			4.6	10	6.91 ± 0.01 ^{aA}	6.91 ± 0.07 ^{aA}	7.08 ± 0.01 ^{aA}
			3.8	10	6.88 ± 0.07 ^{aA}	6.88 ± 0.04 ^{aA}	7.07 ± 0.08 ^{aA}
			2.8	20	6.52 ± 0.02 ^{bB}	6.42 ± 0.03 ^{bB}	6.97 ± 0.01 ^{aA}
			2.3	20	5.44 ± 0.09 ^{bC}	6.33 ± 0.05 ^{aB}	6.32 ± 0.05 ^{aB}
			2.0	20	4.37 ± 0.03 ^{cD}	6.04 ± 0.03 ^{aC}	5.18 ± 0.06 ^{bC}
Duodenum	Pancreati + bile salt	45	5.0	30	4.31 ± 0.08 ^{cD}	5.97 ± 0.01 ^{aC}	4.86 ± 0.04 ^{bD}
Ileum	-	45	6.5	60	4.31 ± 0.01 ^{bD}	5.64 ± 0.01 ^{aD}	4.17 ± 0.05 ^{bE}
Survival (%)					61.22	80.23	57.84

Step 1: Stages to simulate the conditions in the mouth; Steps 2 to 7: stages to simulate the conditions in the esophagus–stomach; Step 8: stage to simulate the conditions in the duodenum; Step 9: stage to simulate the conditions in the ileum. Results log CFU mL^{-1} are expressed as mean ± SD, determined in duplicate. Mean values of different letters, lowercase in columns and uppercase in rows differ significantly ($p < 0.05$) by the Scott–Knott test. Survival at simulated digestion (%) = (Final viable count (log CFU mL^{-1})/Initial viable count (log CFU mL^{-1})) × 100.

Table 5. Antifungal activity of LAB isolates.

Bacteria	Inhibition of Fungi (%)					
	Aspergillus flavus MUM 08.201					
	2 days	**3 days**	**4 days**	**5 days**	**6 days**	**7 days**
L. brevis CCMA1762	40.7 ± 0.7 ^{cA}	25.4 ± 1.3 ^{bB}	22.9 ± 2.5 ^{bB}	19.8 ± 3.3 ^{bC}	2.5 ± 0.3 ^{bD}	4.7 ± 0.2 ^{aD}
L. paracasei CCMA1764	59.2 ± 0.9 ^{aA}	30.9 ± 1.0 ^{aB}	29.7 ± 3.4 ^{aB}	24.8 ± 0.9 ^{aC}	11.2 ± 0.8 ^{aD}	6.5 ± 0.5 ^{aE}
L. pentosus CCMA1768	51.8 ± 2.4 ^{bA}	29.1 ± 0.8 ^{aB}	25.7 ± 1.5 ^{bC}	19.8 ± 1.0 ^{bD}	5.6 ± 0.4 ^{bE}	3.5 ± 0.8 ^{aE}
	Penicillium nordicum MUM 08.16					
	2 days	3 days	4 days	5 days	6 days	7 days
L. brevis CCMA1762	100 ^{aA}	100 ^{aA}	100 ^{aA}	100 ^{aA}	67.6 ± 3.1 ^{bB}	64.8 ± 5 ^{bB}
L. paracasei CCMA1764	100 ^{aA}	100 ^{aA}	100 ^{aA}	100 ^{aA}	100 ^{aA}	100 ^{aA}
L. pentosus CCMA1768	100 ^{aA}	100 ^{aA}	100 ^{aA}	100 ^{aA}	100 ^{aA}	100 ^{aA}

The results are expressed as the means of inhibiting fungal growth for 7 days ± standard deviation ($n = 2$). Mean values of different letters, lowercase in columns and uppercase in rows differ significantly ($p < 0.05$) by the Scott–Knott test.

The fungus *P. nordicum* was more sensitive to the effect of the bacterial supernatants. For the strains *L. paracasei* CCMA1770 and *L. pentosus* CCMA1768, the fungus was inhibited entirely during the incubation period (100% inhibition). On the other hand, when testing the supernatant of the bacterium *L. brevis* CCMA1762, the inhibition remained total and equal ($p > 0.05$) until the fifth day of incubation, then until the 7th day, there was an inhibition of 64.8 ± 5.7%.

The adsorption of AFB1 and OTA by the LAB strains was analyzed (Table 6). The supernatant of LAB showed a similar percentage of mycotoxin elimination. For Aflatoxin B1, the values ranged from 40 ± 1.40% (*L. paracasei* CCMA 1770) to 45 ± 1.12% (*L. brevis* CCMA 1762); for Ochratoxin A, the values ranged from 34 ± 1.45% (*L. paracasei* CCMA 1770) to 40 ± 2.05 (*L. brevis* CCMA 1762).

Table 6. Percentage of AFB1 and OTA eliminated by the LAB culture during adsorption experiments.

Species	Percentage of Mycotoxin Elimination (%)	
	Aflatoxin B1	**Ochratoxin A**
L. brevis CCMA1762	45 ± 1.12	40 ± 2.05
L. paracasei CCMA1770	40 ± 1.40	34 ± 1.45
L. pentosus CCMA1768	43 ± 1.08	38 ± 1.10

The results are expressed as the means of elimination after 7 days of incubation ± standard deviation (n = 2). No statistical differences were found.

4. Discussion

The objective of our research was to analyze the probiotic potential, evaluate the safety, the antifungal activity, and elimination of mycotoxins capacity of LAB isolated from Brazilian table olives to explore the potential of these microorganisms for future biotechnological applications and to be used as a bio preservative that ensures food safety. Fourteen LAB were tested; three strains belonging to the *Levilactobacillus brevis* CCMA1762, *Lactiplantibacillus pentosus* CCMA1768, and *Lacticaseibacillus paracasei* subsp. *paracasei* CCMA1770 species showed favorable results for evaluated probiotic properties, including resistance to unfavorable conditions found in the human body, antibacterial activity against pathogens, adhesion capacity in the epithelium, and safety assessment. As far as we know, this is the first report to evaluate the probiotic characteristics of LAB strains isolated from naturally fermented Brazilian table olives.

The probiotic must survive the extremely low pH levels and the presence of bile salts and reach the gut in a viable physiological state to colonize and proliferate in this environment to confer beneficial effects on the host [33,34]. In our work, twelve isolates had survival rates ranging from 84.38 to 100% after exposure to an acidic environment and the presence of 0.3% bile salts (a concentration similar to the small intestine). Consequently, these LAB tolerated the hostile conditions present in the GIT, maintaining viability (>6.64 log CFU mL^{-1}) after 3 h of incubation under acidic and bile conditions.

Usually, *Lactobacillus* spp. are the most resistant to low pH values and show great adaptation in different food matrices, mainly since these bacteria belong to a species that occurs naturally in several fermented products [35]. Therefore, the maximum pH limit in table olives should be approximately 4.3 [36]. Thus, bacteria isolated from the fermentation of olives can survive in acidic environments and aren't inhibited in growth by pH 2.0 [37].

One of the main desirable properties to consider in the search for new strains with probiotic potential is related to adhesion to epithelial cells, which consequently results in colonizing the gastrointestinal tract, helping probiotic bacteria in the competition and proliferation in the intestine [38]. The adhesion of microorganisms to epithelial cells is related to both hydrophobic properties and the ability to auto aggregate, which influences cell surface interactions. The interaction between microorganisms and human epithelial cells is enhanced by surface hydrophobicity. In addition, the adhesion mechanism is facilitated based on the capacity to auto-aggregate [39]. In this study, the hydrophobicity of the LAB species for n-hexadecane ranged from 11.54 ± 1.5% to 31.23 ± 1.7%, whereas the auto-aggregation varied from 68.19 to 88.83%. These percentages of hydrophobicity and auto-aggregation are higher than those found in other studies with LAB species [40,41]. In addition, the high auto-aggregation capacity (values greater than 80%) of LAB isolated from table olive was confirmed [37]. The isolates *L. paracasei* CCMA1770, *L. brevis* CCMA1762, and *L. pentosus* CCMA 1768 exhibited high values for both hydrophobicity and auto-aggregation. Some researchers have suggested a correlation between these two properties [18,42].

For in vitro evaluation, the adhesion capacity of probiotics was frequently evaluated using the Caco-2 cell line. These cells resemble mature enterocyte cells in the small intestine of humans and form crypts, forming a homogeneous typical epithelial monolayer [43–45]. However, the strains' adhesion percentage on Caco-2 cells did not differ ($p > 0.05$) from the positive control strain *L. paracasei* LBC-81. The adherence values found were similar to those of other studies for LAB isolated from fermented foods [41,46].

Various studies have reported a correlation between aggregation and adhesion capacities [47,48]. According to our results, the strains *L. brevis* CCMA1762, *L. paracasei* CCMA1770, and *L. pentosus* CCMA 1768 had good adhesion capacities in Caco-2 cells and more excellent auto-aggregation properties; thus, this correlation between adherence and auto-aggregation was confirmed in our study.

Another important functional characteristic associated with probiotic LAB is its coaggregation abilities with pathogenic microorganisms, which may prevent intestinal colonization by pathogenic bacteria, representing an important host defense mechanism against infections [49,50]. All selected strains could co-aggregate with EPEC and *S*. enteritidis (Figure 3). The coaggregation percentages ranged from 28.57 ± 0.53 to 64.79 ± 2.0% in the case of *S.* Enteritidis S64 and 22.371.7 to 42.95 ± 2.4% in the case of *E. coli* (EPEC). CDC 055 showed significant differences ($p < 0.05$) between the tested strains and the reference strain *L. paracasei* LBC-81.

Further, safety-associated selection criteria are important parameters that should be carefully evaluated before using microorganisms as probiotics [51]. The strains used in the present study showed no hemolytic activity, DNAse, and gelatinase, all of which are important criteria in the safety assessment of probiotics for human use.

The tested strains also displayed the capability to inhibit pathogenic bacteria, including *S. aureus*, *S.* Enteritidis, and *L. monocytogenes*, in the well-diffusion technique and high antifungal activity against *P. nordicum* in the poisoned food technique. The nature of the inhibitory substance, nevertheless, remains unknown. It is known that probiotics could exert competitive inhibition against pathogens. Moreover, it can confer antagonism against potentially harmful microorganisms, thereby preventing colonization of the host mucosa [52]. In addition, various species of LAB can produce compounds with antimicrobial activities, for example, organic acids (acetic and lactic acid), antifungal peptides, low-molecular-weight compounds, and antibacterial peptides (bacteriocins) [53].

A probiotic pathway across the human digestive tract is not easy. Several physiological barriers can affect the survival of probiotics, such as low pH, presence of enzymes and bile salts, peristaltic movement, etc. [54]. Therefore, the probiotic candidate should withstand the stressful conditions of the human digestive system and exercise functional properties. Out of the fourteen LAB in this study, three different LAB species were subjected to simulated gastrointestinal conditions (Table 4). The survival rate of in vitro digestion was 57.84% for *L. paracasei* CCMA1770, 61.22% for *L. pentosus* CCMA1768, and 80.23% for *L. brevis* CCMA1762. After exposure to simulated gastric fluid, the final cell count ranged from 4.17 ± 0.05 to 5.64 ± 0.01 log CFU mL^{-1}.

The number of probiotic bacteria that guarantee a beneficial effect in humans is not standardized and is dependent on the tension and effect [55]. In our study, the survival rate is high. Conversely, in a food item that contains a more significant number of microorganisms, these would easily reach the intestine in large quantities, resulting in the expected beneficial effects of probiotics.

Using isolated lactic acid bacteria in different environments with a probiotic and antifungal capacity of lactic acid bacteria (LAB) is a strategy to obtain functional and bio preservative strains for food/feed industries [56]. Furthermore, studies related to biological control indicate that microorganisms, such as bacteria, can mitigate mycotoxins, preventing their presence in food, both for humans and animals [57]. In addition, the ability of lactic acid bacteria to remove mycotoxins has been studied by other authors [58,59], including the mycotoxins analyzed in our work Aflatoxin B1 [60] and Ochratoxin [61]. Our tested strains showed probiotic potential, antifungal activity, and mycotoxin adsorption capacity. Several studies have also demonstrated the probiotic properties combined with the antifungal activity of lactic acid bacteria [16,56].

Research of microorganisms from fermented foods, such as olives, is significant as it can identify microorganisms with interesting biotechnological properties [62]. Several studies have applied such microorganisms in different fermentations to evaluate the technical abilities of these isolates, and satisfactory results have been found [37,63,64]. Based on our

results, we discovered new possibilities for using LAB isolated from olives fermentation. These strains can characterize a product as a potential probiotic and potentially be used to develop biological control formulations to minimize contamination by mycotoxigenic fungi, and both hypotheses contribute to health and food security.

5. Conclusions

The exploration of fermented Brazilian table olives was relevant for isolating new LAB strains with probiotic and antifungal potential that can affect performances similar to commercial probiotics and food biopreservation. Out of fourteen LAB isolates from table olives, three isolates, namely, *L. paracasei* CCMA1770, *L. pentosus* CCMA1768, and *L. brevis* CCMA1762, showed potential properties that allow for their use as probiotics and antifungal strategy. These strains were capable of auto-aggregation, coaggregation with pathogens, exhibiting cellular hydrophobicity, tolerating low pH and bile salts, and antimicrobial activity against pathogens, bacteria, and fungi mycotoxigenic. Furthermore, the strains displayed adhesion to Caco-2 human epithelial cells, a high survival rate during simulated digestion, and mycotoxin adsorption capacity. These results indicated that LAB strains from naturally fermented Brazilian table olives are potentially probiotic and antifungal candidates. Therefore, other complementary tests are necessary to demonstrate functionality and safety for the consumer, prove their use in food, and contribute to scientific advances related to probiotics and biopreservation.

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