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Probiotic growth-stimulating capacity and antimicrobial activities of aqueous extracts of *Lentinus crinitus* (L.) Fr (polyporales, basidiomycota)☆

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ABSTRACT

Lentinus crinitus (L.) Fr is a wild macrofungus that is popular as antimicrobial and various biological activities. This study aims to determine the capacity growth stimulation of *Lactobacillus paracasei* and antimicrobial activity of aqueous extracts of *L. crinitus* obtained from wild basidiomata, mycelial biomass by liquid fermentation and spent mushroom substrate obtained by solid-state fermentation. The antimicrobial activity was investigated against bacterial and fungal pathogens and growth stimulation *L. paracasei* probiotic bacterium. The total carbohydrate and β-glucan contents of the extracts were determined using colorimetric analysis. The aqueous extracts obtained showed inhibition against *Fusarium oxysporum.*, P*enicillium* sp., *Rhizopus oryzae*, *Aspergillus niger*, *Escherichia coli* and *Salmonella typhimurium*. The aqueous extract obtained from wild basidiomata, and mycelial biomass showed the highest percentage of stimulation of *L. paracasei* growth in 48 h. The extracts obtained from *L. crinitus* have antimicrobial potential and stimulating capacity of the probiotic *Lactobacillus paracasei*. Additionally, different biotechnological techniques such as liquid and solid-state fermentation can be used to obtain aqueous extracts.

1. Introduction

Lentinus crinitus (L.) Fr is a wild macrofungus with pantropical and neotropical distribution used as food by ethnic communities in Brazil, Colombia, Peru and Venezuela [1–3] and biological activities have been reported, including antimicrobial [4–6], antitumor [7],

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antioxidant [8,9], bioremediating potential [10–13], lignin biodegradation [14], dye decolourisation and ligninolytic enzymes production [15–21].

The first scientific reports of biological activities are related to the antimicrobial activity of metabolites or extracts obtained from submerged culture and other more recent studies related to the antimicrobial activity of basidiomata of *L. crinitus* wild or cultivated by solid culture, as well as mycelial biomass obtained from submerged culture [4,22]. However, there are no detailed reports on aqueous extracts from *L. crinitus* obtained by different biotechnological methods and probiotic growth-stimulating capacity with potential use as prebiotics.

The aqueous extracts from mushrooms can be used as a source of dietary fiber with prebiotic effects [23,24]. This potential has been evaluated in fractions containing β-glucans from the *Pleurotus* genus, which showed selective prebiotic stimulation of strains of *Lactobacillus, Bifidobacterium*, and *Enterococcus* [25], as well as improvements in the survival of microorganisms in dairy products and in different farming systems during storage periods [26]. These mushroom aqueous extracts have synergistic effects with peptides and amino acids in *in-vitro* conditions, achieving beneficial effects in the host, lowering the pH, and suppressing potential pathogens, such as *Clostridium* spp., *Staphylococcus* spp., and *Enterococcus* spp [27].

Additionally, antimicrobial activity of these compounds is broad. Their application could be of interest in agriculture and the food industry, mainly because they can be used against phytopathogenic fungi that are responsible for numerous diseases and disorders in agricultural plants, which result in severe global reduction of food production, significant economic losses, decreased quality of life, and the potential for severe life-threatening disease [28,29].

Aqueous extracts can be obtained directly from basidiomata collected in the field or using biotechnological tools such as submerged fermentation (SmF) and solid-state fermentation (SSF) have been used for the transformation of rice husk and other agricultural waste using strains of mushrooms, which can give it a high added value and obtain metabolites or products with food, feed, or pharmacological uses [30–32].

The SSF can be described as a process in which organisms grow on solid materials or within porous solid particles in the absence of water, the result of which is an enriched product, with a higher content of proteins, vitamins, and other components than the original substrate [33]. This process has been used to take advantage of waste generated from agro-industries to produce food from strains of edible fungi [34]; or to generate spent waste with specific characteristics to be used in food, feed, compost production or to obtain bioactive metabolites [35–37]. On the other hand, the SmF involves the formation of pellets in a liquid medium and can generated various products or metabolite obtained from mycelial biomass or the product of liquid fermentation such as intrapolysaccharides and exopolysacharides, respectively; or other metabolites such as phenols and sesquiterpenes [38,39].

The application of these biotechnological tools is an alternative to obtain different bioproducts from residual biomass and provide innovative solutions in health, food, soil and water contamination, biological control, among other applications [40]. Therefore, the objective of the present study was to determine the capacity growth stimulation of *Lactobacillus paracasei and* antimicrobial activity of aqueous extracts of *L. crinitus* obtained from wild basidiomata, mycelial biomass by liquid fermentation and spent mushroom substrate obtained by solid state fermentation.

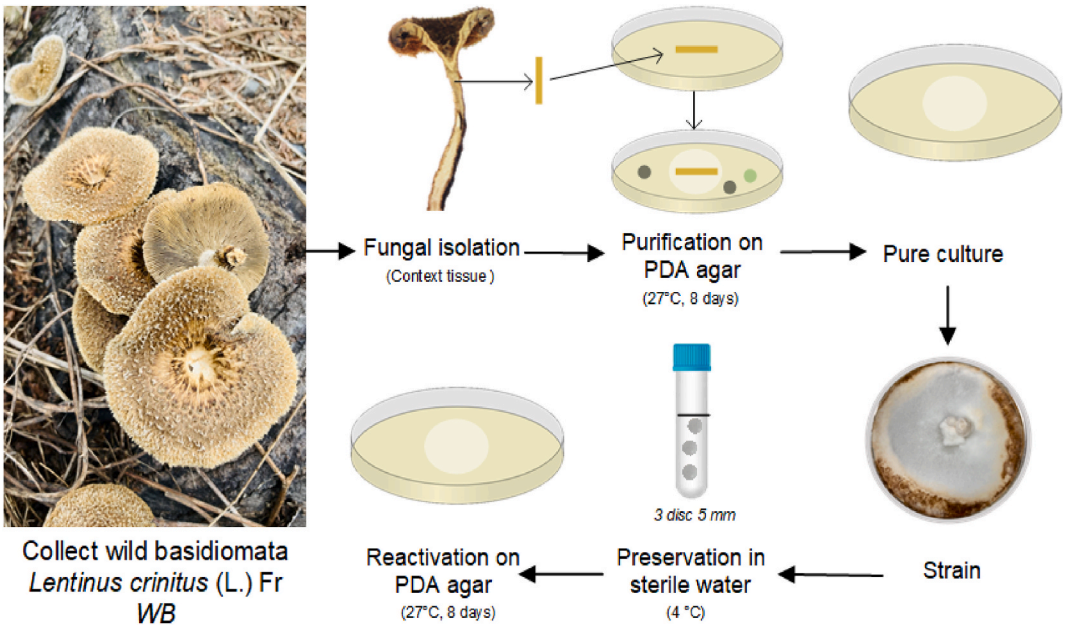

Fig. 1. Fungal isolation from collected wild basidiomata of Lentinus crinitus (L.) Fr.

2. Material and methods

2.1. Basidiomata collection

Basidiomata of L. *crinitus* were collected in Combeima Canyon in the region of Tolima in Colombia (4°33′25.8″N 75°19′34.4″W; 1900 m a.s.l). Basidiomata were photographed *in situ*, removed with a knife, deposited in paper bags with the respective collection number and taken to the laboratory. Morphological identification was made from macroscopic and microscopic characteristics. A macroscopic description using mycological guides was then performed [41–44]. Sections of the basidiomata were prepared in microscope slides with 3% KOH, Red Congo or Cotton Blue and Melzer's reagent (IKI) [42,45]). The specimen voucher (LRD 1) was deposited and preserved in the Fungario Universidad del Tolima (FUT).

2.2. Fungal strain

Strain was obtained from the context tissues of the basidiome of *L. crinitus* on Potato Dextrose Agar (PDA, Merck, Darmstadt, Germany) medium in Petri dishes under 27 °C for 8 days (Fig. 1). The strain was maintained in PDA slants at 4° [46]. Fresh strain was prepared on PDA plates at 27 °C for 8 days and utilized as the inoculum for the precultures (Fig. 1).

3. Substrates

Three types of substrates generated by the local agroindustry were used: rice husk (RH), orange peel (OP), and rice bran (RB). These substrates were dried at 40 °C for 48 h in a stove (Memmert, Schwabach, Germany). Three treatments were performed by combining the substrates, which were then used for the growth of *L. crinitus* in SSF and SmF (Table 1). In a study prior to this work, the proximal composition and the major and minor elements of the substrates were reported [47].

3.1. Solid-state fermentation (SSF)

The mycelia of *L. crinitus* were reactivated in PDA (Potato Dextrose Agar) medium and three 5-mm discs were taken and aseptically inoculated over wheat grains (previously washed, hydrated, and sterilized in bottles with stoppers). The flasks were incubated in the dark at 25–28 °C and a relative humidity of 70–80%, until the mycelium invaded the whole seed (used as inoculum to be sown on the substrates). Three combinations of the substrate were chosen (T1, T2, T3 and substrate without mushroom inoculum) and four replicates were made. Substrates were cut into 2-cm particles and water was added until a moisture content of 60% (wet basis) was reached. Then, 300 g of each combination were packed in polypropylene bags and autoclaved at 120 °C and 1 atm for |15min. Once the bags had cooled to 28–30 °C, they were inoculated with 5% of the wheat seeds invaded with *L. crinitus* (Fig. 2). The bags were closed with a PVC ring and cotton plug to allow gas exchange and were incubated in total darkness at 25–26 °C [48,49]. The spent mushroom substrate was stored at 4 °C for its subsequent preparation of aqueous extracts. For each substrate combination, controls were included (no substrate inoculation).

3.2. Submerged fermentation (SmF)

Erlenmeyer flasks (300 mL), sterile water (150 mL), and 6 g of the substrate for each treatment were used (T1, T2, T3). Substrates were ground in an electric mill. Additionally, treatment with Sabouraud Dextrose Broth (BD, Merck) was used (T4). The flasks were autoclaved at 15 psi for 60 min. When the flasks had cooled 25–28 °C, three quadrants (5 mm thick) of the *L. crinitus* mycelium were inoculated. After that, the flasks were shaken (CERTOMAT MO II, Sartorius, Goettingen, DE) at 200 rpm, for 20 days. The mycelial biomass was filtered and washed with distilled water to remove the particles of insoluble material (Fig. 2). The mycelial biomass was deposited in a Petri dish and dried in an oven at 40 °C for 48 h for subsequent preparation of the aqueous extracts [50].

3.3. Preparation of aqueous extracts

Collected wild basidiomata (WB), the mycelia obtained (MB) by SmF, spent substrate (SMS) obtained for SSF, and the substrates (S) without mushroom inoculum were dried at 30 °C, 12 h and macerated with liquid nitrogen. A 5-mg aliquot of each material was homogenized with 50 mL of deionized water, placed in a water bath (100 °C), and stirred for 8 h. The solids were removed by centrifugation (Centrifuge Z 326 K, Hermle LaborTechnik, Germany) at 2000g for 15 min and the supernatant was lyophilized

Table 1
Different combinations of the substrates.

Treatment	Substrates
T1	RH + OP
T2	OP + RB
T3	RH + OP + RB

RH, rice husk; OP, orange peel; RB, rice bran.

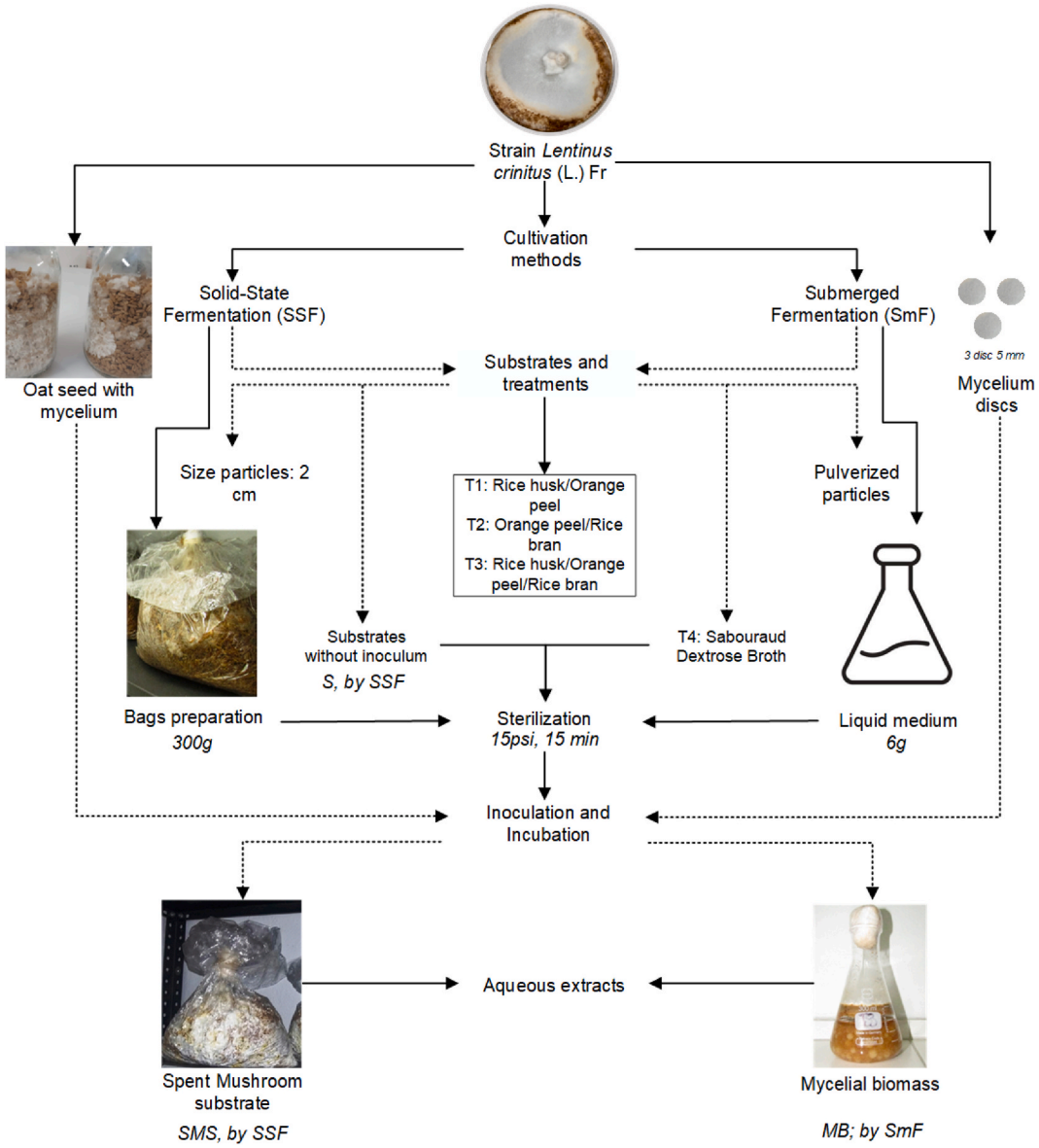

Fig. 2. Biotechnological cultivation methods to obtain spent mushroom substrate and mycelial biomass.

[51–54]. The lyophilized extract was resuspended in deionized water to determine the content of total carbohydrates and β-glucans, as well as to prepare different extract concentrations to evaluate the antimicrobial and growth stimulation of *Lactobacillus paracasei* (Fig. 3). All absorbance measurements were carried out in seven replicates for each *L. crinitus* sample.

3.4. Determination of total carbohydrate content

The carbohydrate content of *L. crinitus* samples was determined using the anthrone-sulfuric acid method, using glucose as a standard solution [55]. Quantification was carried out using a set of 96-well microplates and measured at an optical density of 630 nm in a microplate spectrophotometer (Multiskan GO/UV, Thermo Fischer Scientific, Vantaa, Finland).

3.5. Determination of the β-glucan content

β-Glucan determination was performed using a colorimetric method [56,57]. This method uses Congo Red as a complexing agent and β-glucan as a binder. The complex formed by this interaction generates a bathochromic change in the maximum absorption of Congo Red. To prepare the complexing agent, 100 mL of phosphate-buffered saline (PBS) at pH 7.2 and 0.017 g of Congo Red were

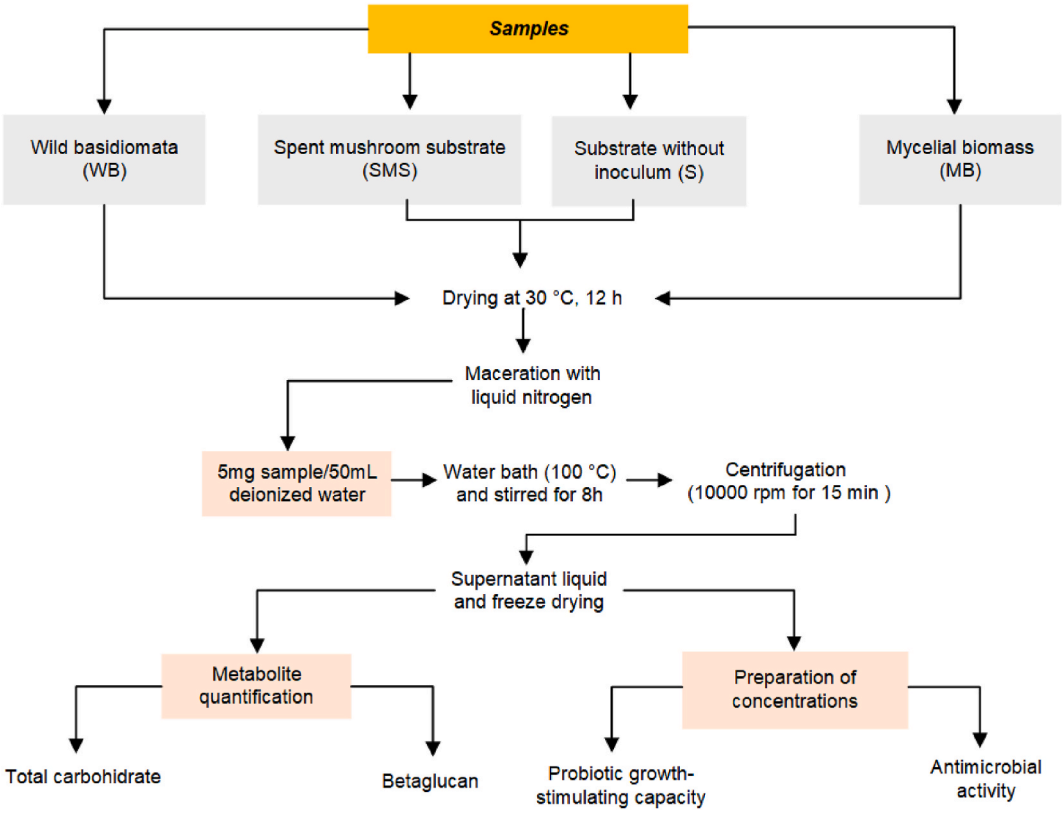

Fig. 3. Process for obtaining aqueous extracts of wild basidiomata, spent mushroom substrate, substrates without inoculum, and mycelial biomass.

mixed. A calibration curve was constructed by using a barley β-1,3-D-glucan standard solution (Sigma-Aldrich, St Louis, MO, USA), at a concentration range of 48–192 mg L^{-1}. The standard solutions were prepared by dissolving in 1 M NaOH and neutralizing with HCl. The reaction mixture contained 180 μL of complexing agent and 120 μL of Barley β-1,3-D-glucan standard solution at different concentrations for a final volume of 300 μL in a 96-well microplate. The absorbance was measured at 523 nm was measured in a 96-well microplate spectrophotometer (Multiskan GO/UV) and all standard solution concentrations were prepared in seven replicates and compared with that of a blank reaction mixture (without standard β-glucan). The amount of β-glucan present in the *L. crinitus* samples was quantified using the constructed calibration curve. All absorbance measurements were carried out in seven replicates for each *L. crinitus* sample.

3.6. Antibacterial activity

The antibacterial activity was assessed against four bacterial strains such as E*scherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC29213), *Bacillus cereus* (NCIMB7464), and *Salmonella typhimurium* (NCTC6017) [58]: Bacterial strains were cultured overnight at 37 °C on Brain Heart Infusion Nutritious broth (BHI, Merck, Darmstadt, Germany) and adjusted to a final concentration of 1×10^8 colony forming units (CFU)·mL^{-1} (0.5 nephelometric turbidity units - McFarland scale) and used as an inoculum [58]. The inhibitory effect on bacterial growth was evaluated using a 96-well microplate spectrophotometer (Multiskan GO/UV). The wells were filled with 50 μL of the bacterial inoculum, 100 μL of liquid medium (BHI, Merck), and 50 μL of the aqueous extract of *L. crinitus* at different concentrations. A negative control was used comprising 100 μL of medium, 50 μL of the inoculum, and 50 μL of sterile water. Contamination controls were also used, which included 200 μL of medium. Oxytetracycline at 2000 mg L^{-1} was used as a positive control. Finally, all samples were incubated at 37 °C for 24 h. The optical density was measured after 0, 6, 18, and 24 h of incubation at a wavelength of 460 nm. The percentage of growth inhibition was calculated for each well using the optical density and the following formula (Eq. (1)):

$$INH = OD_{NC} - OD_T / OD_{NC} \times 100 \quad (1)$$

where *INH* is the percentage of growth inhibition (%), OD_{NC} is the optical density of negative control and OD_T is the optical density of aqueous extract of *L. crinitus*.

3.7. Antifungal activity

The antifungal activity was assessed against four fungi strains such as *Fusarium* sp., P*enicillium* sp., *Rhizopus oryzae*, and *Aspergillus niger* [59]. Fungal spores from each strain were prepared at a final concentration of 1×10^4 conidia·mL^{-1} and used as an inoculum. The inhibitory effect of fungal growth was evaluated using a 96-well microplate spectrophotometer (Multiskan GO/UV). The wells were filled with 50 μL of the fungi inoculum, 100 μL of Sabouraud Dextrose Broth (BD, Merck), and 50 μL of the aqueous extract of *L. crinitus* at different concentrations. A negative control was used comprising 100 μL of medium, 50 μL of the inoculum, and 50 μL of sterile water. Contamination controls were also used, including 200 μL of medium and Ketoconazole at 2000 mg L^{-1} as a positive control. Finally, the samples were incubated at 30 °C for 48 h. The optical density was measured after 0, 18, 24, 36, and 48 h of incubation at wavelength of 595 nm. The percentage of growth inhibition was calculated using the same equation as that for the antibacterial activity described above.

3.8. Lactobacillus paracasei: maintenance conditions and growth stimulation

A probiotic strain of *Lactobacillus paracasei* was grow on Rogosa medium (MRS Agar, Oxoid, Basingstoke, England). One colony was transferred to Rogosa SL Broth (Oxoid) and kept under anaerobic conditions. The Rogosa medium (MRS) was prepared at a pH of 5.4 ± 0.2 with the following composition (g/L): Tryptone, 10; yeast extract, 5; Tween, 80; Potassium phosphate, 6; citric acid, 2; sodium acetate, 17; magnesium sulfate, 0.57; manganese sulfate, 0.12; ferrous sulfate, 0.034; and glucose, 20 [60]. The stimulation of *L. paracasei* growth was determined using the method proposed by Nowak [60]. The test was carried out in a 96-well microplate spectrophotometer (Multiskan GO/UV). Rogosa broth media (100 μL) without glucose was supplemented with 50 μL of the aqueous extracts of *L. crinitus* (at different concentrations) and 50 μL of the inoculum of *L. paracasei* (adjusted to 0.5 in the McFarland scale). The negative control contained 100 μL of MRS medium with glucose (1.5%), 50 μL of sterile water, and 50 μL of the inoculum of

Table 2
growth inhibition (INH) of pathogenic fungi after 48 h of incubation with aqueous extracts obtained from the wild basidiomata (WB), the mycelial biomass (MB; by SmF), spent mushroom substrate (SMS, by SSF), and the substrates without inoculum (S; by SSF).

Sample	Concentration (mg·L^{-1})	*R. oryzae*	*Penicillium* sp	*F.oxysporum*	*A. niger*
B	**4250**	45.634 ± 2.98 a	-	-	34.41 ± 0.43 a
	2125	30.73 ± 2.417 b	–	–	27.47 ± 3.01 b
	1062.5	10.13 ± 5.09 c	–	–	14.45 ± 1.08 c
	531.3	–	–	–	–
***M*-SmF T1**	**2500**	71.48 ± 2.72 a	-	33.37 ± 2.52 a	37.01 ± 4.85 a
	1250	72.43 ± 3.39 a	–	21.21 ± 4.62 b	22.66 ± 3.5 b
	625	64.25 ± 2.63 b	–	17.39 ± 4.44 bc	22.35 ± 3.83 b
	312.5	16.874 ± 1.49 c	–	12.7 ± 2.79 c	–
***M*-SmF T2**	**2500**	73.23 ± 2.18 a	-	24.8 ± 0.31 a	32.69 ± 3.83 a
	1250	71.08 ± 2.48 ab	–	21.71 ± 2.2 b	29.74 ± 2.55 a
	625	70.015 ± 1.60 b	–	18.74 ± 2.78 b	24.11 ± 3.89 bc
	312.5	63.78 ± 2.89 c	–	3.62 ± 1.42 c	15.57 ± 2.42 c
***M*-SmF T3**	**2500**	72.89 ± 1.69 a	-	9.91 ± 2.54	32.59 ± 1.97 a
	1250	70.15 ± 1.78 a	–	–	29.52 ± 1.76 a
	625	57.025 ± 4.00 b	–	–	21.83 ± 6.41 a
	312.5	44.104 ± 3.23 c	–	–	9.69 ± 1.95 b
***M*-SmF Saboraud T4**	**625**	75.30 ± 1.862 a	-	30.21 ± 2.67 a	53.89 ± 4.12 a
	312.5	74.28 ± 2.911 a	–	32.09 ± 0.790 a	50.39 ± 2.95 a
	156.3	65.12 ± 4.908 b	–	32.64 ± 5.08 a	53.07 ± 2.47 a
	78.1	57.037 ± 7.23 c	–	32.69 ± 2.54 a	50.43 ± 3.63 a
SS SFF T1	**2500**	72.25 ± 1.750 a	-	-	–
	1250	66.26 ± 2.35 b	–	–	–
	625	36.4 ± 5.7 c	–	–	–
	312.5	31.47 ± 1.48 c	–	–	–
SS SFF T2	**3593**	–	35.61 ± 2.97 a	-	43.46 ± 2.77 a
	1796	–	30.04 ± 5.76 a	–	39.57 ± 4.83 a
	898	–	-	–	–
	449	–	–	–	–
***SS*–SFF T3**	**2500**	37.55 ± 1.66 a	–	-	–
	1250	31.95 ± 3.76 a	–	–	–
	625	–	-	–	–
	312.5	–	–	–	–
***S*-SFF T1**	**4000**	16.51 ± 1.54	–	-	–
***S*-SFF T2**	**4000**	–	–	–	–
***S*-SFF T3**	**4000**	–	-	–	–
Control +	**2000**	73.38 ± 4.08	68.02 ± 2.05	45.73 ± 4.13	52.7 ± 1.25

The values are mean ± S.E.M; n = 7, $p < 0.05$. One-way Anova followed by Bonferroni post hoc test. WB, Wild Basidiomata; MB, Mycelial Biomass; S, Substrate; SmF, Submerged Fermentation; SSF, Solid State Fermentation; T1, Rice husk/Orange peel; T2, Orange peel/Rice bran; T3, Rice husk/Orange peel/Rice bran; T4, Sabouraud Dextrose Broth.

L. paracasei. Finally, the samples were incubated at 37 °C during for 48 h. The optical density was measured after 0, 6, 12, 24 and 48 h of incubation at a wavelength of 600 nm. The percentages of *L. paracasei* growth was calculated for each well using the optical density and the following formula (Eq. (A.2)):

$$LGW = \mathrm{OD}_T - \mathrm{OD}_{NC}/\mathrm{OD}_T \times 100 \quad \text{(A.2)}$$

where *LGW* is the percentage increase in *L. paracasei* growth (%), OD_{NC} is the optical density of the negative control, and OD_T is the optical density of the aqueous extract of *L. crinitus.*

3.9. Statistical analysis

The resulted were expressed as the measurement averages standard ± deviation. To study the differences between treatments and samples analysis of variance (ANOVA) was used. A significance level of 95% ($p \leq 0.05$) was considered- The Bonferroni's multiple comparison test was performed to determine the differences between the means of the obtained values. The statical analysis was performed with the R software package (http://www.R-project.org).

4. Results

The antimicrobial activity of the aqueous extracts against the tested microorganisms are shown Table 2. The positive control inhibited the growth of *R. oryzae* (73.4%), *Penicillium* sp. (68%), *F. oxysporum* (45.7%), and *A. niger* (52.7%). The aqueous extracts obtained from mycelial biomass by SmF-T4 a the concentration of 625 mg L^{-1} showed the highest percent growth inhibition of *R. oryzae* (75.2%), *F. oxysporum* (30.21%) and *A. niger* (53.9%). Aqueous extracts of mycelial biomass obtained by SmF with T1, T2 and T4 generated percent inhibition (71.5%–72.9%) against *A. niger* in the concentration of 2500 mg L^{-1}. Extracts aqueous obtained from

Table 3
growth inhibition (INH) of bacteria after 24 h of incubation with aqueous extracts obtained from the wild basidiomata (WB), the mycelial biomass (MB; by SmF), spent mushroom substrate (SMS, by SSF), and the substrates without inoculum (S; by SSF).

Sample	Concentration (mg·L^{-1})	*B. cereus*	*E. coli*	*S. aureus*	*S. typhimurium*
B	**4250**	–	24.83 ± 3.10 a	–	56.93 ± 4.86 a
	2125	–	20.11 ± 2.45 b	–	9.43 ± 2.19 b
	1062.5	–	3.37 ± 2.81 c	–	8.98 ± 2.70 b
	531.3	–	2.19 ± 1.18 c	–	–
***M*-SmF T1**	**2500**	–	22.72 ± 4.66 a	–	21.03 ± 2.39 a
	1250	–	23.03 ± 1.31 a	–	15.92 ± 2.36 b
	625	–	21.54 ± 3.46 a	–	10.66 ± 2.99 c
	312.5	–	20.1 ± 6.11 a	–	9.29 ± 1.74 c
***M*-SmF T2**	**2500**	–	29.98 ± 7.75 a	–	28.6 ± 2.07 a
	1250	–	23.82 ± 6.2 ab	–	27.01 ± 1.36 a
	625	–	24.62 ± 2.77 ab	–	23.83 ± 0.50 b
	312.5	–	24.51 ± 1.84 b	–	20.06 ± 2.19 c
***M*-SmF T3**	**2500**	–	2.67 ± 2.62	–	53.98 ± 1.09 a
	1250	–	–	–	46.44 ± 5.36 b
	625	–	–	–	34.42 ± 2.01 c
	312.5	–	–	–	29.73 ± 2.67 c
***M*-SmF Saboraud T4**	**625**	–	8.55 ± 2.16 a	–	38.33 ± 2.65 a
	312.5	–	7.91 ± 2.68 ab	–	38.32 ± 1.85 a
	156.3	–	6.79 ± 3.92 ab	–	35.19 ± 2.29 b
	78.1	–	3.08 ± 2.27 b	–	29.9 ± 4.19 c
SS SFF T1	**2500**	–	–	–	46.94 ± 4.50 a
	1250	–	–	–	29.14 ± 3.390 b
	625	–	–	–	15.13 ± 4.08 c
	312.5	–	–	–	11.84 ± 2.97 c
SS SFF T2	**3593**	–	–	–	48.46 ± 4.66 a
	1796	–	–	–	25.11 ± 4.14 b
	898	–	–	–	22.74 ± 5.9 bc
	449	–	–	–	17.15 ± 4.49 c
***SS*–SFF T3**	**2500**	–	–	13.3 ± 1.09 a	59.96 ± 2.73 a
	1250	–	–	9.24 ± 1.67 a	57.78 ± 5.03 a
	625	–	–	–	48.97 ± 5.59 b
	312.5	–	–	–	31.62 ± 4.89 c
***S*-SFF T1**	**4000**	–	–	–	–
***S*-SFF T2**	**4000**	–	–	–	–
***S*-SFF T3**	**4000**	–	–	–	–
Control +	**2000**	10.7 ± 6.86	56.3 ± 2.63	14.7 ± 1.77	56.27 ± 2.62

The values are mean ± S.E.M; $n = 7$, $p < 0.05$. One-way Anova followed by Bonferroni post hoc test. WB, Wild Basidiomata; MB, Mycelial Biomass; S, Substrate; SmF, Submerged Fermentation; SSF, Solid State Fermentation; T1, Rice husk/Orange peel; T2, Orange peel/Rice bran; T3, Rice husk/Orange peel/Rice bran; T4, Sabouraud Dextrose Broth.

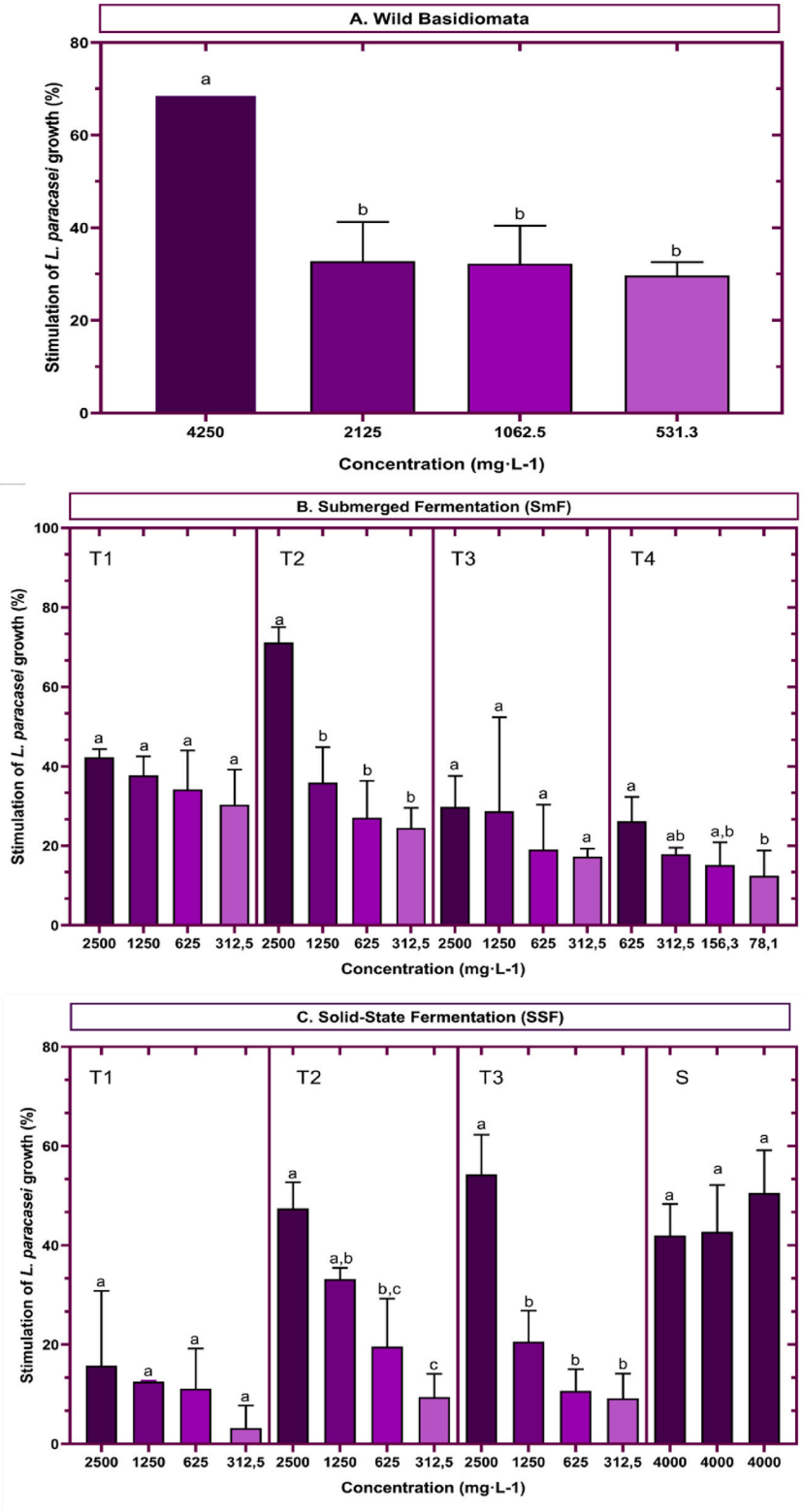

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Fig. 4. Stimulation of L. paracasei growth (LGW) at 48 h of incubation with aqueous extracts obtained from: (A), the wild basidiomata (WB); (B), the mycelial biomass (MB; by SmF) and (C), spent mushroom substrate (SMS, by SSF) and the substrates without inoculum (S; by SSF). The values are mean ± S.E.M; n = 7, $p < 0.05$. One-way Anova followed by Bonferroni post hoc test. WB, Wild Basidiomata; MB, Mycelial Biomass; S, Substrate; SmF, Submerged Fermentation; SSF, Solid State Fermentation; T1, Rice husk/Orange peel; T2, Orange peel/Rice bran; T3, Rice husk/Orange peel/Rice bran; T4, Sabouraud Dextrose Broth. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

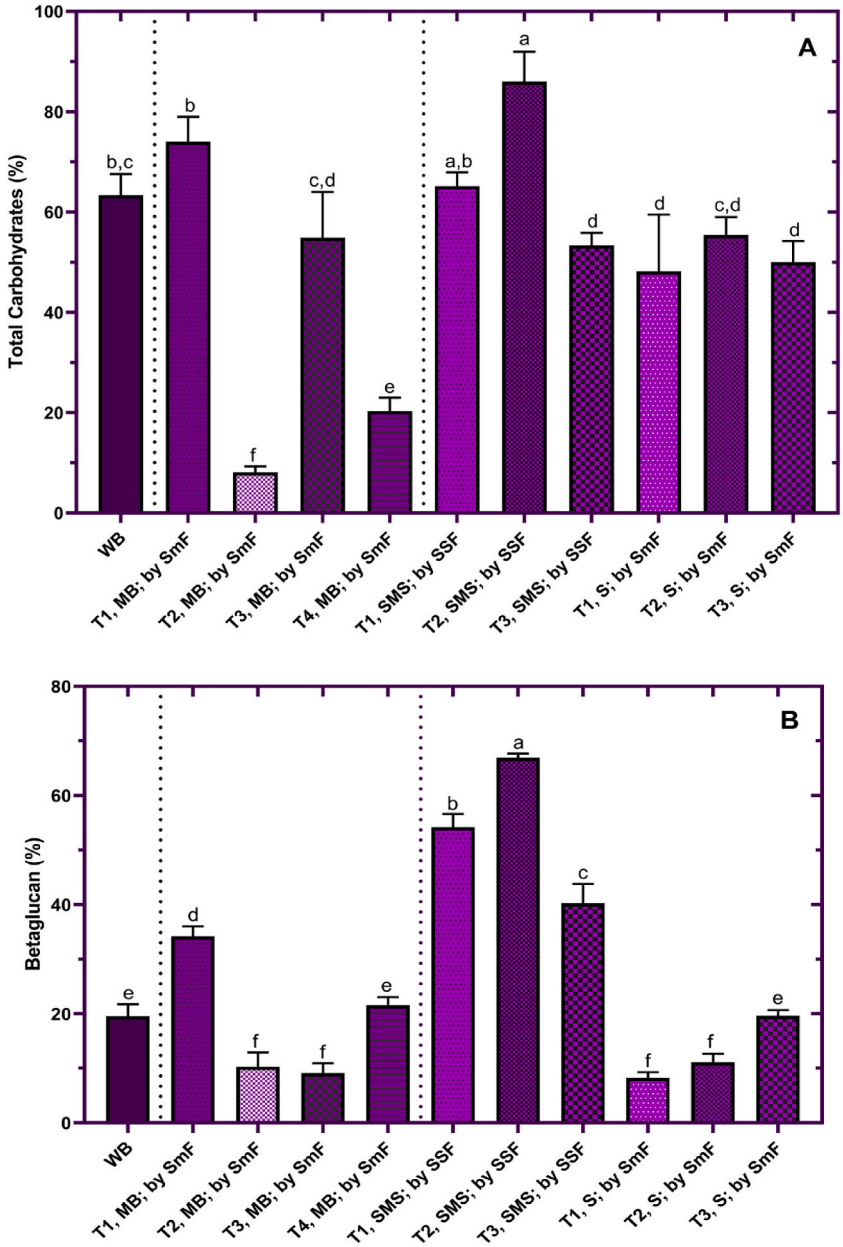

Fig. 5. Total carbohydrate (A) and β-glucan (B) contents of the aqueous extracts obtained from the wild basidiomata (WB), the mycelial biomass (MB; by SmF), spent mushroom substrate (SMS, by SSF), and the substrates without inoculum (S; by SSF). The values are mean ± S.E.M; n = 7, $p < 0.05$. One-way Anova followed by Bonferroni post hoc test. WB, Wild Basidiomata; MB, Mycelial Biomass; S, Substrate; SmF, Submerged Fermentation; SSF, Solid State Fermentation; T1, Rice husk/Orange peel; T2, Orange peel/Rice bran; T3, Rice husk/Orange peel/Rice bran; T4, Sabouraud Dextrose Broth. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the spent mushroom substrate by SSF with T1 inhibited the growth of *R. oryzae* by 72.2% at the highest concentration (2500 mg L^{-1}). The extracts obtain by the SSF (substrate without inoculum) didn't show percent growth inhibition for the four evaluated pathogenic fungi, except extracts obtain by *S*-SFF T1 (16.51%) with *R. oryzae*.

Taken together, Table 3 shows the percent inhibition of the aqueous extracts at different concentrations against the four bacteria evaluated. The aqueous extracts obtained from wild basidiomata, mycelia biomass by SSF and SmF in T3 showed the highest percent inhibition against *S. typhimurium.* None of the extracts showed antibacterial activity against *B. cereus* and *S. aureus*. Only the aqueous extracts obtained wild basidiomata, SmF in T1, SmF in T2 show percent inhibition against *E. coli.* For all the antibacterial assays, the extracts obtain by the SSF (substrate without inoculum) did inhibit the growth of the four evaluated bacteria. The positive control inhibited the growth of *B. cereus* (10.7%), *E. coli* (56.3%), *S. aureus* (14.7%) and *S. typhimurium* (56.3%). For both the antimicrobial and antifungal tests, in some cases there was a relationship between the concentration and the percentage of inhibition. As the concentration of aqueous extracts increased, the percentage of inhibition increased.

The aqueous extracts stimulated the growth of *L. paracasei* (Fig. 4). All of them (at the highest concentration) showed stimulation of the probiotic between 15.77% and 71.2% at 48 h, particularly the aqueous extracts from mycelial biomass obtained by SmF with T2 (71.2%), wild basidiomata (68.6%), and spent mushroom substrate with T3 (54.3%) at the highest concentrations evaluated. The extracts obtain by the SSF (substrate without inoculum) show percent stimulation (42%).

Total carbohydrate and β-glucan contents from the wild basidiomata, mycelial biomass (by SmF), spent mushroom substrate and substrate (by SSF) are shown on Fig. 5. The quantity of carbohydrate and β-glucan were significantly high ($p < 0.05$) in the in the spent mushroom substrate (obtained by SSF) with T2 and (86.02%, 56.96%) The content of polysaccharides and β-glucans was different in the combinations of the agroindustrial substate and the type of fermentation methodology used.

5. Discussion

The carbohydrate and β-glucan contents were different depending on the sample type. In solid-state fermentation, higher production of carbohydrates and β-glucans was obtained in the medium containing orange peel and rice bran (T2). There was a higher production of these compounds for solid fermentation, which probably reflected the carbohydrates of both the fungus and the spent mushroom substrate. Previous studies suggested that the content of these compounds varies according to the culture conditions in which the fungal strain has been grown, particularly the effect of the C/N ratio on the biosynthesis of polysaccharides in the fungal cell wall and other factors such as extraction condition [38,61–63]. In the present study, the content of β-glucans was within the ranges reported for some culturable and edible species, such as *Lentinula edodes* (25.0%), *Agaricus bisporus* (8.6%), and *Pleurotus ostreatus* (24.2%) [64]. Aqueous extracts obtained by SmF showed higher percentages of inhibition against fungi and bacteria than those obtained by SSF. This confirms that submerged fermentation can produce this type of extracts in a shorter time (20 days) compared with

Table 4
Antimicrobial activity reported for Lentinus crinitus (L.) Fr (Polyporales, Basidiomycota).

Sample	Extract or metabolite	Activity	Region or Country	Year	Reference
Culture broth/ Submerged Fermentation	Crude extract with ethyl acetate. Most active compounds: 1-desoxy-hypnophilin and 6,7 epoxy-4 (15)-hirsutene-5-ol	Antimicrobial activity against: *Bacillus cereus* DSM 318, *Staphylococcus aureus* ATCC 13709, *Escherichia coli* ATCC 9637, *Candida albicans* ATCC 10231, *C. tropicalis* DSM 1346, *Rhodotorula glutinis* DSM 70398	Ethiopia	1994	[4]
Culture broth/ Submerged Fermentation	Crude extract with ethyl acetate. Chromamones	Used of different carbon source in the medium for the formation of antibiotic metabolites	East African	1995	[66]
Synthesis	(±)-1-desoxy-hypnophilin:	Antimicrobial activity	East African	2000	[67,68]
Wild Basidiomata	Hydroalcoholic cold extracts	Antifungal activity against *Fusarium* sp	Brazil	2014	[65]
Basidiomata/Solid State Fermentation	Hydromethanolic extract	Antimicrobial activity against *Listeria monocytogenes*, *Salmonella enterica*, *Penicillium ochrochloron*, *Aspergillus ochraceus* and *Trichoderma viride.*	Brazil	2021	[69]
Basidiocarp pileus and stipe/Solid State Fermentation	Methanolic extracts	Antimicrobial activity against *Bacillus cereus, Enterobacter cloacae, Escherichia coli, Listeria monocytogenes, Micrococus luteus, Pseudomonas aeruginosa, Salmonella enterica, Staphylococcus aureus, Aspergillus fumigatus, Aspergillus niger, Aspergillus versicolor, Penicillium aurantiogriseum, Penicillium ochrochlonron, Talaromyces funiculosus and Trichoderma virens.*	Brazil	2021	[5]
Mycelial biomass/ Submerged Fermentation	Methanolic extracts	Antimicrobial activity against *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Escherichia coli, Salmonella enterica, Enterobacter cloacae, Aspergillus fumigatus, Aspergillus niger, Aspergillus versicolor, Talaromyces funiculosus, Penicillium aurantiogriseum and Trichoderma virens.*	Brazil	2022	[70]

solid fermentation (30 days) and obtain extracts with better bioactivities. It is important to highlight that the extracts of the substrates without inoculum of *L. crinitus* didn't showed antifungal and antibacterial activity at the concentration evaluated.

In the literature, there are some reports of the evaluation of the extracts of metabolites of *L. crinitus* with antimicrobial activity obtained from wild basidiomata, liquid fermentation or basidiomata obtained in solid state fermentation (Table 4). One of them, corresponds to the first report of this activity antimicrobial activity against pathogens such as S. *aureus, E. coli, A. niger* [4]. A more recent investigation evaluated the inhibitory activity of aqueous extracts of mushrooms of *L. crinitus*, inhibited more than 92% of the sporulation of conidia of *Fusarium* sp [65]. These results agree with those obtained in the present study, although the percentages of inhibition are lower (24.8%-33.37) at the highest concentrations. Further, extracts of *L. crinitus* showed high percentages of inhibition of *E. coli, S. typhimurium, R. oryzae, F. oxysporum and A. niger.* A more recent investigation evaluated the antimicrobial activity of *L. crinitus* basidiocarp, where the minimum bactericidal and fungicidal concentration values similar to or lower than those verified by commercial antibiotics or food additives used as preservatives [6] and other investigation show the *L. crinitus* basidiocarps are an alternative to antimicrobials against foodborne pathogens and food spoilage microorganisms [5]. There are no reports of aqueous extracts of *L. crinitus* obtained from the spent mushroom substrate obtained from solid fermentation.

Aqueous extracts obtained by liquid fermentation with unconventional carbon sources could be used to treat infections caused by bacteria that have become resistant to conventional drugs, thereby avoiding a worldwide public health problem [71]. Furthermore, they can be used to counteract the phytopathogenic fungi that cause food decomposition, which generating different levels and types of rot in several crops, such as corn, potatoes, tomatoes, and strawberries, causing significant economic damage. In addition, the bioactivity of the aqueous extracts might be caused by other parameters that were not evaluated in the present study, such as the degree of branching and solubility of the compounds [72].

The growth stimulation of the evaluated probiotic suggested that the aqueous extracts could act as a carbon source for bacterial growth. In addition, these extracts have great potential for use as ingredients in functional products, such as dietary products, including juices or beers, because they can increase the viability of a bacterial inoculum [60,73,74]. Extracts with a greater stimulatory effect on *L. paracasei*, showed inhibitory effects on the evaluated pathogenic bacteria, such as *S. typhimurium* and *E. coli*; however, it will be necessary to evaluate their prebiotic potential *in vivo*, as well as their possible immunomodulatory effect or other biological activities.

6. Conclusion

The study shows the content of total carbohydrates and beta-glucans of aqueous extracts obtained from wild basidiomata, mycelial biomass and spent mushroom substrate of *L. crinitus.* Also, the results showed that *R. oryzae, F. oxysporum, A. niger*, *E. coli* and *S. typhimurium* are inhibited by the aqueous extracts of L. *crinitus,* thereby representing a potential source of macrofungal antimicrobial agents. Further study is required to isolate antimicrobial compounds from *L. crinitus* for development of antimicrobial agents. In addition, the aqueous extracts obtained stimulated the growth of *L. paracasei,* this being the first study to evaluate this activity. This result together with the antimicrobial activity could be used as the starting point for deepen the evaluation of aqueous extracts to be added to food additives or their prebiotic activity.

Author contribution statement

Lina Rocio Dávila Giraldo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Paula Xioma Villanueva Baez: Performed the experiments; Analyzed and interpreted the data.

Henry A. Vaquiro: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hector Suárez Mahecha: Walter Murillo Arango: Jonh Jairo Méndez Arteaga: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. Material/referenced in article.

Declaration of competing 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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