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CIÊNCIAS BIOLÓGICAS:

Gênese na formação multidisciplinar

Alana Maria Cerqueira de Oiveira
(Organizadora)

Atena Editora
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APRESENTAÇÃO

O Livro “Ciências biológicas: Gênese na formação multidisciplinar 2”, traz ao leitor vinte capítulos de relevada importância na área de Genética, Citogenética, Imunologia, Parasitologia, Química medicinal, Saúde pública e Ecologia. Entretanto, caracteriza-se como uma obra multidisciplinar que engloba diversas áreas da Ciências biológicas.

Os capítulos estão distribuídos em temáticas que abordam de forma categorizada e multidisciplinar a Ciências biológicas , as pesquisas englobam estudos de: mapeamentos genético, citogenético, sequenciamento, genética e educação ,análises forenses , doenças genética, eugenesia clássica, engenharia genética, análise por PCR, cultura de células de linfoma e leucemia, saúde mental, resposta imune, vacinação contra a covid-19, vírus Sars-Cov-2, métodos de extração de lipídios ,levantamento taxonômico, morfologia vegetal, eficiência de inseticidas , química medicinal, cromatografia líquida de alta eficiência (CLAE), espectroscopia de infravermelho (IV) e espectrometria de massas (EM), problemática ambiental e de saúde pública, poluentes emergentes e biodiesel.

A obra foi elaborada primordialmente com foco nos profissionais, pesquisadores e estudantes pertencentes às áreas de Ciências biológicas e Ciências da Saúde e suas interfaces ou áreas afins. Entretanto, é uma leitura interessante para todos aqueles que de alguma forma se interessam pela área.

Cada capítulo foi elaborado com o propósito de transmitir a informação científica de maneira clara e efetiva, em português, inglês ou espanhol. Utilizando uma linguagem acessível, concisa e didática, atraindo a atenção do leitor, independente se seu interesse é acadêmico ou profissional.

O livro Ciências biológicas: Gênese na formação multidisciplinar 2”, traz publicações atuais e a Atena Editora traz uma plataforma que oferece uma estrutura adequada, propícia e confiável para a divulgação científica de diversas áreas de pesquisa.

Alana Maria Cerqueira de Oliveira

SUMÁRIO

CAPÍTULO 14

EFFECT OF LACTIC ACID BACTERIA ON *Fusarium verticillioides* GROWTH AND FUMONISIN B_1 DETOXIFICATION

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ABSTRACT: *Fusarium verticillioides* is a primary corn pathogen with global importance because it causes economic losses and risks to human and animal health due to fumonisin production. This study aimed to evaluate the effect of lactic acid bacteria (LAB) strains on *F. verticillioides* growth and fumonisin B_1 (FB_1) detoxification. The antifungal activity of seven LAB strains was evaluated against five *F. verticillioides* strains. *Lactobacillus plantarum* BG112 and *Lactobacillus reuteri* LR-92 showed the highest antifungal activity against *F. verticillioides* 97L strain. *L reuteri* LR-92 cells caused morphological alterations such as pore formation. Moreover, cell-free supernatant (CFS) from both LAB was effective in reducing conidial germination and mycelial growth in addition to inducing morphological changes in conidia. The supernatant possibly had a fungicidal effect since there was no mycelial development. Furthermore, all strains showed FB_1 removal ability, and the greatest reductions were shown by nonviable cells of *L. reuteri* LR-92 (88.0%) and viable cells of *L. fermentum* ATCC 9339 (78.0%). LAB strains showed antifungal activity and *in vitro* FB_1 detoxification ability. These bacteria are promising for developing new biological control formulations to minimize fumonisin contamination, thus contributing to improved food safety.

KEYWORDS: Antifungal activity; *Lactobacillus reuteri*; *Lactobacillus plantarum*; Mycotoxin adsorption.

EFEITO DE BACTÉRIAS LÁTICAS NO CRESCIMENTO DE *Fusarium verticillioides* E NA DETOXIFICAÇÃO DE FUMONINA B_1

RESUMO: *Fusarium verticillioides* é um patógeno primário de milho de importância mundial, pois causa prejuízos econômicos e riscos à saúde de seres humanos e animais devido à produção de fumonisinas. Este estudo teve como objetivo avaliar o efeito de cepas de bactérias lácticas (BAL) no crescimento de *F. verticillioides* e na detoxificação da fumonisina B_1 (FB_1). A atividade antifúngica de sete cepas de BAL foi avaliada contra cinco cepas de *F. verticillioides*. *Lactobacillus plantarum* BG112 e *Lactobacillus reuteri* LR-92 apresentaram a maior atividade antifúngica contra a cepa *F. verticillioides* 97L. A cepa *L. reuteri* LR-92 causou alterações morfológicas, como a formação de poros. Além disso, o sobrenadante livre de células (SLC) de ambas as BAL foi eficaz na redução da germinação de conídios e do crescimento micelial, além de induzir alterações morfológicas nos conídios. O SLC possivelmente teve efeito fungicida, uma vez que não houve desenvolvimento micelial. Além disso, todas as cepas apresentaram capacidade de remoção de FB_1, sendo que a maior redução da concentração de FB_1 foi apresentada por células não viáveis de *L. reuteri* LR-92 (88,0%) e células viáveis de *L. fermentum* ATCC 9339 (78,0%). As cepas de BAL apresentaram atividade antifúngica e capacidade de detoxificação de FB_1 *in vitro*. Essas bactérias são promissoras para o desenvolvimento de novas formulações de controle biológico para minimizar a contaminação por fumonisinas, contribuindo para a melhoria da segurança de alimentos.
PALAVRAS-CHAVE: Atividade antifúngica; *Lactobacillus reuteri*; *Lactobacillus plantarum*; adsorção de micotoxinas.

1 I INTRODUCTION

Corn (*Zea mays* L.) is one of the most produced cereal grains worldwide. In 2018/2019, the total worldwide production was 1 billion tons (USDA, 2019), and Brazil is the third largest producer, with 102 million tons for the 2019/2020 harvest season (COMPANHIA NACIONAL DE ABASTECIMENTO, 2020). Corn is the staple food in several countries and is the main ingredient of animal feed (GARCÍA-LARA; SERNA-SALDIVAR, 2019). However, the nutritional characteristics of this cereal grain make it susceptible to contamination by *Fusarium verticillioides*, which is considered the main corn phytopathogen (ARIAS *et al.*, 2016; COUNCIL FOR AGRICULTURAL SCIENCE AND TECHNOLOGY, 2003). Corn infection by this filamentous fungus results in great economic losses due to decreased production in the field and grain contamination by mycotoxins such as fumonisins (COUNCIL FOR AGRICULTURAL SCIENCE AND TECHNOLOGY, 2003).

Ingestion of fumonisin-contaminated grains is related to the increased incidence of esophageal (KHAN; PHULUKDAREE; CHUTURGOON, 2018) and liver cancer (ARUMUGAM *et al.*, 2019) and neural tube defects in humans (MARASAS *et al.*, 2004). In animals,

fumonisins are associated with several mycotoxicoses, such as leukoencephalomalacia in equines (VENDRUSCOLO *et al.*, 2016), pulmonary edema in pigs (PÓSA *et al.*, 2016) and decreased body weight gain, alterations in immune function and liver pathology in poultry (TESSARI *et al.*, 2006). Among the fumonisins, fumonisin B_1 (FB_1) is the most prevalent and most toxic analog (COPPOCK; DZIWENKA, 2014). FB_1 has been classified in group 2B, meaning that it is possibly carcinogenic to humans (INTERNATIONAL AGENCY FOR RESEARCH ON CANCER, 2002).

Conventional fungicides have been widely used to control *F. verticillioides* contamination in corn; however, consumer concern about this practice is increasing because of the negative effects on the environment (RATHORE; NOLLET, 2012) and human health (WORLD HEALTH ORGANIZATION, 1990). Biological control methods are an attractive alternative to chemical agents, as they do not cause adverse effects on soil and are not harmful to human health.

Lactic acid bacteria (LAB) are promising biocontrol agents due to their antifungal activity against some fungal species (KHARAZIAN *et al.*, 2017; SIEDLER *et al.*, 2019) and their ability to detoxify mycotoxins (JUODEIKIENE *et al.*, 2018; CHLEBICZ; ŚLIŻEWSKA, 2019).

The antifungal mechanism of LAB has been associated with the production of several bioactive metabolites, including organic acids (acetic, lactic, propionic, succinic, formic and butyric acid), phenyllactic acid, fatty acids, and peptides (cyclic dipeptides and bacteriocins) (BLACK *et al.*, 2013; KWAK; LIU; KANG, 2018; LUZ *et al.*, 2017; SIEDLER *et al.*, 2019).

Another important feature of LAB is the ability to reduce mycotoxin levels. Some studies have shown that the probable mechanism of *in vitro* FB_1 detoxification is mycotoxin binding to the bacterial cell wall (NIDERKORN *et al.*, 2009; PIZZOLITTO; SALVANO; DALCERO, 2012). Other studies have shown that some microorganisms can produce enzymes that degrade mycotoxins to less toxic intermediate compounds (HEINL *et al.*, 2010; TUPPIA *et al.*, 2016).

Considering the potential of LAB for mycotoxin detoxification and the few published studies relating LAB to antifungal activity against *F. verticillioides* and FB_1 detoxification, the aim of this study was to evaluate the *in vitro* effect of LAB on *F. verticillioides* growth and FB_1 detoxification.

2 | MATERIAL AND METHODS

2.1 Microorganisms and culture conditions

Seven LAB strains were used for the antifungal activity screening and FB_1 removal experiments: *Lactobacillus plantarum* ATCC 14917, *L. plantarum* ATCC 94917, and *L. fermentum* ATCC 9339 (American Type Culture Collection); *L. paracasei* and *L. gasseri*, belonging to the Culture Collection of the Department of Food Science and Technology—

State University of Londrina; and *L. reuteri* Lyofast LR-92 (Sacco) and *L. plantarum* Lyofast BG112 (Sacco) (commercial cultures). The LAB strains were cultured in the De Man–Rogosa–Sharpe (MRS) broth at 37 °C for 24 h and subcultured at least three times. The strains were maintained as frozen stock cultures at –20 °C in MRS supplemented with 25% (v/v) glycerol.

F. verticillioides strains (97L, 97K, 103F, 113F and 103G) were provided by the Mycological Culture Collection of the Department of Food Science and Technology, State University of Londrina. The fungal strains were grown on PDA (potato dextrose agar) at 25 °C for 7 days and stored at 4 °C.

All of the *F. verticillioides* strains produced fumonisins (FB_1 + FB_2), as follows: 97K (4051 $\mu g\ g^{-1}$), *97L (3230* $\mu g\ g^{-1}$), *103F* (5120 $\mu g\ g^{-1}$), *113F (724* $\mu g\ g^{-1}$) *and 103G (225* $\mu g\ g^{-1}$).

2.2 Screening of antifungal activity

Antifungal activity was evaluated by an agar diffusion assay according to Motomura *et al*. (1996). Sterile Petri dishes were inoculated with a suspension of *F. verticillioides* strains (10^5 conidia mL^{-1}) and 20 mL of PDA.

After agar solidification, an aliquot (100 µL) of MRS broth with LAB cell concentrations adjusted to 3.0 x 10^9 CFU mL^{-1} was inoculated in 5-mm-diameter holes in the center of the plate (AMMOR *et al.*, 2006). Then, plates were incubated at 25 °C for 7 days, and the inhibition zone was measured. The inhibition zone was defined as the diameter of the circle without fungal growth surrounding the hole. All tests were performed in triplicate.

2.3 Effect of pH, acetic acid and lactic acid concentration on antifungal activity

The agar diffusion assay was performed according to De Muynck *et al*. (2004) with some modifications. Antifungal activity was evaluated using sterile MRS broth with different concentrations of acetic acid and lactic acid (10%, 20%, 30%, 40% and 50% (w/v)). In addition, MRS broth with the pH adjusted to 1, 2, 3, 4, 5 and 6 using hydrochloric acid (5 mmol L^{-1}) was evaluated. An aliquot (100 mL) of MRS broth was added to 5-mm holes in the center of the PDA agar previously inoculated with 10^5 conidia mL^{-1} *F. verticillioides*. All assays were performed in triplicate.

2.4 Determination of the minimum inhibitory concentration (MIC) of lactic acid and acetic acid for fungal growth

The minimum inhibitory concentrations of lactic and acetic acid were determined for the five *F. verticillioides* strains according to the Clinical and Laboratory Standards Institute (CLSI) guideline (CLSI, 2010) with some modifications. The MIC was defined as the lowest acid concentration that causes a specified reduction in visible fungal growth. Lactic and acetic acid were used at 0.4, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0% (w/v) and 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.9% (w/v), respectively. An aliquot (100 mL) of MRS broth was added to the holes (5 mm) in the center of the PDA previously inoculated with 10^6 conidia mL^{-1} *F.*

verticillioides. All assays were performed in triplicate.

2.5 Effect of cell-free supernatant on *F. verticillioides* mycelial growth and morphology

The two species (*L. plantarum* BG112 and *L. reuteri* LR-92) that showed the best results in the screening of antifungal activity, were selected to investigate the mechanisms involved in the antifungal effect of the *F. verticillioides* 97L strain. LAB strains were cultured in MRS broth at 37 °C for 24 h. The bacterial culture was centrifuged at 6500 ***g*** for 10 min, and the cell-free supernatant (CFS) was filter sterilized (0.22-mm pore size membranes; Millipore). The experiment was performed in PD broth previously inoculated with *F. verticillioides* 97L conidial suspensions (10^5, 10^6 and 10^7 conidia mL^{-1}) mixed with the cell-free supernatants in a 1:1 ratio and incubated at 25 ºC and 180 rpm for 24 h for microscopic analysis of conidial germination percentage and for seven days for biomass estimation and assessment of mycelial morphology alteration. In addition, a coinoculation assay was performed with *F. verticillioides* 97L and *L. plantarum* BG112 or *L. reuteri* LR-92 cultures (10^8 CFU mL^{-1}) to evaluate morphological changes. A control without cell-free supernatant was performed using saline solution (0.85% NaCl). The experiments were performed in triplicate.

2.6 Conidial germination inhibition assay

Conidial germination was determined by counting at least 100 conidia per replicate (germinated and nongerminated) using a light microscope at 400X (CHEN *et al.*, 1999). A conidium was considered germinated when the germ tube was longer than the conidial diameter (DE CAL; MELGAREJO, 1999). The results were expressed as the percentage of conidial germination inhibition, calculated according to the following formula:

$$\% \text{ conidial germination inhibition} = \frac{(cc - ct)}{cc} X100$$

Where:

cc: number of germinated conidia in the control sample

ct: number of germinated conidia in the treatment sample

2.7 Biomass estimation

Estimation of fungal biomass was performed by the gravimetric method. The culture from the assay described in section 2.5 was filtered through a preweighed No. 1 Whatman filter and dried in an oven at 70 ºC to a constant weight. The weight of the treatment sample was compared with that of the control sample, and the percentage of mycelial growth inhibition was calculated as follows:

$$\% \text{ mycelial growth inhibition} = \frac{(\text{control weight} - \text{treatment weight})}{\text{control weight}} X100$$

2.8 Scanning electron microscopy assay (SEM)

F. verticillioides coinoculated with L. plantarum BG112 *or L. reuteri* LR-92 *cells* and *F. verticillioides* conidia treated with CFS (as described in section 2.5) were fixed with 2.5% glutaraldehyde solution in 0.1 mol L^{-1} sodium phosphate buffer (pH 7.2) for 90 min. After the fixation period, they were treated with 2% osmium tetroxide in 0.1 mol L^{-1} sodium phosphate buffer, subjected to dehydration in a graded ethanol series (20, 30, 50, 70, 80, 90% and 100%) and additionally dehydrated at the critical point, mounted on a metallic support and metallized with gold film. Following processing, samples were analyzed in a scanning electron microscope, model FEI Quanta 200.

2.9 Fumonisin B_1 detoxification assay

FB_1 detoxification assay was performed according to Hernandez-Mendoza *et al.* (2009), Niderkorn, Boudra, Morgavi (2006), and Pizzolitto, Salvano and Dalcero (2012we propose that the mechanism involved in the removal of FB 1 is a physical adsorption (physisorption), with some modifications. The final concentration of LAB cells was standardized at 3.0×10^9 CFU mL^{-1} after centrifugation (5000 ***g***, 10 min, 4 °C), followed by washes with phosphate-buffered saline (PBS, pH 7.2) and ultrapure water. In order to evaluate the effect of cell viability on detoxification, heat-inactivated LAB cells (121 ºC for 15 min) were also used. The preparation procedure was the same as that for viable cells (without heat treatment). The resulting pellet was suspended in FB_1 standard (Sigma, St. Louis, MO, USA) (2.5 mg mL^{-1}) diluted in PBS (pH 7.2) to a final concentration of 2.5 mg mL^{-1}. The experiment was carried out in 50-mL Falcon tubes at 37 ± 1 °C. Positive controls were prepared in 50-mL Falcon tubes containing 2 mL of FB_1 solution (2.5 mg mL^{-1}) and processed in the same way as the samples. After incubation, FB_1 determination in the supernatant was performed by high-performance liquid chromatography (HPLC).

The percentage of FB_1 removed was calculated according to the following formula:

$$FB_1 \text{ removed } (\%) = 100 \times 1 - \frac{(\text{Peak area of } FB_1 \text{ in the supernatant})}{(\text{Peak area of positive control})}$$

2.10 Quantification of fumonisin B_1 by HPLC

An aliquot of 1 mL of test supernatant was mixed with 1 mL of methanol: water (3: 1, v/v), and 1 mL of the mixture was cleaned up using an anion exchange cartridge (Sep-Pak Accell Plus QMA, Waters).

Fumonisin B_1 was quantified according to Shephard *et al.* (1990) as modified by Ueno *et al.* (1993). A 200 mL aliquot was dissolved in acetonitrile: water (1: 1, v/v) and

derivatized with o-phthaldialdehyde (OPA; 40 mg of OPA in 1 mL of methanol, 5 mL of 0.1 mmol L^{-1} sodium tetraborate and 50 mL of 2-mercaptoethanol).

Analysis was performed with a reversed-phase isocratic HPLC system (Shimadzu LC-10 AD pump and RF-10A XL fluorescence detector) using a Nucleosil 100-5 C18 column (4.6 x 250 mm, Macherey-Nagel GmbH & Co.). The excitation and emission wavelengths were 335 and 450 nm, respectively. The mobile phase consisted of CH_3OH: 0.1 mol L^{-1} NaH_2PO_4 (80: 20, v/v) with the pH adjusted to 3.3 (with orthophosphoric acid). The flow rate was 1.0 mL min^{-1} at 25 °C. The detection limit for FB_1 was 27.5 ng mL^{-1}.

The average recovery rate of FB_1 from the positive control sample with 2.5 mg mL^{-1} was 88.3%.

2.11 Statistical Analysis

Differences in the mean FB_1 removal (%) among different LAB strains and viable and nonviable (heat-inactivated) cells were evaluated statistically using the Student's t test for two groups and ANOVA followed by the Tukey's test ($p<0.05$) for multiple comparisons. Statistical analysis was performed by Statistica software, version 7 (Stat Soft, Inc., Tulsa, OK, USA, 2007).

3 I RESULTS

3.1 Evaluation of antifungal activity

The antifungal activity was based on the inhibition zone diameter and was classified according to Rouse et al. (2008) as follows: (-) no inhibition, very weak inhibition (diameters from 1 to 15 mm), moderate inhibition (diameters from 16 to 30 mm), medium inhibition (diameters from 31 to 45 mm) and strong inhibition (diameters greater than 45 mm) (Table 1).

Microorganism	*Fusarium verticillioides* strains				
	97L	97K	103F	113F	103G
	Inhibition zone diameter (mm)*				
L. plantarum ATCC 14917	6.0	5.0	5.7	6.0	-
L. plantarum ATCC 94917	5.0	5.7	5.3	6.0	-
L. fermentum ATCC 9339	3.8	4.0	3.3	4.0	-
L. paracasei	10.7	6.3	7.3	7.0	-
L. gasseri	11.3	5.7	5.7	5.3	-
L. reuteri LR-92	30.0	-	-	-	4.0
L. plantarum BG112	23.7	-	-	7.7	6.3

* Inhibition zone diameter correspond to the means of triplicate analysis

(-) no inhibition, very weak inhibition (diameters from 1 to 15 mm), moderate inhibition (diameters from 16 to 30 mm), medium inhibition (diameters from 31 to 45 mm) and strong inhibition (diameters greater than 45 mm)

Table 1. Antifungal activity of lactic acid bacteria against five *F. verticillioides* strains (97L, 97K, 103F, 113F and 103G).

The LAB strains showed different degrees of inhibition against the five F. verticillioides strains. L. reuteri LR-92 and L. plantarum BG112 showed the highest antifungal activity against F. verticillioides 97L, with inhibition diameters of 30.0 mm and 23.7 mm, respectively (moderate inhibition).

3.2 Effect of pH, acetic acid and lactic acid concentration on antifungal activity

The antifungal activity of LAB against *F. verticillioides strains* was characterized by evaluating the effect of pH, different concentrations of lactic and acetic acid (Table 2) on mycelial growth. Medium at pH 1 inhibited the growth of the 97L, 103F, 113F and 103G strains.

The five *F. verticillioides* strains were susceptible to different concentrations of acetic acid and lactic acid.

Parameters		*Fusarium verticillioides* strains				
		97L	97K	103F	113F	103G
		Inhibition zone diameter (mm) *				
Acetic Acid Concentration (%)	10	26	18	15	20	15
	20	39	23	38	24	25
	30	55	26	28	31	34
	40	55	38	40	41	37
	50	55	47	49	43	37
Lactic Acid Concentration (%)	10	10	5	7	4	5
	20	13	6	5	7	5
	30	15	9	5	8	8
	40	17	9	10	10	12
	50	17	11	13	12	13
pH of culture medium	1	6	0	3	2	2
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0

* Inhibition zone diameter correspond to the means of triplicate analysis

Table 2. Effect of different concentrations of acetic acid, lactic acid and pH on antifungal activity.

3.3 Minimal inhibitory concentrations (MIC) of acetic acid and lactic acid for *F. verticillioides* strains

The MIC values of these acids against *F. verticillioides* (Table 3) indicate that the 97L strain was the most susceptible.

	MIC % (m/v)	
Strains	Lactic acid	Acetic acid
97L	0.4	0.2
97K	1.0	0.4
103F	2.0	1.0
113F	2.0	0.5
103G	2.0	0.4

Table 3. Minimum inhibitory concentrations of lactic and acetic acid against *F. verticillioides* strains (97L, 97K, 103F, 113F and 103G).

3.4 Effect of cell-free supernatant on *F. verticillioides* mycelial growth and morphology

The antifungal effect of cell-free supernatants (CFSs) from *L. plantarum* BG112 and *L. reuteri* LR-92 against *F. verticillioides* 97L (Table 4) was evaluated by conidial germination and mycelial growth assays and conidial morphology alteration. The CFSs from the LAB showed a fungicidal effect against *F. verticillioides* 97L, as evidenced by the inhibition of conidial germination and mycelial growth. In addition, the mycelial morphology of *F. verticillioides* 97L after treatment with CFS was investigated by SEM (Fig. 1). SEM micrographs of the control (without treatment) demonstrated normal hyphae (Fig. 1A) with homogeneous, intact structures with long monophialides and showed extracellular material resembling a biofilm around the hyphae. However, fungus treated with CFS from *L. plantarum* BG112 (Fig. 1C) and *L. reuteri* LR-92 (Fig. 1D) for seven days showed structural modifications of fungal conidia, and fungal mycelia were not visible. The CFS caused irreversible damage to conidia, resulting in cell mortality and inhibition of hyphal growth. Conidia showed deformation with wrinkled surfaces and were collapsed, most likely because of the lack of cytoplasm (Fig. 1C and D). In contrast, morphological alterations, including distorted and wrinkled hyphae with pores on the surface, were observed only in cells treated with *L. reuteri* LR-92 (Fig. 1B).

	Percentage of conidial germination inhibition (%)			Percentage of mycelial growth inhibition (%)		
F. verticillioides (conidia/mL)	10^5	10^6	10^7	10^5	10^6	10^7
Treatment						
CFS *L. plantarum* BG112	96%	98%	98%	100%	100%	100%
CFS L *reuteri* LR-92	97%	99%	97%	100%	100%	100%

Table 4. Effect of cell-free supernatant of *L. plantarum* BG112 and *L. reuteri* LR-92 on the conidial germination and mycelial growth of *F. verticillioides* 97L.

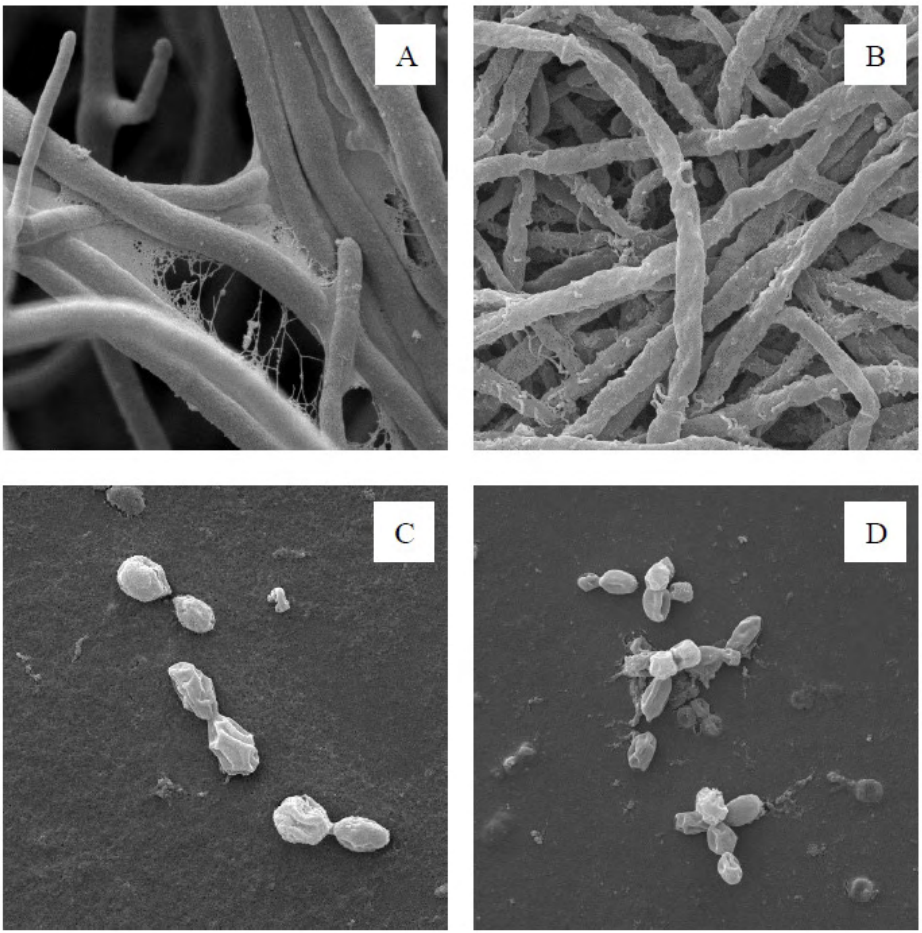

Fig. 1. Scanning electron micrographs of *F. verticillioides* 97L after seven days of culture at 25ºC. Untreated (fungal control), 8000x (A); mycelia treated with *(10^8 CFU/mL) L. reuteri* LR-92 cells (B); conidia treated with cell-free supernatant (CFS) from *L. plantarum* BG112, 8000X (C); and conidia treated with CFS from *L. reuteri* LR-92, 8000x (D).

3.5 Effect of LAB strains on fumonisin B_1 detoxification

The ability of LAB strains to remove FB_1 from PBS solution is shown in Table 5. The percentage of FB_1 removed by the viable cells ranged from 30.1% (*L. gasseri*) to 78.0% (*L. fermentum* ATCC 9339). *L. fermentum* ATCC 9339 showed the highest FB_1 removal ability (78.0%), but there was no significant difference ($p>0.05$) from that of *L. reuteri* LR-92 (71.9%). Heat-inactivated (nonviable) cells showed FB_1 removal percentages ranging from 49.5% (*L. plantarum* BG112, *L. plantarum* ATCC 94917) to 88.0% (*L. reuteri* LR-92). *L. reuteri* LR-92 (88.0%), *L. paracasei* (80.7%), *L. fermentum* ATCC 9339 (80.7%) and *L. gasseri* (72.9%) strains showed the highest FB_1 removal ability, differing significantly ($p<0.05$) from that of other LAB strains.

	% FB_1 removal*	
Microorganism	Viable cells†	Nonviable cells by heat treatment†
L.plantarum ATCC 14917	35.6 ± 5.8^{deB}	50.9 ± 1.6^{bB}
L.plantarum ATCC 94917	46.2 ± 5.8^{cdB}	49.5 ± 4.9^{bB}
L.fermentum ATCC 9339	78.0 ± 1.9^{aA}	80.7 ± 0.8^{aA}
L.paracasei	57.2 ± 0.9^{bcB}	80.7 ± 1.9^{aA}
L.gasseri	30.1 ± 4.2^{eB}	72.9 ± 4.7^{aA}
L.reuteri LR-92	71.9 ± 3.1^{abB}	88.0 ± 0.1^{aA}
L.plantarum BG112	41.7 ± 5.2^{deB}	49.5 ± 3.4^{bB}

*Results correspond to the means and SD (standard deviation) from triplicate analysis

†Means followed by the different lowercase letters (a-e) in the same column or by the different uppercase letters (A-B) in the same line differ significantly ($p<0.05$) by the Tukey test and the t-Test, respectively.

Table 5. Fumonisin B_1 removal (%) by viable and nonviable cells of LAB strains at 2.5 mg mL^{-1} FB_1 concentration.

In the comparison of FB_1 removal ability (%) by the same strain, heat-inactivated cells of the *L. reuteri* LR-92, *L. paracasei*, and *L. gasseri* strains showed the highest removal ability ($p<0.05$). The other strains showed no significant difference ($p>0.05$) in the mean FB_1 removal (%) between the viable and heat-inactivated cells.

4 I DISCUSSION

Interest in biological control as an alternative method to conventional fungicides has been growing in recent years. LAB are considered promising biological control agents

because they are generally regarded as safe (GRAS) and several studies on natural fungal antagonists of lactic acid bacteria (LAB) have been reported (JUODEIKIENE *et al.*, 2018; TAROUB *et al.*, 2019). In the present study the antifungal activity of LAB strains was evaluated against *F. verticillioide*s strains. Among seven LAB strains, *L. plantarum* BG112 and *L. reuteri* LR-92 showed the highest antifungal activity (Table 1). These results are in line with those of Laitila *et al*. (2002), Lavermicocca *et al*. (2000), Oliveira *et al*. (2015) and Schmidt *et al*. (2018), who demonstrated the inhibitory activity of these LAB strains against some *Fusarium* species. Laitila *et al*. (2002) demonstrated the efficiency of *L. plantarum* E76 against *F. avenaceum*, *F. culmorum*, *F. oxysporum* and *F. graminearum*. Lavermicocca *et al*. (2000) evaluated the antifungal activity of *L. plantarum* 21B and reported inhibition against *Penicillium expansum* IDM/FS2, *Aspergillus flavus* FTDC3226, and *Fusarium graminearum* IDM623. Oliveira *et al*. (2015) and Schmidt *et al*. (2018) showed the strong inhibitory capacity of *L. reuteri* R29 against *F. culmorum*.

Antifungal activity of LAB (Tables 2 and 3) is probably associated with the production of lactic acid and acetic acid. These results are in accordance with those of Plocková *et al*. (2001), who reported that the antifungal activity of *L. reuteri* CCM3625 was associated with the production of a mixture of lactic acid (0.9% w/w), acetic acid (0.2% w/w) and succinic acid (0.2% w/w). In the same study, Plocková *et al*. (2001) evaluated the ability of *L. rhamnosus* VT1 and *L. reuteri* CCM3625 to control mold growth (*Fusarium* sp., *Aspergillus* sp. and *Penicillium* sp.). The most sensitive was *Fusarium* sp., whose growth was completely inhibited by both bacteria. Moreover, *L. reuteri* LR-92, which was used in this study, has been previously characterized as a lactic acid-, acetic acid-, succinic acid- and reuterin-producing strain (MAURO; GARCIA, 2019).

CFSs from *L. plantarum* BG112 and *L. reuteri* LR-92 inhibited growth (Table 4) and caused morphological alteration of *F. verticillioides* 97L conidia (Fig. 1C and 1D), indicating that antifungal activity is related to the production of bioactive compounds. Deepthi *et al*., (2016) also reported conidial and mycelial growth inhibition and alterations in the conidial morphology of *Fusarium proliferatum* after treatment with CFS from *Lactobacillus plantarum* MYS6. Similarly, CFS from *L. plantarum* MYS44 suppressed conidial germination and mycelial growth of *Aspergillus parasiticus*, in addition to inducing morphological changes and disruptions in the hyphal wall (POORNACHANDRA RAO *et al*., 2019). Growth inhibition and hyphal deformation of *Penicillium chrysogenum* was also observed in an experiment with *Lactobacillus casei* AST18 (LI *et al*., 2014). The results presented in this study (Fig. 1) demonstrate that the antifungal compounds produced by the selected lactic acid bacteria can alter *F. verticillioides* conidial and hyphal morphology. Conidial germination is a critical phase in phytopathogen development; therefore, the action of antifungal compounds at this stage can reduce fungal pathogenicity, because conidia are essential for asexual reproduction and fungal development (BORAH *et al*., 2016). Previous studies have shown that lactic acid bacteria can produce several antifungal compounds, including organic

acids such as lactic acid, acetic acid, phenyllactic acid and propionic acid (CROWLEY; MAHONY; VAN SINDEREN, 2013; LE LAY *et al.*, 2016), peptides (GUPTA; SRIVASTAVA, 2014), reuterin (SCHMIDT *et al.*, 2018), fatty acids and hydroxy fatty acids (BLACK *et al.*, 2013). The antifungal activity of LAB is a complex phenomenon because these bacteria are capable of producing different antifungal compounds that often act in synergy (LAITILA *et al.*, 2004).

Several researchers have reported the ability of LAB to detoxify mycotoxins, including fumonisins (TUPPIA *et al.*, 2016), aflatoxins (CHLEBICZ; ŚLIŻEWSKA, 2019), deoxynivalenol (DON) (FRANCO *et al.*, 2011), ochratoxin A (LUZ *et al.*, 2018), and patulin (HATAB; YUE; MOHAMAD, 2012).

The FB_1 removal percentages (Table 5) were higher than those reported by Pizzolitto, Salvano and Dalcero (2012), probably because these authors used lower cell concentrations (6.5×10^8 cell mL^{-1}) and different LAB species. Pizzolitto, Salvano and Dalcero (2012) evaluated the ability of *Lactobacillus acidophilus* 24 to remove FB_1 from PBS solution.

Niderkorn, Boudra, Morgavi (2006) evaluated the removal of DON and fumonisin B_1 and B_2 from MRS broth (pH 4.0) by twenty-nine LAB and propionic acid bacteria (PAB). Mycotoxin removal differed among the strains, and the highest values were obtained for DON (55%), FB_1 (82%) and FB_2 (100%). Binding was the probable mode of action because no toxin derivatives were detected, and the removal ability was not impaired in nonviable bacteria.

Niderkorn *et al*. (2009) investigated the cell wall components of lactic acid bacteria (LAB) and the functional groups of fumonisin (FB) involved in the LAB-FB interaction. The authors suggested that the peptidoglycan (PG) and tricarboxylic acid in LAB and FB, respectively, were the main components involved in the interaction. Other similar studies also indicated mycotoxin binding to the bacterial cell wall as the main mechanism involved, and the highest removal percentage was obtained using nonviable cells (HASKARD *et al.*, 2001; FRANCO *et al.*, 2011).

Heating of viable cells can cause protein denaturation or the formation of Maillard reaction products. Furthermore, heat treatment may decrease the peptidoglycan thickness and/or increase pore sizes. These changes would be responsible for mycotoxin binding to structures that were not available in the intact cell, therefore improving the detoxification process (EL-NEZAMI *et al.*, 2002; NIDERKORN, BOUDRA, MORGAVI, 2006).

The variability in the detoxification ability can be explained by differences in the molecular structure of peptidoglycans, which vary among bacterial species (NIDERKORN *et al.*, 2009). In this study, the FB_1 removal percentages (Table 5) were statistically different ($p<0.05$) among bacterial species, but strains of the same species (*L. plantarum*) showed similar results ($p>0.05$). Binding of mycotoxin by the bacterial cell wall is a possible mechanism involved in FB_1 removal.

5 | CONCLUSION

L. plantarum BG112 and *L. reuteri* LR-92 strains showed antifungal activity against toxigenic *F. verticillioides*. CFS from both bacteria inhibited conidial germination and mycelial growth and caused morphological changes, indicating that these strains are promising as biocontrol agents, since many commercial synthetic antifungals have similar effects. In addition, some LAB strains reduced the FB_1 concentration *in vitro*. These results demonstrate the potential of LAB for biological control of *F. verticillioides* to minimize fumonisin contamination, thus contributing to improved food safety.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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