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THE *IN VITRO* ANTIFUNGAL ACTIVITY OF *LACTOBACILLUS* SPP. AGAINST *ASPERGILLUS OCHRACEUS* GROWTH AND ITS OCHRATOXIN A PRODUCTION

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ABSTRACT

This study was aimed to monitor the inhibitory effect of selected species of the genus *Lactobacillus* on growth and ochratoxin A production of the microscopic filamentous fungus *Aspergillus ochraceus* (SLO-B-245). Nine species of the genus *Lactobacillus* were used in this study, namely *L. bifermentatus. L. perolens, L. reuteri, L. paracasei, L. paraplantarum, L. plantarum, L. salivarius, L. fructivorans* and *L. pentosus.* All strains were isolated from dairy products and their identification was performed by MALDI TOF MS (Bruker Daltonics, Germany, Maldi Biotyper). The antifungal activity was carried out using the overlay technique. Solid culture media inoculated with bacterial and fungal inocula were cultivated under aerobic conditions at 37 ºC for 8 days. Diameters (Ø mm) of the grown fungal colonies were measured every two days. To determine the inhibition of ochratoxin A production, the thin-layer chromatography method (TLC) was used. *L. plantarum* and *L. paraplantarum* were able to inhibit the growth of *A. ochraceus* (SLO-B-245) completely (100% inhibition), while *L. fructivorans* appeared to be the least effective (10.73%). Interestingly, the latter one was able to completely inhibit or partially suppress ochratoxin A production. We hope that our results can contribute to the search for harmless substances for the biological control of fungi in food. Lactobacilli and similar organisms could be very promising alternatives for food preservation.

Keywords: *Aspergillus*, ochratoxin A, antifungal activity, *Lactobacillus* spp., biopreservation

INTRODUCTION

Contamination of food, feed and other agricultural commodities by microscopic filamentous fungi leads to significant economic losses worldwide. Among *Aspergillus* species are one of the most common microscopic fungi causing degradation of stored food and feed. In addition, some species of the genus *Aspergillus* pose a major security risk due to their ability to produce mycotoxins **(Oliveira *et al.*, 2014)**. Some of them are toxic and may have carcinogenic, teratogenic or mutagenic properties that can cause various acute or chronic conditions in humans and animals. High incidence of contamination caused by microscopic filamentous fungi and their products - mycotoxins in food and feed, their ability to colonize different substrates and the lack of effectiveness of existing control measures require more effective action to be taken to eliminate these microorganisms **(Hassan *et al.*, 2016)**. At present, the food industry depends on chemical preservatives (such as benzoic, propionic, and sorbic acids), which can extend the shelf life of foods and control the growth of toxigenic fungi. On the other hand, there is a growing awareness of the health risks of consumers associated with the use of chemical preservatives **(Luz *et al.*, 2017)**. The demand for foods that do not contain these synthetic substances has also been increasing recently. In addition, the use of synthetic preservatives is beginning to be limited due to the resistance development in microscopic filamentous fungi **(Muhialdin *et al.*, 2020)**. In recent years, there has been a growing interest in research and development of natural antimicrobial agents capable of inhibiting the growth of spoilage fungi in food **(Ribes *et al.*, 2018)**. Replacing synthetic food chemicals by natural compounds promotes production of healthier food products that are protected against microscopic spoilage **(Gamba *et al.*, 2015; Hati *et al.*, 2018)**. One of the substitutions is the use of certain microorganism that must be non-toxic and easy to handle growth manifestations on simple media. Lactic acid bacteria (LAB) represent such an alternative as they are known to be a potential source of various secondary metabolites, such as bacteriocins, organic acids, fatty acids, protein compounds, cyclic dipeptides, hydrogen peroxide and other compounds **(Siedler *et al.*, 2019)**. These bioactive metabolites can be used to control pathogenic and food degrading microorganisms **(Muhialdin *et al.*, 2020)**. Several studies have reported interesting antifungal potential of some LABs strains **(Rouse *et al.*, 2008, Gerez *et al.*, 2009; Muhialdin *et al.*, 2016; Luz *et al.*, 2017; Coton *et al.*, 2018; Alaoui *et al.*, 2021; Abouloifa *et al.*, 2021)**, in addition, LAB are marked with GRAS status (Generally recognized as safe) and have been designed for use in a variety of food model systems including bread and yeast, dairy products, or in fruits and vegetables **(Abouloifa *et al.*, 2021)**. So, the aim of this study was to monitor the antifungal effect of selected types of lactobacilli on the growth of *Asperillus ochraceus* isolated from bread and its ability to produce ochratoxin A.

MATERIAL AND METHODS

Antagonistic microorganisms - identification, culture conditions and inoculum preparation

Overall, nine strains of the genus *Lactobacillus* were used in this study as antagonistic microorganisms, specifically *L. bifermentatus. L. perolens, L. reuteri, L. paracasei, L. paraplantarum, L. plantarum. L. salivarius, L. fructivorans* and *L. pentosus*. All strains were obtained previously by isolation from dairy products and their identification was performed by MALDI TOF MS (Bruker Daltonics, Germany, Maldi Biotyper) as described in **Hleba *et al.* (2017)**. All the strains were cultivated in MRS broth (HiMedia, India) under aerobic conditions at 37 °C for 48 hours in order to obtain bacterial suspensions for inoculums preparation. The inoculums containing 10^6 CFU/ml were prepared by diluting the lactobacilli suspensions in sterile phosphate saline and adjusting the density to 0.5 McFarland units by densitometer.

Fungal strain - identification, growth condition and inoculum preparation

Aspergillus ochraceus (SLO-B-245) was used to determine the inhibitory effect of lactobacilli. This strain was previously isolated from bread samples and identified as *A. ochraceus* ITS: MH517572 **(Císarová *et al.*, 2020)**. Prior to fungal inoculum preparation, *A. ochraceus* (SLO-B-245) was grown on Sabourad Dextrose Agar (SDA) (HiMedia, India) at 37 °C for 5-7 days in the dark. The grown colonies were resuspended in 1 ml of sterile phosphate saline containing Tween 80 and homogenized. Obtained suspension was diluted in sterile phosphate saline to a final concentration 2.5 x 10^6 CFU/ml by adjusting the density to 0.9 McFarland units.

Screening of *Lactobacillus* spp. antifungal activity in *in vitro* condition

The antifungal activity of the 9 lactobacilli strains was carried out using the overlay technique as described by **Magnusson and Schnürer (2001)** with some modifications. The lactobacilli strains were cultivated on MRS agar (HiMedia,

India) in Petri dishes (Ø 60 mm) . The sterile Whatman filter paper (Ø 12 mm) was placed in the middle of the prepared MRS agar plates and the lactobacilli inoculum (100 µl) was applied on it. Plates were incubated for 72 hours under aerobic conditions at 37 °C, subsequently overlaid with 2 ml of SDA medium and inoculated by 20 µl of *A. ochraceus* (SLO-B-245) inoculum (applied in the center of the SDA medium) (Fig. 1). Inoculated plates were cultivated under aerobic conditions at 37 ºC for 8 days. The control treatment contained sterile Whatman filter paper without bacterial inoculum. Diameters (Ø mm) of the grown colonies were measured every two days (on days 2, 4, 6, and 8 of cultivation) using a digital caliper. The obtained data was expressed as the percentage growth inhibition (PGI) according to the equation:

$$\text{Percentage growth inhibition (\%)} = \frac{rA2}{rB2}\ x\ 100$$

where rA = radius of the inhibition zone; rB = Petri dish radius.

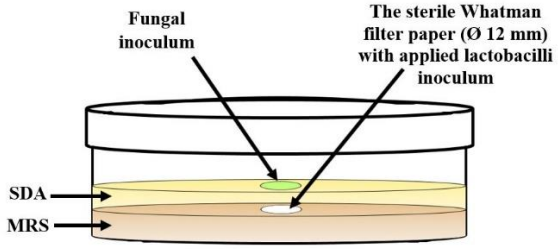

Figure 1 The scheme of used method (overlay technique) for tested antifungal activity of lactobacilli

Antitoxigenic effect of *Lactobacillus* spp.

After 8 days of cultivation, the ability of selected species of *Lactobacillus* spp. to affect the production of ochratoxin A (OTA) by *A. ochraceus* (SLO-B-245) strain was tested. Only lactobacilli that did not inhibit the growth of *A. ochraceus* (SLO-B-245) completely were used in this assay. The qualitative TLC (thin layer chromatography) method according to **Samson *et al*. (2002)**, described in **Hlebová *et al*. (2021)** was used. The obtained spots were compared with the relevant standard of tested mycotoxin (OTA) (Sigma-Aldrich, Germany) and visualization of ochratoxin A was performed under a UV lamp at 366 nm, where ochratoxin A appears as a blue fluorescent spot **(Samson *et al*., 2002)**.

Statistical evaluation

All experiments were performed in triplicates. The percentage of growth inhibition (PGI%) was calculated using the MS Office Excel 2016 program and the obtained averages of fungal colony treated by lactobacilli strains were evaluated by using the Stagraphics centurion XVI (version 16.1.11) (one-way ANOVA and Tukey HSD 95% multiple range test ($p<0.05$).

RESULTS AND DISCUSSION

Antifungal activity of the tested lactobacilli

Microscopic fungi are ubiquitous organisms that can grow almost everywhere, however their presence in goods results into the greatest losses in food industry. In food products they cause not only spoilage but also loss of nutrients, discoloration, and odor change, and, of course, they are able to produce some highly toxic secondary metabolites - mycotoxins **(Hassan *et al*., 2016)**. Nowadays the consumers concern about the safety of food has increased as well as the need for finding alternatives suitable for food preservation without the use of synthetic preservatives **(Luz *et al*., 2017; Luz et al., 2020)**. Lactic acid bacteria (LAB) attract much attention as they produce various substances with antifungal properties. LAB are considered as "green preservatives" because of their ability to inhibit the growth of microscopic fungi in food. Organic acids are still considered to be the major metabolites of LABs that dramatically affect fungal growth by inhibiting the mycelial growth. Organic acids in protonated or undissociated form are lipophilic and therefore easily diffuse across the fungal cell membrane and accumulate in the cytoplasm **(Sadiq *et al*., 2019)**.

In this study, the antifungal effect of nine lactobacilli strains (*L. bifermentatus. L. perolens, L. reuteri, L. paracasei, L. paraplantarum, L. plantarum. L. salivarius, L. fructivorans* and *L. pentosus*) was tested against ochratoxigenic fungus *A. ochraceus (*SLO-B-245). The results (Tab. 1) showed that all tested lactobacilli possess the ability to inhibit the growth of *A. ochraceus* (SLO-B-245) when compared to control sets. All studied strains caused significant differences ($P < 0.05$) on the mycelial growth of *A. ochraceus (*SLO-B-245). *L. plantarum* and *L. paraplantarum* were the most effective species of the set as they inhibited the growth completely (0.00 ± 0.00 mm; PGI 100%) after cultivation period (8 days). Similar results obtained **Luz *et al*. (2017).** They determined inhibition effect of the cell free supernatants (CFSs) containing peptides obtained from four strains of lactobacilli (*Lactobacillus rhamnosus* CECT 278T, *Lactobacillus johnsoni* CECT 289, *Lactobacillus plantarum* CECT 749, *Lactobacillus delbrueckii bulgaricus* CECT 4005) on the growth of *A. parasiticus* and *P. expansum*. CFS obtained by *Lactobacillus plantarum* showed the highest inhibition activity. High inhibitory effectiveness of *L. plantarum* S61 was published by **Abouloifa *et al*. (2021).** They observed high antifungal activity against tested molds (*Aspergillus niger, Penicillium* sp., *Fusarium oxysporum*, *Rhizopus* sp.) and yeasts (*Candida pelliculosa* and *Rhodotorula* sp.) in all tested lactobacilli strains (*L. plantarum, L. pentosus* and *L. brevis*). But strain *L. plantarum* S61 delayed the growth of *P. digitatum* for 15 days.

Table 1 The inhibition effect of lactobacilli on the growth of *Aspergillus ochraceus* (SLO-B-245) (mean colony diameter in mm±SD) on the SDA during cultivation period (2nd, 4th, 6th, and 8th cultivation day) at 37±1°C in the dark

Tested lactobacilli	Days of cultivation				Growth inhibition (%)
	2	4	6	8	
	mean colony diameter in mm±SD				
L. bifermentatus	10.19±0.16^{c}	17.19±2.49^{b}	23.86±3.38^{bc}	37.19±7.67^{b}	38.02
L. perolens	4.30±3.76^{abc}	17.26±9.12^{b}	27.26±9.12^{c}	37.26±9.12^{b}	38.23
L. reuteri	10.01±0.1^{bc}	18.43±2.42^{b}	25.10±3.68^{c}	35.10±3.68^{b}	41.50
L. paracasei	3.15±1.20^{ab}	9.82±3.69^{ab}	9.82±3.69^{a}	9.82±3.69^{a}	83.63
L. paraplantarum	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	100
L. plantarum	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	100
L. salivarius	5.31±1.12^{abc}	15.31±1.12^{b}	25.31±1.12^{c}	35.31±1.12^{b}	41.15
L. fructivorans	20.23±3.76^{d}	30.23±3.76^{c}	40.23±3.76^{d}	53.56±2.01^{c}	10.63
L. pentosus	2.18±2.15^{a}	11.81±2.72^{b}	11.81±2.72^{ab}	11.81±2.72^{a}	80.32
Control	10.37±4.48^{c}	36.53±3.02^{c}	46.53±7.35^{d}	59.67±0.57^{c}	NT

Legend: data in the column followed by different letters are significantly different in 95.0% Tukey HSD test, $P < 0.05$; SD – standard deviation; NT – not tested

According to our results, *L. paracasei* (3.15±1.20 mm; PGI 83.63%) and *L. pentosus* (2.18±2.15; PGI 80.32%) possess a strong inhibitory effect on the growth of *A. ochraceus* at the beginning of cultivation (Fig. 2). After 4 days of cultivation, the growth of *A. ochraceus* was stopped by the action of these two species compared to the control sets with the maximum colony diameter size of 9.82 mm when treated by *L. paracasei* and 11.81 mm in case of *L. pentosus*. Potent antifungal activities of *L. pentosus* (against *C. albicans, C. tropicalis* and *C. krusei*) were publishedby **Aarti et al. (2018)**. **Barrios-Roblero *et al.* (2019)** studied the antifungal and antisporulation activity of *Lactobacillus plantarum* (3 strains), *L. paracasei* (4 strains) and *L. pentosus* (3 strains) against the phytopathogen *Colletotrichum gloeosporioides*, which causes anthracnose in papaya. They found that all tested strains have a stronger inhibition effect on the germination of *C. gloeosporioides* by least 60% and completely inhibited its mycelial growth. Very good antifungal activity of *L. paracasei* (against *A. niger*) was observed by **Yujia *et al*. (2022)**. They also investigated the effect of growth substrate concentration on lactobacilli inhibition activity. They demonstrated that the concentration of 6.0% exhibited a higher inhibition of *A. niger*. Positive effect of increasing substrate concentration on the growth rate of microorganisms and the yield of bacteriocin was previously published by **Zacharof and Lovitt (2013)**. Bacterial growth and bacteriocin production was stopped when the concentration of the substrate decreased. So, the antifungal activity of lactobacilli is influenced by many factors.

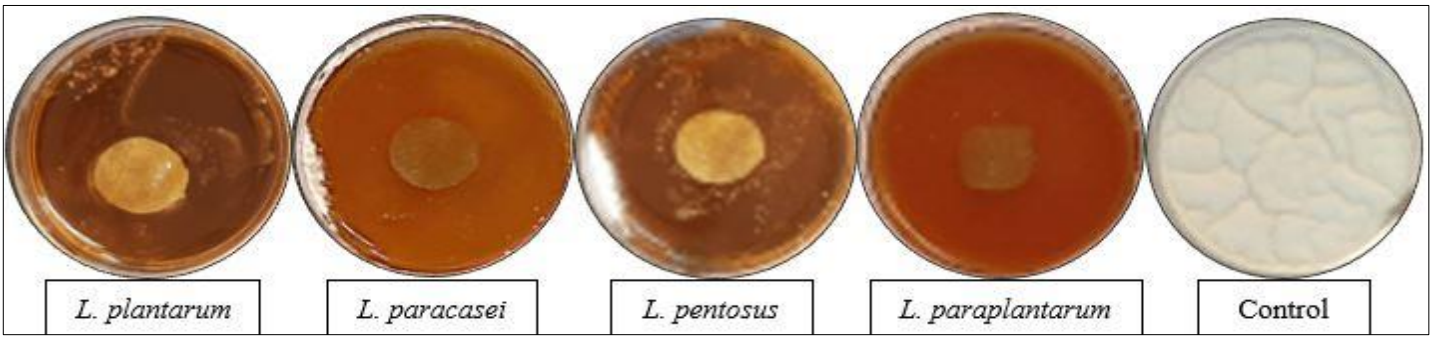

Figure 2 Inhibitory effect of some tested species of the genus *Lactobacillus* on the growth of *A. ochraceus* (SLO-B-245) after 8 days of cultivation in the dark at 37±1°C

The other tested lactobacilli had a moderate inhibitory effect on the growth of *A. ochraceus* with a PGI ranging from 38.02% (*L. bifermentatus*, *L. perolens*) to 41.50%. Interestingly, the species *L. salivarius* (5.31±1.12 mm) and *L. perolens* (4.30±3.76 mm) inhibited the growth of *A. ochraceus* most significantly till the second day of cultivation. Many studies reported a broad antifungal activity spectrum of these *Lactobacillus* species making them suitable for application in food industry **(Le Lay *et al.*, 2016; Schmidt *et al.*, 2018; Bakhski *et al.*, 2021; Chen *et al.*, 2021)**.

In our study, the least effective species was *L. fructivorans*, showing only 10.73% growth inhibition of *A. ochraceus* (SLO-B-245) at the end ofcultivation time. This strain even stimulated the growth of *A. ochraceus* (SLO-B-245) until the second day of cultivation compared to the control set (Fig. 3, Fig. 4). Similar results obtained **Inglin *et al.* (2015)** when testing 504 lactobacillus isolates including *L. fructivorans* against different bacteria and fungi strains. They found that *L. fructivorans* does not exhibit any inhibitory activity against both, bacteria, and fungi, respectively.

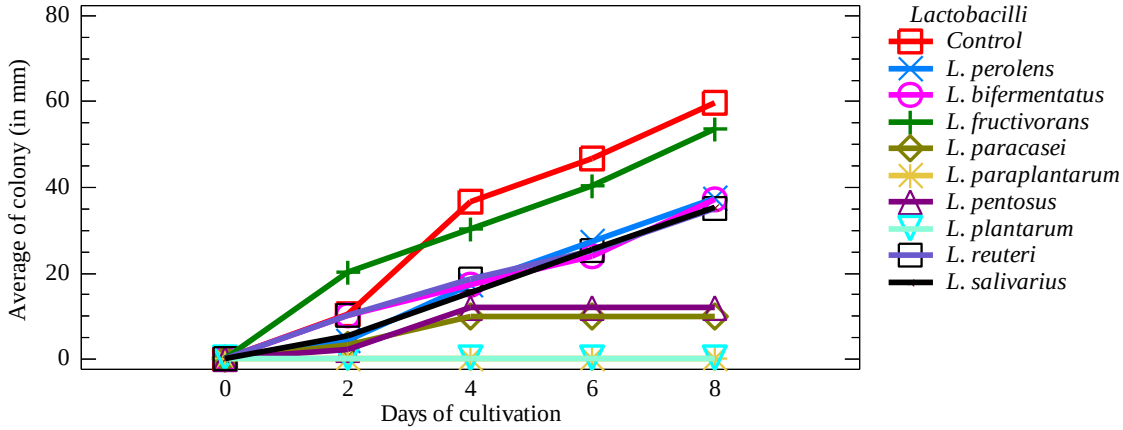

Figure 3 The antagonistic effect of the tested *Lactobacillus* spp. on the growth of *A. ochraceus* (SLO-B-245) (n=3) during 8 days of cultivation in the dark (in mm) at 37±1°C

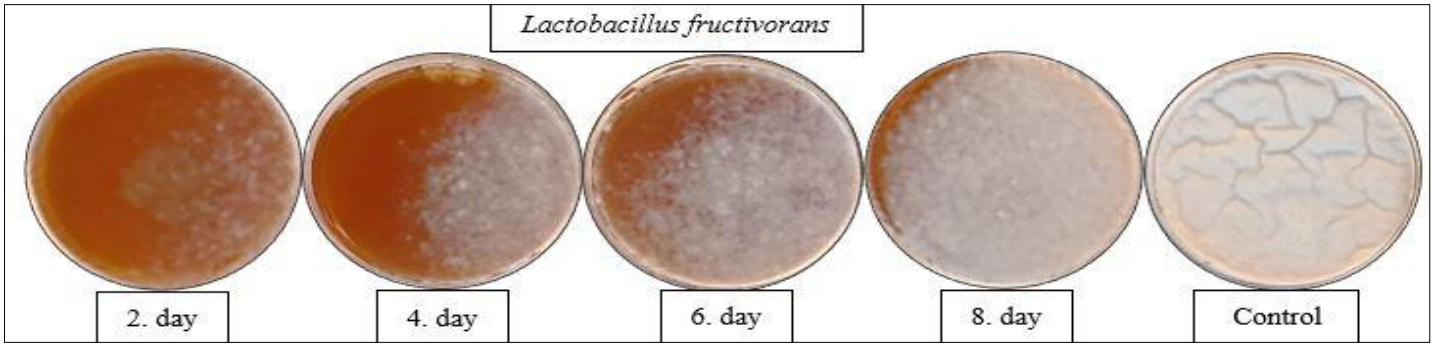

Figure 4 Effect of *Lactobacillus fructivorans* on the growth of *A. ochraceus* (SLO-B-245) during all cultivation days (2nd, 4th, 6th, and 8th day) in the dark at 37±1°C compared to the control

Inhibitory effect of *Lactobacillus* spp. on mycotoxin production by *A. ochraceus* (SLO-B-245)

Contamination of food by microscopic filamentous fungi and their toxins represents a fundamental safety risk for humans and animals **(Uzsáková and Hlebová, 2021)**. Therefore, in our work we also monitored the potential ability of lactobacilli to inhibit ochratoxin A production in *in vitro* conditions by using the TLC method. Only those colonies of *A. ochraceus* (SLO-B-245) were chosen for this assay, which were not completely inhibited by lactobacilli in previous experiment. The results showed that lactobacilli acted differently on the production of ochratoxin A (Tab. 2, Fig. 5). The most effective lactobacilli, inhibiting the production of ochratoxin A completely, showed to be the following species: *L. perolens*, *L. paracasei*, *L. pentosus* and *L. fructivorans*. *L. fructivorans* was able to completely suppress the production of ochratoxin A by *A. ochraceus* despite its inability of fungal growth inhibition. **Piotrowska and Zakowska (2005)** also observed the ability of five species of the genus *Lactobacillus* (*L. plantarum* BS, *L. brevis*, *L. acidophilus* CH–5, *L. rhamnosus* GG and *L. sanfranciscensis*) to inhibit the production of ochratoxin A. In their study the most effective species was *L. rhamnosus* GG, which was able to reduce the production of ochratoxin A by 89%. The inhibition of ochratoxin A production using lactobacilli was also monitored by **Li et al. (2021)**. Their results showed that *Lactobacillus brevis* 8–2B strain significantly inhibited the growth of *Aspergillus carbonarius* as well as its production of OTA.

Ragoubi et al. (2021) found out that the species *L. delbrueckii* and *L. acidophilus* were able to reduce not only OTA and aflatoxin B_1 production but also zearalenone and deoxynivalenol production by 30 to 57% except. Lactic acid bacteria can remove mycotoxins in two ways, either by adsorption through the cell wall or by mycotoxin molecules degradation resulting into less toxic compounds. From safety point of view, adsorption is considered to be a more effective method as the degradation process can create some even more toxic metabolites than the original mycotoxins. Furthermore, various studies have proven that the complex mycotoxin–the cell wall of BMK shows reduced rate of adhesion to mucous membranes **(Vinderola and Ritieni, 2015)**. In addition to antifungal and

antitoxinogenic properties of lactic acid bacteria, which include, for example, the genera *Lactobacillus* or *Bifidobacterium*, there are many other beneficial effects possessed by these bacteria, boosting consumers' health **(Uzsáková and Hlebová, 2021)**.

Table 2 Effect of *Lactobacillus* spp. on the ochratoxin A biosynthesis in tested *A. ochraceus* (SLO-B-245) (n = 3) after treatment

Tested lactobacilli	OA
L. bifermentatus	2/3
L. perolens	0/3
L. reuteri	2/3
L. paracasei	0/3
L. paraplantarum	nt
L. plantarum	nt
L. salivarius	3/3
L. fructivorans	0/3
L. pentosus	0/3
Control	3/3

Legend: nt – not tested (mycelial growth was completely inhibited by lactobacilli), OA – ochratoxin A, ** - number of mycotoxins produced isolates, * - number of tested isolates

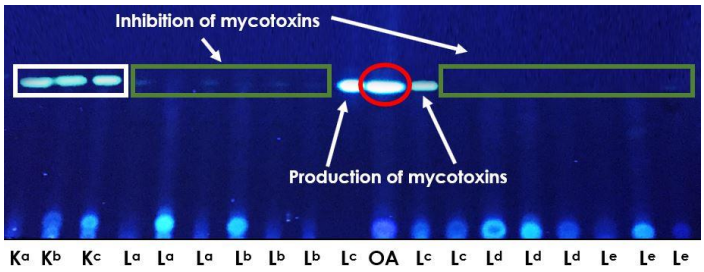

Figure 5 The inhibitory effect of some species of lactobacilli on the production of ochratoxin A by the strain *A. ochraceus* (SLO-B-245) tested by the TLC method under UV light 366 nm; K^{abc} – Control, L^{a} – *L. perolens*, L^{b} – *L. fructivorans*, L^{c} – *L. reuteri*, L^{d} – *L. paracasei*, L^{e} - L. *pentosus*, * - repetitions

CONCLUSION

Results obtained in this study proved the antagonist effect of LAB (lactobacilli isolated from dairy products) against filamentous fungus *A. ochraceus* (SLO-B-245) as well as the ability to inhibit or suppress its ochratoxin A production. Antifungal activity assay revealed that two species were able to inhibit the growth of *A. ochraceus* (SLO-B-245) completely (100%) during all days of cultivation (8 days), namely *L. plantarum* and *L. paraplantarum*. The least effective species was *L. fructivorans* possessing no inhibitory effect on the growth of the microscopic fungus compared to the control; however, it was able to completely inhibit the production of ochratoxin A. The production of ochratoxin A was completely inhibited also by the species *L. perolens*, *L. paracasei* and *L. pentosus*. The second and the third species showed at the same time a high rate of growth inhibition of *A. ochraceus* (SLO-B-245). Our study revealed some lactobacilli strains with promising properties such as antifungal activity and ability to inhibit/suppress mycotoxin production. In conclusion, our results emphasize the importance of research on lactic acid bacteria due to their application potential in food protection and biopreservation as well as biological control agents in food against microscopic filamentous fungi and their mycotoxins.

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REFERENCES

Aarti, C., Khusro, A., Varghese, R., Arasu, M. V., Agastian, P., Al-Dhabi, N. A., ... & Choi, K. C. (2018). In vitro investigation on probiotic, anti-Candida, and antibiofilm properties of Lactobacillus pentosus strain LAP1. *Archives of Oral Biology*, *89*, 99-106. https://doi.org/10.1016/j.archoralbio.2018.02.014

Abouloifa, H., Gaamouche, S., Rokni, Y., Hasnaoui, I., Bellaouchi, R., Ghabbour, N., ... & Asehraou, A. (2021). Antifungal activity of probiotic Lactobacillus strains isolated from natural fermented green olives and their application as food bio-preservative. *Biological Control*, *152*, 104450. https://doi.org/10.1016/j.biocontrol.2020.104450

Alaoui, K., Chafik, Z., Arabi, M., Abouloifa, H., Asehraou, A., Chaoui, J., & Kharmach, E. Z. (2021). In vitro antifungal activity of Lactobacillus against potato Late blight Phytophthora infestans. Materials Today: Proceedings, 45, 7725-7733. https://doi.org/10.1016/j.matpr.2021.03.338

Bakhshi, M., Salari, S., Almani, P. G. N., & Afshari, S. A. K. (2021). Evaluation of the antifungal activity of Lactobacillus reuteri against Candida species. *Gene Reports*, *25*, 101369. https://doi.org/10.1016/j.genrep.2021.101369

Barrios-Roblero, C., Rosas-Quijano, R., Salvador-Figueroa, M., Gálvez-López, D., & Vázquez-Ovando, A. (2019). Antifungal lactic acid bacteria isolated from fermented beverages with activity against Colletotrichum gloeosporioides. *Food Bioscience*, *29*, 47-54. https://doi.org/10.1016/j.fbio.2019.03.008

Císarová, M., Hleba, L., Medo, J., Tančinová, D., Mašková, Z., Čuboň, J., ... & Klouček, P. (2020). The in vitro and in situ effect of selected essential oils in vapour phase against bread spoilage toxicogenic aspergilli. *Food Control*, *110*, 107007. https://doi.org/10.1016/j.foodcont.2019.107007

Coton, M., Lebreton, M., Salas, M. L., Garnier, L., Navarri, M., Pawtowski, A., ... & Mounier, J. (2018). Biogenic amine and antibiotic resistance profiles determined for lactic acid bacteria and a propionibacterium prior to use as antifungal bioprotective cultures. *International Dairy Journal*, *85*, 21-26. https://doi.org/10.1016/j.idairyj.2018.05.001

Gamba, R. R., Caro, C. A., Martínez, O. L., Moretti, A. F., Giannuzzi, L., De Antoni, G. L., & Peláez, A. L. (2016). Antifungal effect of kefir fermented milk and shelf life improvement of corn arepas. *International Journal of Food Microbiology*, *235*, 85-92. https://doi.org/10.1016/j.ijfoodmicro.2016.06.038

Gerez, C. L., Torino, M. I., Rollán, G., & de Valdez, G. F. (2009). Prevention of bread mould spoilage by using lactic acid bacteria with antifungal properties. *Food control*, *20*(2), 144-148. https://doi.org/10.1016/j.foodcont.2008.03.005

Hassan, Y. I., Zhou, T., & Bullerman, L. B. (2016). Sourdough lactic acid bacteria as antifungal and mycotoxin-controlling agents. *Food Science and Technology International*, *22*(1), 79-90. https://doi.org/10.1177/1082013214565722

Hati, S., Patel, N., Sakure, A., & Mandal, S. (2018). Influence of whey protein concentrate on the production of antibacterial peptides derived from fermented milk by lactic acid bacteria. *International Journal of Peptide Research and Therapeutics*, *24*(1), 87-98. https://doi.org/10.1007/s10989-017-9596-2

Hleba, L., Kmeť, V., Tóth, T., & Kačániová, M. (2017). Resistance in bacteria and indirect beta-lactamase detection in E. coli isolated from Culex pipiens detected by matrix-assisted laser desorption ionization time of flight mass spectrometry. *Journal of Environmental Science and Health, Part B*, *52*(1), 64-69. https://doi.org/10.1080/03601234.2016.1229466

Hlebová, M., Hleba, L., Medo, J., Uzsakova, V., Kloucek, P., Bozik, M., ... & Čuboň, J. (2021). Antifungal and antitoxigenic effects of selected essential oils in vapors on green coffee beans with impact on consumer acceptability. *Foods*, *10*(12), 2993. https://doi.org/10.3390/foods10122993

Chen, G., Chen, D., Zhou, W., Peng, Y., Chen, C., Shen, W., ... & Yuan, Q. (2021). Improvement of metabolic Syndrome in High-Fat Diet-Induced Mice by Yeast β-Glucan Is Linked to Inhibited Proliferation of Lactobacillus and Lactococcus in gut microbiota. *Journal of agricultural and food chemistry*, *69*(27), 7581-7592. https://doi.org/10.1021/acs.jafc.1c00866

Inglin, R. C., Stevens, M. J., Meile, L., Lacroix, C., & Meile, L. (2015). High-throughput screening assays for antibacterial and antifungal activities of Lactobacillus species. *Journal of microbiological methods*, *114*, 26-29. https://doi.org/10.1016/j.mimet.2015.04.011

Le Lay, C., Mounier, J., Vasseur, V., Weill, A., Le Blay, G., Barbier, G., & Coton, E. (2016). In vitro and in situ screening of lactic acid bacteria and propionibacteria antifungal activities against bakery product spoilage molds. *Food Control*, *60*, 247-255. https://doi.org/10.1016/j.foodcont.2015.07.034

Li, L., Yang, B., Humza, M., Geng, H., Wang, G., Zhang, C., ... & Liu, Y. (2021). A novel strain Lactobacillus brevis 8-2B inhibiting Aspergillus carbonarius growth and ochratoxin A production. *LWT*, *136*, 110308. https://doi.org/10.1016/j.lwt.2020.110308

Luz, C., Izzo, L., Ritieni, A., Mañes, J., & Meca, G. (2020). Antifungal and antimycotoxigenic activity of hydrolyzed goat whey on Penicillium spp: An application as biopreservation agent in pita bread. *Lwt*, *118*, 108717. https://doi.org/10.1016/j.lwt.2019.108717

Luz, C., Saladino, F., Luciano, F. B., Mañes, J., & Meca, G. (2017). In vitro antifungal activity of bioactive peptides produced by Lactobacillus plantarum against Aspergillus parasiticus and Penicillium expansum. *LWT-Food Science and Technology*, *81*, 128-135. https://doi.org/10.1016/j.lwt.2017.03.053

Magnusson, J., & Schnürer, J. (2001). Lactobacillus coryniformis subsp. coryniformis strain Si3 produces a broad-spectrum proteinaceous antifungal compound. *Applied and environmental microbiology*, *67*(1), 1-5. https://doi.org/10.1128/aem.67.1.1-5.2001

Muhialdin, B. J., Algboory, H. L., Kadum, H., Mohammed, N. K., Saari, N., Hassan, Z., & Hussin, A. S. M. (2020). Antifungal activity determination for the peptides generated by Lactobacillus plantarum TE10 against Aspergillus flavus in maize seeds. *Food Control*, *109*, 106898. https://doi.org/10.1016/j.foodcont.2019.106898

Muhialdin, B. J., Hassan, Z., Bakar, F. A., & Saari, N. (2016). Identification of antifungal peptides produced by Lactobacillus plantarum IS10 grown in the MRS broth. *Food Control*, *59*, 27-30. https://doi.org/10.1016/j.foodcont.2015.05.022

Oliveira, P. M., Zannini, E., & Arendt, E. K. (2014). Cereal fungal infection, mycotoxins, and lactic acid bacteria mediated bioprotection: From crop farming to

cereal products. *Food microbiology*, *37*, 78-95. https://doi.org/10.1016/j.fm.2013.06.003

Piotrowska, M., & Zakowska, Z. (2005). The elimination of ochratoxin A by lactic acid bacteria strains. *Polish Journal of Microbiology*, *54*(4), 279-286.

Ragoubi, C., Quintieri, L., Greco, D., Mehrez, A., Maatouk, I., D'Ascanio, V., ... & Avantaggiato, G. (2021). Mycotoxin removal by Lactobacillus spp. and their application in animal liquid feed. *Toxins*, *13*(3), 185. https://doi.org/10.3390/toxins13030185

Ribes, S., Fuentes, A., Talens, P., & Barat, J. M. (2018). Prevention of fungal spoilage in food products using natural compounds: a review. *Critical reviews in food science and nutrition*, *58*(12), 2002-2016. https://doi.org/10.1080/10408398.2017.1295017

Rouse, S., Harnett, D., Vaughan, A., & Sinderen, D. V. (2008). Lactic acid bacteria with potential to eliminate fungal spoilage in foods. *Journal of Applied Microbiology*, *104*(3), 915-923. https://doi.org/10.1111/j.1365-2672.2007.03619.x

Sadiq, F. A., Yan, B., Tian, F., Zhao, J., Zhang, H., & Chen, W. (2019). Lactic acid bacteria as antifungal and anti-mycotoxigenic agents: a comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, *18*(5), 1403-1436. https://doi.org/10.1111/1541-4337.12481

Samson, R. A., Hoekstra, E. S., Lund, F., Filtenborg, O., & Frisvad, J. C. (2004). Methods for the detection, isolation and characterisation of food-borne fungi. *Introduction to food-and airborne fungi*, (Ed. 7), 283-297. https://doi.org/10.1007/978-1-4684-8453-3_8

Schmidt, M., Lynch, K. M., Zannini, E., & Arendt, E. K. (2018). Fundamental study on the improvement of the antifungal activity of Lactobacillus reuteri R29 through increased production of phenyllactic acid and reuterin. *Food Control*, *88*, 139-148. https://doi.org/10.1016/j.foodcont.2017.11.041

Siedler, S., Balti, R., & Neves, A. R. (2019). Bioprotective mechanisms of lactic acid bacteria against fungal spoilage of food. *Current opinion in biotechnology*, *56*, 138-146. https://doi.org/10.1016/j.copbio.2018.11.015

Uzsáková, V., Hlebová, M. (2021) Antifungálny a antitoxinogénny potenciál baktérií mliečneho kvasenia. In: SciCell Magazín – Odborný online magazín, 2021, ISSN 2585–9137

Vinderola, C. G., & Ritieni, A. (2014). Role of probiotics against mycotoxins and their deleterious effects. *Journal of Food Research* (4), 1-10. https://doi.org/10.5539/jfr.v4n1p10

Yujia, X. I. E., Peng, X., Li, Z., Wang, J., Li, M., Xiao, S., & Zhang, S. (2022). Production and fermentation characteristics of antifungal peptides by synergistic interactions with Lactobacillus paracasei and Propionibacterium freudenii in supplemented whey protein formulations. *LWT*, 113632. https://doi.org/10.1016/j.lwt.2022.113632

Zacharof, M. P., & Lovitt, R. W. (2013). Low molecular weight liquid media development for Lactobacilli producing bacteriocins. *Journal of Chemical Technology & Biotechnology*, *88*(1), 72-80. https://doi.org/10.1002/jctb.3892