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The efficiency of lactic acid bacteria against pathogenic fungi and mycotoxins

Adam Perczak[1], Piotr Goliński[1], Marcin Bryła[2], and Agnieszka Waśkiewicz[1]

Department of Chemistry, Poznan University of Life Sciences, Poznań[1], Department of Food Analysis, Prof. Waclaw Dabrowski Institute of Agricultural and Food Biotechnology, Warsaw[2], Poland

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Mycotoxins are produced by some fungal species of the genera *Aspergillus*, *Penicillium*, and *Fusarium* and are common contaminants of a wide range of food commodities. Numerous strategies are used to minimise fungal growth and mycotoxin contamination throughout the food chain. This review addresses the use of lactic acid bacteria, which can inhibit fungal growth and participate in mycotoxin degradation and/or removal from contaminated food. Being beneficial for human and animal health, lactic acid bacteria have established themselves as an excellent solution to the problem of mycotoxin contamination, yet in practice their application in removing mycotoxins remains a challenge to be addressed by future research.

KEY WORDS: Aspergillus*; biological methods;* Fusarium*; inhibition; LAB;* Penicillium

Mycotoxin contamination of feed and food is a significant issue worldwide. Mycotoxins are a large group of secondary metabolites produced by the *Aspergillus*, *Penicillium*, and *Fusarium* genera, and pose serious risks for human and animal health (1-4). Fungal growth and mycotoxin production may occur in the field and/or during storage, if the temperature and humidity are favourable (4-8).

The main sources of mycotoxins are cereal grains (including wheat, barley, oats, corn, and rice) and their products, nuts, almonds, fruits, coffee, spices, and legumes (5, 6, 9-11) (Table 1).

Table 1 *The main sources of mycotoxins*

Mycotoxin	Species of fungi	Main source	References
Aflatoxins	*Aspergillus flavus, Aspergillus parasiticus*	peanuts, oilseed cereals, cow's milk, sorghum, spices	(12-18)
Ochratoxins	*Aspergillus ochraceus, A. sulphureus, Penicillum verucosum*	cereal grain storage, feed, grapes, wine, coffee	(19-23)
Trichothecenes	*Fusarium poae, F. sporotrichioides, F. acuminatum, F. equiseti*	wheat, barley, maize, oat, buckwheat, sorghum, feed	(1, 24-34)
Zearalenone	*Fusarium graminearum, F. culmorum, F. cerealis*	maize, wheat, barley, oat, sorghum, rice, pea, feed	(1, 26, 35-40)
Fumonisins	*Fusarium verticillioides, F. proliferatum*	maize and other cereals and their products, asparagus	(41-44)
Patulin	*Penicillum expansum, P. cyclopium*	apples, pears, apple juice	(45-47)

Correspondence to: Agnieszka Waśkiewicz, Department of Chemistry, Poznan University of Life Sciences, Wojska Polskiego 75, Poznań, Poland, E-mail: *agat@up.poznan.pl*

As they enter the food chain, mycotoxins can also contaminate eggs, milk, and meat and accumulate in different organs or tissues (9, 11, 48).

Long-term exposure to mycotoxins has also been associated with carcinogenic, mutagenic, teratogenic, oestrogenic, haemorrhagic, immunotoxic, nephrotoxic, hepatotoxic, neurotoxic, and immunosuppressive adverse health effects (48-51).

Because of these risks, the EU has set down limits for several important mycotoxins in food and feed: aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FBs), zearalenone (ZEA), trichothecenes [principally deoxynivalenol (DON), T-2, and HT-2 toxins], and patulin (PAT) (9).

A number of studies have investigated the options of eliminating these compounds (52-54), and arrived at the conclusion that the best way to solve the problem is to prevent mycotoxin formation. To do that, the following rules need to be observed: (i) plant materials should be stored in a cold and dry environment, (ii) plants should be harvested without delay, (iii) crops should be rotated in the field to prevent adaptation of pathogenic microorganisms to a specific monoculture, and (iv) agricultural crops should be handled carefully to prevent mechanical damage, which renders them vulnerable to contamination (52, 53, 55).

There where prevention fails, chemical, physical, or biological methods of detoxification step in (56-58). Some of these methods, such as the use of ozone (59-62), alkaline hydrogen peroxide (63), or gamma irradiation (64, 65) have more promising results than ammoniation (66) or heat treatment (67-69).

However, contamination with several mycotoxins at the same time lessens the efficiency of detoxification, as some mycotoxins are less sensitive to the method than others.

Recently, a new approach to the removal of mycotoxins emerged, and microorganisms such as propionic fermentation bacterium *Saccharomyces cerevisiae*, and lactic acid bacteria (LAB) have come into focus. This article reviews the current uses of the latter as promising probiotics in mycotoxin removal.

Lactic acid bacteria

Lactic acid bacteria (LAB) are Gram-positive, nonsporulating, air and acid tolerant, organotrophic, fermentative rods or cocci producing lactic acid. They do not use oxygen as an electron acceptor. Not possessing catalase, they synthesise superoxide dismutase, removing reactive oxygen species. All the lactic acid bacteria are anaerobic, while some of them tolerate low levels of oxygen in the environment. Currently, only a few are considered to be probiotic, and, together with prebiotics, these have been used in nutrition and treatment of people and farm animals, such as pigs. When homofermentative, LAB ferment 85 % of glucose into lactic acid. In heterofermentation (70) the yield is 50 % plus ethanol and CO_2.

There are a variety of industrially important genera, including *Lactococcus, Enterococcus, Oenococcus, Pediococcus, Streptococcus, Leuconostoc*, and *Lactobacillus* species (71). Yet, LAB have been used to preserve food and beverages since the beginnings of agriculture (72). Different strains of LAB have been passed down from generation to generation through culinary traditions and fermented food. Currently, LAB play a significant role in the world food production, performing major bioconversions in fermented dairy products, vegetables, and meat. They are also essential for the production of silage, coffee, wine, cocoa, sourdough, and many indigenous fermented foods (73-75). LAB improve flavour, texture, and shelf-life of food products (76).

LAB inhibit fungal growth

Lactic acid bacteria have the ability to control the growth of various fungi. Inhibition of toxigenic fungi has been demonstrated many times over (77-81). Generally, this antagonistic effect is owed to low-molecular-weight compounds produced by the LAB, such as organic acids (acetic and lactic acid), hydrogen peroxide, proteinaceous compounds, reuterin, hydroxyl fatty acids, and phenolic compounds (Table 2). Organic acids can be native to food or added to it. They are products of carbohydrate metabolism and are safe to use for food preservation. Lactic acid lowers pH, which inhibits the growth of various microorganisms or even kills susceptible bacteria (89). In heterofermentation, LAB can produce acetic acid and trace amounts of propionic acid, both of which have a higher content of undissociated forms at a given pH of the lactic acid. In addition to their effect on the fungus membrane, they also inhibit the absorption of amino acids (89). Low pH also increases the antifungal activity of various salts of propionic acid (90). A particularly interesting component involved in the inhibition of fungal growth is reuterin, a compound of glycerol fermentation produced by various LAB genera under anaerobic conditions (91). Reuterin suppresses the activity of ribonuclease, the enzyme involved in the biosynthesis of DNA (98). It inhibits the growth of the *Fusarium* and *Aspergillus* species. Therefore, to enhance these effects, simply add glycerol to LAB cultures.

Lactic acid bacteria can produce various types of fatty acids that improve the sensory quality of fermented products. One such fatty acid, caproic acid, has a strong antifungal activity. It may be synergistic with propionic, butyric, or valeric acids (92).

The best period of incubation to inhibit the growth of toxin-forming fungi is about 48 h and the best temperature is from 25 to 30 °C (93). These conditions favour the production of organic acids, which in turn, inhibit the growth of pathogenic fungi.

Table 2 *Antifungal compounds produced by LAB*

LAB	Compounds	References
Lactobacillus reuteri	Reuterin	(82)
L. plantarum	Peptids – cyclo (Leu-Pro), cyclo (Phe-Pro)	(83)
Pediococcus acidilactici	Phenolic compounds	(84)
L. reuteri	Acetic acid, phenyllactic acid	(85)
L. plantarum	3-phenyllactic acid	(86)
L. paracasei subsp. paracasei	Lactic acid, propionic acid, acetic acid, succinic acid, hydroxyphenyllactic acid, 3-phenyllactic acid	(86)
L. plantarum	3,6-bis(2-methylpropyl)-2,5-piperazinedion	(87)
L. plantarum	2-hydroxy-4 methylpentanoic acid	(77)

Removal of mycotoxins with the LAB

Numerous studies have demonstrated that many LAB species can remove mycotoxins. Removal efficiency ranges from small amounts to almost complete removal (94-99). The most efficient species are *Lactobacillus rhamnosus, L. acidophilus, L. plantarum, L. lactis, Streptococcus thermophilus*, and *Bifidobacterium bifidum.* Each species acts differently and on different mycotoxins. The most versatile seems to be *L. rhamnosus*, which efficiently removes several mycotoxins at once (96, 100-103). Reduction is even higher at pH 4 (100). Other crucial parameters include LAB cell viability and mycotoxin concentrations (104). There are several mechanisms of removal, but the most efficient is binding to the bacterial cells (105). LAB cell surfaces bind various molecules such as toxins and metal ions (106, 107). Their cell walls contain peptidoglycan matrices, neutral polysaccharides, teichoic and lipoteichoic acid, and a protein S layer. However, binding is based on the adsorption capacity of mycotoxins to the cells and not on enzyme activity. This is where peptidoglycan and exopolysaccharides play an important role (108). In fact, thermally inactivated LAB exhibit higher removal capacity, due to changes on the cell surface. Mycotoxin binding is permanent only if the LAB are dead, whereas the living bacteria may release some of the mycotoxin content with time (109). Bueno et al. (110) proposed a mathematical model to illustrate the attachment of AFB_1 to LAB and *S. cerevisiae*, taking into account two processes: adsorption and desorption. This model shows that AFB_1 binds to a number of sites in LAB.

Another method of mycotoxin removal is adhesion (111). Its efficiency correlates with the bacterial concentration, but some of the toxin content is released with time and is therefore not permanent.

Table 3 lists the LAB that can remove mycotoxins.

Aflatoxins

This group of compounds is formed mainly by the species *Aspergillus flavus* and *A. parasiticus*, commonly found in soil and in stored agricultural produce (137-139). *A. parasiticus* often contaminates oilseeds and produces B_1, B_2, G_1, and G_2 aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2), while *A. flavus* is usually found on cereals and produces only the B_1 and B_2 aflatoxins (13, 18) (Table 1). AFB_1 in dairy mammal feed is strongly associated with aflatoxin M_1 (AFM_1) in milk (104). Other, less common species include *A. nomius*, *A. toxicarius, A. tamarii, A. pseudotamari*, and *A. bombycids* (15, 140, 141).

Conditions favouring aflatoxin production are humidity above 13 % and temperature between 24 and 37 °C (142), which are mostly encountered in the countries with subtropical and tropical climates (143-145). In recent years, aflatoxins in maize have also been reported in southern Europe. This is probably due to climate change and adaptive abilities of the *Aspergillus* spp. (146).

Agricultural commodities get contaminated with aflatoxigenic fungi before and at the harvest, processing, transport, and storage (147), especially peanuts, cereals, and their products (148, 149), as well as animal feeds (16, 150-152).

Motameny et al. (117) investigated the removal of AFB_1 from a gastrointestinal model with *L. rhamnosus, L. plantarum*, and *L. acidophilus* and found that *L. plantarum* was the most successful (28 %), followed by *L. acidophilus* (22 %) and *L. rhamosus* (18 %). Elsanhoty et al. (120) compared the ability of viable and heat-treated *L. acidophilus*, *L. rhamnosus*, *L. sanfranciscensis,* and *Bifidobacterium angulatum* to remove AFBs (AFB_1, AFB_2, AFG_1, and AFG_2) from PBS liquid medium. Among the four tested strains, *L. rhamnosus* was the most efficient in the initial binding of all these aflatoxins and confirmed superior efficiency after 4 washes, which suggests that it forms the most stable complexes with these aflatoxins.

Hernandez-Mendoza et al. (124) studied the binding of AFB_1 by *Lactobacillus reuteri* and *L. casei* at different pH (6, 7.2, and 8) and incubation time (0, 4, and 12 h). Both strains showed the highest AFB_1-binding capacity at pH 7.2 after 4 and 12 h of incubation (67.8 and 55.6 % for *L. casei* and 80 and 80 % for *L. reuteri*, respectively).

Corassin et al. (112) compared the AFB_1-binding ability of *L. delbrueckii* spp. *bulgaricus, L. rhamnosus, and B. lactis* in combination with heat-killed *S. cerevisiae*. This combination ensured complete mycotoxin binding (100 %).

Khoury et al. (113) compared the AFM_1-binding efficiency of *L. bulgaricus* and *S. thermophilus* in PBS. *L.*

Table 3 *The ability of lactic acid bacteria to reduction of mycotoxins*

Mycotoxins	Bacteria	Matrices	References
Aflatoxin	*Lactobacillus bulgaricus*	phosphate buffer saline, skim milk; UHT skim milk; milk and yogurt	(94) (112) (113)
	Lactobacillus plantarum	phosphate buffer saline, skim milk; silage extract medium; phosphate buffer saline; ruminant gastrointestinal model; maize grain;	(94) (114) (115, 116) (117) (118)
	Lactobacillus gasseri	phosphate buffer saline, skim milk	(94)
	Lactobacillus rhamnosus	phosphate buffer saline, skim milk; UHT skim milk; silage extract medium; phosphate buffer saline, dough, baladi bread; *in vitro* digestion model; phosphate buffer saline; ruminant gastrointestinal model; MRS broth	(94, 119) (112) (114) (120) (121) (122) (117) (100)
	Lactobacillus casei	phosphate buffer saline; female rats; maize grain; *in vitro* digestion model;	(115, 123, 124) (125) (118) (121)
	Lactobacillus fermentum	phosphate buffer saline;	(115, 122)
	Lactobacillus acidophilus	*in vitro* digestion model; ruminant gastrointestinal model; maize grain; phosphate buffer saline, skim milk	(121) (117) (118) (119)
	Lactobacillus brevis	maize grain	(118)
	Lactobacillus delbruekki	maize grain	(118)
	Lactobacillus reuteri	female rats; phosphate buffer saline; phosphate buffer saline, skim milk	(125) (124) (119)
	Lactobacillus johnsonii	phosphate buffer saline; phosphate buffer saline, skim milk	(124) (119)
	Lactobacillus sanfranciscenis	phosphate buffer saline, dough, baladi bread	(120)
	Lactococcus lactis	LAPTg medium; phosphate buffered saline	(126) (116)
	Streptococcus thermophilus	milk and yogurt	(113)
	Enterococcus avium	Phosphate buffer saline, skim milk	(94)
	Enterococcus faecium	LAPTg medium; phosphate buffer saline	(126) (127)
	Pediococcus pentosaceus	phosphate buffer saline, skim milk; phosphate buffer saline	(94) (122)
	Bifidobacterium lactis	phosphate buffer saline, skim milk; UHT skim milk	(94) (112)
	Bifidobacterium bifidum	phosphate buffer saline; phosphate buffer saline, skim milk	(124) (119)
	Bifidobacterium longum	*in vitro* digestion model	(121)
	Bifidobacterium angulatum	phosphate buffer saline, dough, baladi bread	(120)
Ochratoxin A	*Leuconostoc mesenteroides*	MRS agar, PDA agar, coffee meal extract agar	(95)
	Lactobacillus brevis	MRS agar, PDA agar, coffee meal extract agar	(95)
	Lactobacillus plantarum	sodium phosphate buffer; MRS agar, PDA agar, coffee meal extract agar	(128) (95)
	Lactobacillus helveticus	MRS medium	(102)

Mycotoxins	Bacteria	Matrices	References
Ochratoxin A	*Lactobacillus bulgaricus*	sodium phosphate buffer; MRS medium; dried skim milk	(128) (102) (129)
	Lactobacillus casei	yeast medium, MRS broth; MRS medium	(130) (102)
	Lactobacillus lactis	sodium phosphate buffer; MRS medium	(128) (102)
	Lactobacillus plantarum	sourdough MRS medium	(131) (102)
	Lactobacillus brevis	sourdough; MRS medium	(131) (102)
	Lactobacillus rhamnosus	MRS medium	(102)
	Lactobacillus sanfrancisco	sourdough	(131)
	Lactobacillus sanfransciscensis	MRS medium	(102)
	Lactobacillus sakei	yeast medium, MRS broth	(130)
	Lactobacillus acidophillus	sodium phosphate buffer; MRS medium	(128) (102)
	Oenococcus oeni	MLO culture medium	(132)
	Streptococcus salivarius subsp. *thermophilus*	dried skim milk	(129)
	Streptococcus salivarius	yeast medium, MRS broth	(130)
	Bifidobacterium bifidum	dried skim milk	(129)
	Bifidobacterium longum	sodium phosphate buffer	(128)
	Bifidobacterium animalis	sodium phosphate buffer	(128)
Fumonisins	*Lactobacillus paraplantarum*	corn infusion	(133)
	Lactobacillus lactis	corn infusion	(96)
	Lactobacillus bulgaricus	corn infusion	(96)
	Lactobacillus rhamnosus	corn infusion	(96)
	Lactococcus lactis subsp. *cremoris*	corn infusion	(133)
	Leuconostoc mesenteroides	corn infusion	(96)
	Streptococcus thermophilus	corn infusion	(133)
Zearalenon	*Lactobacillus paracasei*	phosphate buffer saline, mice	(97)
	Lactobacillus plantarum	phosphate buffer saline, mice; silage extract medium	(97) (114)
	Lactobacillus rhamnosus	phosphate buffer saline	(102, 134)
	Streptococcus thermophilus	ruminal fluid	(135)
Trichotecenes	*Lactobacillus plantarum*	MRS broth	(98)
	Lactobacillus pentosus	ultrapure water	(104)
	Lactobacillus paracasei	ultrapure water	(104)
	Lactobacillus casei	MRS broth	(98)
	Lactobacillus brevis	MRS broth	(98)
	Lactococcus lactis	MRS broth	(98)
Patulin	*Lactobacillus rhamnosus*	apple juice; phosphate buffer saline	(103) (136)
	Lactobacillus acidophilus	MRS broth	(99)
	Lactobacillus delbrueckii ssp. *lactis*	phosphate buffer saline; MRS broth	(136) (99)
	Lactobacillus plantarum	MRS broth	(99)
	Enterococcus faecium	apple juice; phosphate buffer saline	(103) (127)
	Bifidobacterium bifidum	phosphate buffer saline	(136)
	Bifidobacterium animalis	phosphate buffer saline	(136)

bulgaricus showed the highest binding efficiency (87.6 %). The same species was also used to investigate its AFM_1 binding in yogurt processing over 6 h. Again, *L. bulgaricus* won with 58.5 %, over *S. thermophilus* which bound 37.7 % of AFM_1. It was also found that the binding efficiency increased with time.

Sezer et al. (116) investigated the efficiency of LAB (*L. lactis* and *L. plantarum*) and their bacteriocins in removing AFB_1 from liquid culture. *L. plantarum* was more efficient than *L. lactis* (46 % vs 27 %, respectively), but efficacy was even higher when combined with bacteriocins. When the two strains were combined, AFB_1 removal reached 81 %.

Zinedine et al. (100) studied LAB efficiency in removing AFB_1 from the Moroccan sourdough bread. The winner was *L. rhamnosus* with 44.89 % AFB_1 removal at pH 6.5 and 30 °C.

Ochratoxin A

There are three major OTA-producing species, *Aspergillus ochraceus*, *A. carbonarius*, and *Penicillium verrucosum* (153, 154). Other species reported to produce OTA include *A. niger, A. sclerotioniger, A. lacticoffeatus, A. foetidus, A. westerdijkiae, A. steynii* and *A. tubingensis* (155, 156).

OTA is common in stored cereal grain, starch-rich food such as cereals (including wheat, barley, maize, rice, oat, and rye), and edible legume seeds (20). It does not attack plants during vegetation, save for the grapevines (23) (Table 1).

A number of studies investigated its removal by LAB (95, 102, 128-130, 132). Piotrowska and Żakowska (102) reported removal by *L. rhamnosus* as high as 87.5 %. *L. acidophilus* removed 70.5 %, *L. lactis* 59.6 %, *L. brevis* 56.2 %, *L. plantarum* 56.2 %, *L. sanfranciscensis* 52.0 %, *L. helveticus* (31.0 %), *L. delbrueckii subsp. bulgaricus* 28.3 %, and *L. casei* 16.6%. In another study, Piotrowska and Żakowska (131) investigated OTA removal from flour. *L. plantarum* was the most efficient (56 % removal), followed by *L. sanfrancisco* (51.0 %). A combination of *L. plantarum, L. sanfrancisco, L. brevis,* and *S. cerevisiae*, however, yielded even higher removal of 68 % after 40 h of incubation.

Fuchs et al. (128) examined the reactions and the relationship between the amount of added mycotoxins (500 and 1000 ng) and LAB species in a liquid medium. Even though they did not establish a clear relationship, the most efficient in removing OTA was *L. acidophilus* (97 %), followed by *Bifidobacterium longum* (58 %), *L. plantarum* (44 %), *L. lactis* (34 %), *L. casei* (31 %), and *L. bulgaricus* (29 %).

Mateo et al. (132) tested three factors to investigate the dynamics of OTA removal: *Oenococcus oeni* (10 strains), OTA level in medium (2 and 5 $\mu g\ L^{-1}$), and incubation time (0, 5, 10, and 14 days). All ten strains eliminated OTA from the medium but the highest reduction was 63 % after 14 days of incubation with the 124M strain in a medium spiked with 2 $\mu g\ L^{-1}$ of OTA and 58 % after 10 days of incubation with the 6G strain in a medium spiked with 5 $\mu g\ L^{-1}$ of OTA.

In another experiment Kapetanakou et al. (130) used *Streptococcus salivarius, Lactobacillus sakei*, and *L. casei* to reduce varying amounts of OTA, taking into account pH. Reduction increased slightly with the amount of added mycotoxins. The highest removal of 20 % was observed for the two *Lactobacillus* species at pH 5. The best result for *S. salivarius* was about 10 % at pH 4.

Fumonisins

Fumonisins have been identified and described relatively recently. They were first isolated from the strain *Fusarium verticillioides* (formerly *F. moniliforme*) in 1988 in South Africa (158). Other producers of fumonisins are *F. proliferatum*, *F. napiforme*, *F. oxysporum*, *F. dlamini*, *F. nygamai*, and *Aspergillus niger* (which produce fumonisins B_2, B_4, and B_6 but not B_1) (44, 159-161).

Of the 28 fumonisin analogues, only three are natural contaminants of food and feed: FB_1 (which makes 70-80 % of the three fumonisins), FB_2 (15-25 %), and FB_3 (3-8 %) (162). Fumonisins typically contaminate maize crops, but were also reported in other cereals (42) and asparagus (163) (Table 1).

Niderkorn et al. (96) tested the ability of several bacterial species to remove FB_1 and FB_2 from a medium at pH 4. FB_1 was best removed by *Leuconostoc mesenteroides* (82 %), *Pediococcus pentosaceus* (79 %), *L. plantarum* (74 %), and *L. rhamnosus* (74 %). FB_2 was completely (100 %) removed by *L. lactis*, whereas *L. mesenteroides, S. thermophilus, P. pentosaceus, L. casei, L. helveticus, L. bulgaricus, L. plantarum,* and *L. rhamnosus* removed over 90 %. Niderkorn et al. (133) also combined *L. paraplantarum*, *S. thermophilus*, and various treatment methods to eliminate FB_1 and FB_2. The best binding result (37 %) was observed with *S. thermophilus* in trichloroetic acid. Under the same conditions *L. paraplantarum* bound 19 % of the mycotoxin. With HCl *S. thermophilus* bound 24 %. Binding with other treatments did not exceed 15 %. FB_2 binding rate was much higher than that of FB_1, and the highest was observed with trichloroacetic acid (76 % for *S. thermophilus* and 65 % for *L. paraplantarum*) and HCl (65 % for *S. thermophilus* and 51 % for *L. paraplantarum*). These findings indicate that the method of detoxification, pH, and bacterial concentration play the key role in fumonisin removal. Methods that degrade cell wall surface structures increase the mycotoxin binding area. Binding can be further improved by increasing the concentration of peptidoglycans.

Zearalenone

Zearalenone (ZEA), also known as the F-2 toxin, is the third most common mycotoxin in plants, maize in particular (1, 26, 35). It is one of the strongest non-steroid oestrogens found in nature (164) produced by certain *Fusarium* species,

mainly *F. graminearum*, *F. culmorum, F. semitectum*, *F. equiseti*, and *F. cerealis* (32, 165, 166). *Fusaria* are among the most pathogenic toxin-forming fungi. Unlike other mycotoxins, ZEA reaches its maximum levels at 16 % humidity and temperature below 25 °C (167), usually before harvest. High levels were also detected in animal feed containing improperly stored maize (26, 32). Apart from maize, zearalenone can contaminate wheat, barley, oat, sorghum, rice, and peas (26, 38, 39, 40) (Table 1).

El-Nezami et al. (134) tested ZEA removal from culturing media with *L. rhamnosus*. In one experiment, it was about 60 % and in another (102) 64 % from phosphate buffer and lipase with heat-killed bacteria. Acid-killed bacteria removed 59 % of the mycotoxin.

Niderkorn et al. (135) tested the ability of *S. thermophilus* to bind ZEA and its metabolites (α- and β-ZOL) in ruminal fluid. Feed (50 % maize grain and 50 % alfalfa hay) alone bound 73 % of ZEA and its metabolites almost immediately and 69 % after 18 h. When *S. thermophilus* was added to the feed, binding rose to 91 % at first and dropped to 67 % after 18 h. When feed was eliminated as an experimental factor, *S. thermophilus* alone bound 83 % and 46 % of ZEA and its metabolites, respectively.

In another study (168), *L. plantarum* was combined with the Tunisian montmorillonite clay as absorbent. Clay alone bound 87 %, of ZEA, *L. plantarum* alone bound 78 %, while the combination bound as much as 94 % after 24 h.

Čvek et al. (111) reported that ZEA binding rose with LAB concentrations in MRS agar (99.12 % for *L. plantarum* and 84.71 % for *L. rhamnosus* at the concentration of 8 $\log_{10}$ CFU mL^{-1}) and dropped with incubation time (60-70 % after 72 h).

Trichothecenes

Fungi producing trichothecenes B (deoxynivalenol and its derivatives as well as nivalenol) mostly affect wheat and other crops (169). They include *Fusarium culmorum* and *F. graminearum*, which are also responsible for the biosynthesis of ZEA (1, 26, 170). Conditions favouring trichothecenes production are 21-25 °C and >0.95 % water activity, depending on *Fusarium* species (32, 171).

The primary sources of deoxynivalenol (DON) in the food chain are cereals, including wheat, barley, maize, and oat (2, 25-27, 30-33). It was also found in buckwheat, sorghum, and processed food such as flour, bread, pasta, beer, and malt (29, 34) (Table 1).

Franco et al. (104) investigated its removal by *L. plantarum, L. pentosus,* and *L. paracasei*. The study was conducted in three variants; (i) with viable cells, (ii) with pasteurised cells, and (iii) with sterilised cells. Sterilised unviable cells showed the best results when used alone; *L plantarum* bound 67 % of the toxin, *L. pentosus* 47 %, and *L. paracasei* 57 %.

Zou et al. (98) investigated the removal of DON and T-2 from MRS agar with *L. lactis, L. brevis, L. casei*, and *L. plantarum* over 0, 24, 48, and 72 h of incubation. The best results were observed at 48 h, while at 72 h the binding rate did not change. *L. plantarum* was the most successful in reducing both DON and T-2 levels (from 1 to about 0.8 μg mL^{-1}).

Patulin

Patulin is the best known mycotoxin, toxic to both plants and animals and associated with fruit and fruit preserves (45-47). It was first isolated from *Penicillium patulum* in 1940. The Joint Food and Agriculture Organization - World Health Organization Expert Committee on Food Additives has limited the maximum tolerable daily intake of this mycotoxin to <0.4 mg kg^{-1} of body weight per day.

Patulin is a dangerous mycotoxin produced under improper storage conditions of various products. Therefore, numerous studies have been conducted to reduce it. Hatab et al. (136) tested the efficiency of viable and unviable *Bifidobacterium bifidum, B. animalis, L. rhamnosus,* and *L. lactis* at 37 °C for 24 h varying the pH. The best results were obtained at pH 4 with unviable bacteria, as follows (in the descending order): *B. bifidum* (54.8 %), *L. rhamnosus* (52 %), *L. lactis* (35.6 %), and *B. animalis* (21.3 %). The same authors (103) also investigated the efficiency of *L. lactis, L. rhamnosus, L. helveticus, B. animails, B. bifidum,* and *Enterococcus faecium* in patulin reduction in apple juice varying two factors: temperature (30 and 37 °C) and patulin concentrations (100, 150, and 200 μg mL^{-1}). The most efficient reduction (about 80 %) was observed with *L. rhamnosus* (strain 6224) at patulin concentration of 100 μg mL^{-1} and temperature of 30 °C.

Hawar et al. (99) reported the highest reduction rate from 100 to about 50 μg mL^{-1} at pH 2 and the lowest at pH 9 (to about 85 μg mL^{-1}). They also found that the reduction rate dropped with higher CFU.

CONCLUSIONS

Many studies have demonstrated varying efficiency of LAB in removing mycotoxins from a variety of matrices. Removal mainly relies on mycotoxin binding to LAB cells and inactivation by antifungal products such as acetic acid.

Rendering LAB cells unviable with high temperature or acids seems to increase their mycotoxin-binding efficiency. This is quite likely related to the LAB cell wall components, mainly peptidoglycans and exopolysaccharides. The binding mechanisms, however, are not yet fully understood, and remain to be investigated by future research.

The most efficient LAB strains could be applied in various cereal products and livestock feed to increase food safety. Washing the products with suitable LAB preparations could also bind and remove mycotoxins. Preparations could also be used in cases of fungal infection in animals. Livestock may be fed these compounds at an early stage of

fungal infection, as they not only provide nutrients but also act as pharmaceuticals.

Despite the promising research findings, several questions need to be answered by future tests. As raw materials are subjected to ever more complex technological processes to meet consumer requirements, these questions include optimal timing, pH, methods for inactivating bacterial cells, and LAB concentrations that would yield best results. Future studies should also focus on identifying the exact mechanisms of mycotoxin binding to render it permanent. In the future, LAB will be used more widely in processing raw food liable to contamination with mycotoxins. At this stage, reducing mycotoxins in practice seems like a challenge to be addressed by new technological schemes.

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Djelotvornost mliječnokiselinskih bakterija protiv patogenih plijesni i mikotoksina

Mikotoksini su sekundarni proizvodi pojedinih vrsta plijesni rodova *Aspergillus*, *Penicillium* i *Fusarium* koji često zagađuju raznovrsne prehrambene proizvode. Stoga se u suzbijanju tih plijesni i zagađenja mikotoksinima primjenjuju mnoge strategije kroz cijeli prehrambeni lanac. U ovome se prikazu raspravlja o primjeni mliječnokiselinskih bakterija, kojima se već stoljećima sprječava razvoj i rast plijesni, a koje sudjeluju i u razgradnji mikotoksina i/ili u njihovu uklanjanju iz zagađene hrane. Budući da su korisne za ljudsko i životinjsko zdravlje, mliječnokiselinske bakterije izvrsno su rješenje problema sa zagađenjem mikotoksinima, ali je njihova primjena još pred izazovima, koje će riješiti buduća istraživanja.

KLJUČNE RIJEČI: Aspergillus; *biološke metode;* Fusarium; *inhibicija; LAB;* Penicillium