Agriculture and Food

I-CRAFT

4th International Conference on Research of Agricultural and Food Technologies (I-CRAFT 2024)

ISBN: 978-625-99572-1-0

ESP 10



PROCEEDINGS BOOK

4th International Conference on Research of Agricultural and Food Technologies

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Impact of Lactic Acid Bacteria on Mold-Produced Mycotoxins in Food Industry Applications

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Abstract. Mycotoxins, produced by molds such as Aspergillus, Penicillium, Fusarium, and Alternaria, pose significant health risks to humans and livestock, including mutagenic, teratogenic, estrogenic, and immunosuppressive effects. Found in crops like barley, nuts, and grains, they contaminate foods during production and storage. While agricultural practices can mitigate contamination, they cannot eliminate it, prompting the need for alternative detoxification methods. One promising solution is the use of lactic acid bacteria (LAB), which exhibit antifungal properties by inhibiting fungal growth, interacting with mycotoxins to deactivate them, or binding them through their cell walls. LAB strains like Lactobacillus plantarum and Lactobacillus gallinarum demonstrate strong antifungal and mycotoxin-binding abilities. Several studies have shown these bacteria can inhibit mold growth and reduce mycotoxins such as aflatoxin B1 (AFB1), deoxynivalenol (DON), and fumonisins (FB1 and FB2) in contaminated silages and food products. LAB strains, particularly postbiotics (compounds produced by LAB), can effectively inhibit mold growth and reduce aflatoxin levels, making them viable natural preservatives for foods like bread and fermented products. LAB postbiotics, including organic acids and antifungal peptides, have shown stability under harsh conditions and can be used to bioconserve raw materials and foods. These natural additives offer a potential replacement for chemical preservatives, providing an eco-friendly approach to preventing fungal contamination and mycotoxin production. LAB's bioprotective capabilities extend to agricultural applications, such as poultry production, where LAB has been shown to reduce mycotoxin residues in animal products. Consequently, LAB strains and their postbiotics hold promise for enhancing food safety and quality.

Keywords: Lactic acid bacteria, Probiotic, Postbiotic, postbiotic metabolites

1. Introduction

Mycotoxins are toxic compounds that pose a serious threat to food safety and public health. These toxins are produced by molds, especially Aspergillus, Penicillium and Fusarium, and can be found in many foodstuffs, especially cereals, nuts, dried fruits and spices. Foods containing mycotoxins can cause acute or chronic effects (mutagenic, teratogenic, estrogenic and immunosuppressive) on the consumer [1]. International criteria and codes of practice to limit exposure to mycotoxins from designated foods were established by the Codex Alimentarius Commission based on thel JECFA decisions. The adverse effects of mycotoxins on human health are closely related to their carcinogenic, hepatotoxic, nephrotoxic and immunosuppressive properties [2]. According to the World Health Organization (WHO), mycotoxins such as aflatoxins are among the major public health problems affecting millions of people worldwide [3]. The presence of mycotoxins in food poses a major threat to food safety and public health in developing countries, while it can cause serious economic losses due to food contamination problems even in developed countries [4]. Uncontrolled hygiene conditions and improper storage and processing techniques in the food production chain can increase mitotoxin contamination, and this has become a common risk that threatens both human and animal health [5]. Since mycotoxins are mostly heat-stable, cooking and other typical heat treatments used in the food industry are unfortunately not effective in removing mycotoxins from contaminated foods [6]. Although there are methods such as applying alkaline ammonia as a chemical process for their degradation, it is not preferred due to the potential side effects on human health and the possibility of changing the properties of food [7]. For these reasons, controlling mitotoxins in the field of food safety is a critical issue that needs to be continuously improved with scientific research and regulations.

1.1 Types of Mitotoxins and Their Effects on Health

Mitotoxins are toxic compounds produced by molds and are considered a common source of contamination in foods. These toxins are produced when molds grow in certain environments and conditions and can have serious effects on human health. Mitotoxin contamination can occur at all stages of agricultural products from production to harvesting, storage and processing, thus posing a significant problem for global food safety [8].

The most common mytoxins include aflatoxins, ochratoxin A (OTA), fumonisins, zearalenone (ZEA), and deoxynivalenol (DON). Each of these toxins is produced by different types of molds and can be found in a variety of foodstuffs.

Aflatoxins: Aflatoxins are produced by molds such as Aspergillus flavus and Aspergillus parasiticus and are found primarily in peanuts, corn, cottonseed, hazelnuts and some grains. Aflatoxin B1 is one of the most potent hepatocarcinogens and has been scientifically proven to increase the risk of liver cancer [9]. In addition, aflatoxins can suppress the immune system and increase susceptibility to other infections [10].

Ochratoxin A (OTA): Produced by Aspergillus and Penicillium species, ochratoxin A is found primarily in cereals, coffee, grapes, and wine [11]. Ochratoxin A is particularly toxic to the kidneys and is nephrotoxic, increasing the risk of kidney cancer with long-term exposure [12].

Fumonisins: Produced by molds of the Fusarium genus, fumonisins are especially common in corn. Fumonisin B1 can have toxic effects on the nervous system and lead to congenital anomalies such as neural tube defects [13]. Fumonisins can also cause toxic effects on the liver and kidneys [14].

Zearalenone (ZEA): Zearalenone is also produced by Fusarium species and can cause hormonal imbalances, especially in animals, due to its estrogenic effects [15]. Long-term exposure in humans can lead to hormonal disorders and reproductive system problems [16].

Deoxynivalenol (DON): Another mitotoxin produced by Fusarium, DON is found especially in grains such as wheat and barley[17]. DON can have toxic effects on the gastrointestinal tract, causing symptoms such as diarrhea, vomiting, and loss of appetite [18].

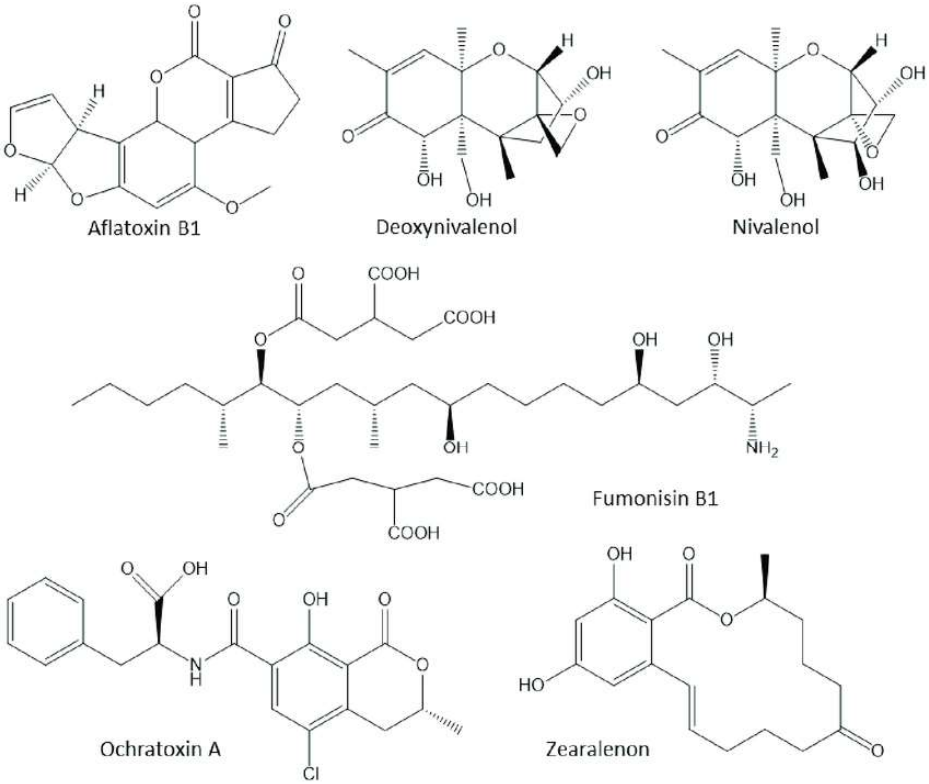

Figure 1. The most common mycotoxins food and feed [19]. Aflatoxin B1 (AFB1) and fumonisin B1 (FB1) are polar mycotoxins that are more easily adsorbed by adsorbents than non-polar mycotoxins. Ochratoxin A, T-2 toxin, deoxynivalenol (DON) and zearalenone (ZEN) are non-polar.

Table 1. Mycotoxins, including their chemical formulas, producing molds, contaminated food sources, effect on health

Mycotoxin/ Chemical Formula	Producing Molds	Contaminated Foods	Health Effects	References
Aflatoxin B1: C17H12O6	*Aspergillus flavus*, *A. parasiticus*	Peanuts, corn, cottonseed, hazelnuts, some grains	Hepatocarcinogen, liver cancer, immune suppression	[9-10]
Ochratoxin A (OTA) C20H18ClNO6	*Aspergillus*, *Penicillium*	Cereals, coffee, grapes, wine	Nephrotoxic, kidney cancer risk	[11-12]
Fumonisin B1 C34H59NO15	*Fusarium*	Corn	Nervous system toxicity, neural tube defects, liver and kidney toxicity	[13-14]
Zearalenone: (ZEA) C18H22O5	*Fusarium*	Grains, animal feed	Estrogenic effects, hormonal imbalances, reproductive issues	[15-16]
Deoxynivalenol (DON) C15H20O6	*Fusarium*	Wheat, barley	Gastrointestinal toxicity, vomiting, diarrhea	[17-18]
Patulin C7H6O4	*Penicillium*, *Aspergillus*, *Byssoclamys*	Apples, apple juice, other fruits	Carcinogenic, mutagenic, immunotoxic effects	[8]

1.2 LAB and Detoxification of Mitotoxins in the Food System

1.2.1 Lactic Acid Bacteria (LAB)

Lactic acid bacteria are Gram-positive, catalase-negative, anaerobic or facultative anaerobic bacteria that are widely used during food fermentation [20]. Some of them are classified as "Generally Recognized as Safe (GRAS)" by the European Food Safety Authority (EFSA) and the Food and Agriculture Organization of the United States (FAO). Therefore, they are suitable for use as probiotics and preservatives in food products [21]. Lactobacillus, Leuconostoc, Pediococcus and Streptococcus are among the most commonly used LAB genera in the food industry. The antimicrobial properties of these bacteria are associated with the production of various compounds such as organic acids, hydrogen peroxide, diacetyl and bacteriocins, but recent studies have shown that LAB may also be a potential solution against mycotoxins and have been considered as one of the promising natural biopreservatives. In addition, due to the GRAS status and Qualified Presumption of Safety (QPS) status provided by FDA and EFSA, LAB have attracted great interest from researchers to investigate their potential as biopreservatives [22].

1.2.2 LAB Activity Against Mycotoxins

LAB can be used as mycotoxin detoxifiers in some foods, including milk, cereals and fermented beverages, to inhibit aflatoxin M1, Fusarium mycotoxins and aflatoxin [23-24]. In selecting LAB to target in food for mycotoxin, its stability and residence time depending on gastrointestinal tract dynamics are important factors, but binding strength is strain specific [25].

Table 2. Effects of lactic acid bacteria (LAB) against mycotoxins and their applications in the food industry.

Food Industry	Food Product	Mycotoxin Type	LAB Type	Mechanism of Action	References
Fermented dairy products	Yogurt, cheese	Aflatoxin B1	*Lactobacillus rhamnosus*	Aflatoxin adsorption during the fermentation process	[26]
Cereal-based fermented products	Corn-based foods, cereals	Zearalenone	*Lactobacillus plantarum*	Biotransformation	[27]
Meat and fermented meat products	Sausage, salami, fermented meat products	Ochratoxin A	*Lactobacillus acidophilus*	Ochratoxin adsorption during fermentation	[28]
Cereal fermented products	Wheat and corn-based breads	Deoxynivalenol (DON)	*Lactobacillus casei*	Adsorption & biotransformation	[29]
Fermented dairy products	Milk and dairy products	Fumonisin B1	*Pediococcus acidilactici*	Fumonisin adsorption during fermentation	[30]
Fruit and vegetable-based fermented products	Apple-based products, juice	Patulin	*Lactobacillus brevis*	Inhibition of mold growth and patulin production	[31]
Combined fermented dairy and meat products	Fermented dairy and meat products	Aflatoxin B1	*Lactobacillus rhamnosus* & *L. acidophilus*	Aflatoxin adsorption through combination	[32]
Fermented meat products	Fermented sausage and salami	Ochratoxin A	*Lactobacillus paracasei*	Ochratoxin biotransformation & adsorption	[33]
Cereal-based fermented products	Wheat and corn-based products	Zearalenone	*Lactobacillus plantarum* & *L. reuteri*	Biotransformation	[34]
Fermented dairy and cereal products	Fermented milk and yogurt products	Aflatoxin B1	*Lactobacillus rhamnosus GG*	Aflatoxin adsorption	[35]

Basically, three mechanisms have been proposed for LAB mycotoxin detoxification. These are:

1. Biotransformation of Mycotoxins: LABs can convert some mycotoxins to less toxic compounds through biotransformation. For example, some LAB strains have been shown to convert zearalenone to zearalenol, which has lower toxicity [36].

2. Adsorption of Mycotoxins: LAB cell wall components can bind mycotoxins and remove them from the food matrix [37]. This binding is especially valid for lipophilic toxins such as aflatoxins. Peptidoglycan, teichoic acid and polysaccharides in the cell wall of LAB are the main components that play a role in this binding process.

3. Production of Antimicrobial Compounds (Postbiotics): LABs can produce antimicrobial compounds such as organic acids and bacteriocins that inhibit the growth of molds (Table 3). Thus, the development of mycotoxin-producing molds in the food environment is limited [38]. Detailed information on the effects of LAB species against mycotoxins and their applications in the food industry is given in Table.2.

Several studies have reported the prevention or control of mycotoxin-producing mold growth by LAB members Lactococcus, Lactobacillus, Pediococcus and Leuconostoc species [39]. Among the LAB members, the antifungal activity of lactobacilli is higher than others, especially Lb. plantarum plays an antifungal role in inhibiting the growth of many fungi [40].

The ability of fermentative bacteria (LAB) and propionic acid bacteria (PAB) to remove Fusarium toxins was evaluated in vitro and reported that the binding ability of selected strains could be used to reduce the bioavailability of toxins such as deoxynivalenol (DON) and fumonisin B1 and B2 (FB1, FB2) in contaminated silages [41-42]. It shows that lactic acid bacteria strains have the potential to inhibit the growth of F. graminearum and remove DON in vitro.

Fusarium ssp. Aspergillus ssp. Penicillium ssp. and Alternaria ssp. have been reported as the main mycotoxin-producing genera [43]. On the other hand, the most important mycotoxins in the European malt and beer industrial chain have been reported to be aflatoxins (AFs), fumonisins (FBs), trichothecenes, ochratoxin A and zearalenone (ZEN) [44-45]. Mycotoxins disrupt the DNA structure by binding to DNA directly or through their metabolites [46].

Table 3. This table highlights how postbiotics produced by lactic acid bacteria (LAB) can be effective in inhibiting the growth of mycotoxin-producing fungi and reducing mycotoxin contamination in foods.

Postbiotic Source	Postbiotic Compound	Mycotoxins Inhibited	Mechanism of Action	References
Lactobacillus brevis	Antifungal peptides (asparagine, glutamine, glycine, alanine, leucine)	Aflatoxin B1	Inhibits growth of Aspergillus spp. and reduces Aflatoxin B1 production	[47]
Lactobacillus paracasei	Antifungal peptides (similar composition to L. brevis)	Aflatoxin B1	Reduces aflatoxin levels in foods and prevents fungal growth	[47]
***Lactobacillus plantarum* K35**	Organic acids (lactic acid, oleic acid, palmitic acid)	Aflatoxin B1	Damages fungal cell walls and cytoplasmic membranes, inhibiting fungal growth	[48]
***Lactobacillus plantarum* AF1**	Peptide compounds (C12H22N2O2, 3,6-bis(2-methylpropyl))	General fungi, including Aspergillus flavus	Disrupts fungal membrane integrity, reducing mycotoxin contamination in foods	[49]
***Lactobacillus rossiae* LD108**	Peptide postbiotics from gluten proteolysis	*Aspergillus japonicus*, *Eurotium repens*, Penicillium spp.	Inhibits fungal growth on bread, reducing potential mycotoxin contamination	[50]
***Leuconostoc mesenteroides* DU15**	Antifungal peptides	Multiple bread spoilage fungi	Prevents fungal contamination in bread products	[51]

Fermented foods have global economic value and are generally considered safe foods. However, the risk of contamination with acid-tolerant fungi is a major problem. Probiotic bacteria as starter cultures or other biocontrols can be selected from those with high aflatoxin content for detoxification of foods. Detection and removal of microbial degradation metabolites and toxicity levels of aflatoxin B1 contaminant in food and feed is an important requirement. Lactic acid bacteria have been used as postbiotics for biodegradation or removal of Aflab1 a toxin in foods, but research is limited because aflatoxin analysis requires special instrumental analysis.

L. brevis and L. paracasei strains producing postbiotic antifungal peptides (both strains contain Asparagine, glutamine, glycine, alanine and leucine amino acids) were tested and some postbiotic peptides from L. brevis and L. paracasei showed 96.31% and 90.43% inhibitory effect on Aspergillus spp growth and aflatoxin B1 production, respectively [47]. As a result of their study, it is seen that these strains can be used as natural preservative additives for the preservation of organic foods. Another study supporting the bioconservative effect of LAB postbiotic peptides was reported by Muhialdin et al. (2018) [51], LAB postbiotic antifungal peptides were obtained from four lactic acid bacteria isolates, Leuc mesenteroides DU15, L. plantarum TE10, L. plantarum IT10, L. plantarum IS10, postbiotics showed a high antifungal effect on 6 fungi mostly associated with bread spoilage at pH 3. Antifungal postbiotics were stable at 121 C and partially lost their activity under proteolytic enzyme treatment. Postbiotic filtrate (10 KDa membrane) showed three times more antifungal activity on fungi. LAB postbiotic peptides can be used as natural additives to preserve bread. Sangmanee & Hongpattarakere (2014) investigated the antifungal effect of multiple antifungal postbiotics obtained from L. plantarum K35 on the growth and related aflatoxin production by Aspergillus flavus TISTR304 [48]. The postbiotic components obtained from L. plantarum were resistant to enzyme treatment (proteinase k, trypsin) and sterilization and catalase treatment (not H2O2 effect). As a result, the antifungal effect was only organic acids; the main postbiotic components were oleic acid (10.52%) and palmitic acid (7.27%) except lactic acid (19.55%) from 2-butyl-4-hexyloctahydro-1H-yne. Other minor antifungal compounds included linoleic acid (2.11%), 2.4-di-tertbutylphenol (1.84%), stearic acid (1.55%), 3-phenyl lactic acid (1.42%), and pyroglutamic acid (1.07%), which were analyzed by GC-MS. The antifungal activity of L. plantarum was due to the synergistic effect. The mode of action of postbiotics was elucidated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Postbiotic compounds caused severe damage to the cell wall and cytoplasmic membrane, leading to a large loss of cytoplasmic content of fungi. Antifungal postbiotics obtained from L. plantarum K35 were extensively studied, and different compounds were discovered in each research study. Yang et al. (2010) identified C12H22N2O2, 3,6-bis(2-methyl propyl)-2,5-piperazinedione with a molecular mass of 226 from L. plantarum AF1 [470], Ström et al. (2002) identified cyclo (L-phe-L-pro), cyclo (L-phe-trans-4-OH-L-pro) and phenyllactic acid from L. plantarum [52]. MiLAB 393 Niku-Paavola et al. (1999) identified benzoic acid, 5-methyl-2,4-imidazolidinedione (methylhydantoin), tetrahydro-4-hydroxy-4-methyl-2H-pyran-2-one (mevalonolactone) and cyclo (glycyl-L-leucyl) from L. plantarum [53]. Studies have yielded positive results indicating that LAB antifungal postbiotics can replace chemical additives. It is promising that LAB postbiotics with this bioprotective potential will be used as effective agents against fungi responsible for aflatoxin production in feeds and foods [48]. The in vitro antifungal activity of bioactive peptides produced by two sourdough species L. rossiae LD108 and L. paralimentarius PB127 against Aspergillus japonicus, Eurotium repens and Penicillium roseopurpureum was tested by Garofalo et al. (2012) [50]. These authors first found the capacity of peptide-postbiotics to inhibit fungal growth. The antifungal properties depended on the peptide postbiotics, which were identified as gluten proteolysis by-products against three fungal cultures. Secondly, the inhibitory activities on mold growth were demonstrated in bread for both species, but only L. rossiae LD108 inhibited mold growth on panettone.

2. Conclusion

Lactic acid bacteria (LAB) stand out as promising biological agents in the food industry to control and reduce mycotoxin contamination. Studies show that LAB can be used against various mycotoxins such as aflatoxin, zearalenone, ochratoxin A, deoxynivalenol, fumonisin and patulin. LAB species prevent the growth of pathogenic microorganisms by lowering the pH level of products during fermentation processes, while at the same time they have the ability to bind and render mycotoxins harmless. This reduces the food industry's dependence on chemical preservatives and creates the potential to offer more natural, clean-label products. In particular, consumer preferences are shifting towards more natural, additive-free foods, further increasing the importance of LAB.

The effectiveness of LAB against mycotoxins may vary depending on the type of food product and production processes. For example, it has been shown that toxins such as aflatoxin B1 can be reduced by up to 80% through adsorption in fermented dairy products, while mycotoxins such as zearalenone are converted to less toxic compounds through biotransformation in cereal-based products. It has been reported that ochratoxin A is reduced by up to 70% through adsorption and biotransformation in meat products, and patulin production is inhibited in fruit-based fermented products. However, the effects of LAB strains may vary depending on the type of mycotoxin, environmental conditions (pH, temperature) and fermentation parameters used. Therefore, the most appropriate LAB strain and fermentation conditions need to be determined for each food type.

For wider applications in the food industry, a better understanding of the mycotoxin control mechanisms of LAB, determination of optimal strains and conditions, and support of innovative approaches in this area are required.

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