



PROSPECTS FOR THE USE OF PROBIOTICS AS ANTAGONISTS OF MYCOTOXIGENIC FUNGI

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Annotation. Microbiological studies of compound feeds used to feed farm animals in the Tashkent region have been initiated. The studies showed a high level of contamination of the compound feeds produced with various opportunistic pathogenic bacteria and fungi. The isolated phytopathogenic fungi are producers of mycotoxins. In the course of the study, lactic acid bacteria were screened for antagonistic activity against phytopathogenic fungi. The use of lactic acid bacteria (LAB) as antagonists of phytopathogenic fungi and as biodegraders of mycotoxins is one of the most promising and environmentally safe approaches to controlling these contaminants.

Keywords: compound feed, food safety, phytopathogenic fungi, mycotoxins, lactic acid bacteria, biodegradation of mycotoxins.

Mycotoxins are secondary metabolites of microscopic fungi of the genera *Aspergillus*, *Fusarium*, *Penicillium* and other taxa, which can contaminate grain, compound feeds, milk and other food products. The most significant mycotoxins in terms of food and feed safety are aflatoxins, ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON), fumonisins and trichothecenes. Their resistance to external factors makes them difficult to remove from contaminated raw materials by traditional physicochemical methods. Therefore, interest in biological detoxification using safe microorganisms and their metabolites has increased in recent years [1]. Mycotoxins differ in chemical structure, toxicity and mechanism of action. A feature common to all mycotoxins is that they are toxic mostly to animals. The most frequently used classification of mycotoxins is based on molecular structure, according to which aflatoxins, trichothecene mycotoxins, ochratoxins, fumonisin, zearalenone and its derivatives, moniliformin, fusarochromanone, patulin and citrinin are distinguished [2–3]. According to WHO, aflatoxin is among the most widespread mycotoxins. It is produced by certain mold species, such as *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi are often found in soil, humus and on cereal crops. They can infect crops such as maize, wheat, rice, peanuts and many nuts. Aflatoxins may also be present in the milk of animals that have consumed contaminated feed. In foods, the most common aflatoxins are B1, B2, G1, G2 and M1 [4, 5]. Other mycotoxins, such as ochratoxin A, are produced by *Aspergillus* and *Penicillium*. This toxin forms during crop storage and can adversely affect animals, causing serious kidney and immune system disorders. Contamination of plant-based food raw materials with mycotoxins is one of the most acute food safety problems, since mycotoxins have carcinogenic, mutagenic, teratogenic and estrogenic effects on the human body, cause reproductive disorders and suppress the immune system [6, 7, 9].

Lactic acid bacteria (LAB) occupy a special place among the microorganisms used to reduce mycotoxin levels, including representatives of the genera *Lactobacillus sensu lato*, *Lactiplantibacillus*, *Lacticaseibacillus*, *Lactococcus*, *Pediococcus*, *Leuconostoc* and *Weissella*. Their advantages stem from their long history of use in fermented foods, their probiotic properties and the generally recognized safety of certain strains. LAB can reduce the toxicological burden of mycotoxins through several mechanisms: adsorption on the cell surface, biotransformation, inhibition of the growth of mycotoxin-producing fungi and suppression of toxin biosynthesis [1]. One of the best-studied mechanisms is the adsorption of mycotoxins by the bacterial cell wall. The interaction of toxins with the cell surface is determined by the properties of peptidoglycan, teichoic acids, polysaccharides and exopolysaccharides. This mechanism is particularly well studied for aflatoxin B₁ (AFB₁). It has been shown that individual strains of *Lactobacillus* and *Lactococcus* are able to bind a significant part of AFB₁ from model solutions. The efficiency of adsorption depends on the strain, bacterial concentration, pH, temperature, contact time and the structure of the mycotoxin itself [10]. However, adsorption does not always mean destruction of the mycotoxin molecule. The bound toxin may retain its chemical structure and be partially released when environmental conditions change. Therefore, when assessing the detoxification potential of LAB, it is necessary to distinguish between physical binding and true biotransformation of the mycotoxin. Studies show that heat- or chemically treated cells of some LAB may have a higher sorption capacity for AFB₁, which indicates an important role of cell wall components [11]. Another promising mechanism is the biological transformation of mycotoxins. In this case, the chemical structure of the toxin is altered by the action of enzymes or metabolites of microorganisms, resulting in compounds of lower toxicity. For aflatoxins, oxidation, reduction and other structural transformations have been described, whereas for OTA, hydrolysis of the amide bond with the formation of less toxic products is considered a promising pathway. At the same time, the presence of a degradation product is not in itself proof of complete detoxification: its chemical identification and toxicity assessment are required [12].

LAB can also act on mycotoxins indirectly, by suppressing mycotoxin-producing fungi. During growth they produce organic acids, hydrogen peroxide, bacteriocins, low-molecular-weight antimicrobial compounds and other metabolites that can limit the development of *Aspergillus*, *Fusarium* and *Penicillium*. Inhibition of fungal growth is accompanied by reduced mycotoxin biosynthesis. In addition, competition for nutrients and changes in substrate pH create unfavorable conditions for the development of toxigenic fungi [5].

Biodegradation (biodegradation) of mycotoxins is the process of breaking down toxic mycotoxin molecules into non-toxic or less toxic compounds with the help of living organisms (bacteria, yeasts, fungi) or their enzymes. LAB biodegraders are specific strains of lactobacilli that are able to neutralize mycotoxins. Some probiotics can modify the structure of aflatoxin AFB₁ and ochratoxin A, converting them into less toxic compounds. For example, *Bacillus subtilis* and *B. licheniformis* produce enzymes (e.g., laccases) that break down aflatoxin and ochratoxin into safe metabolites. Strains of *Lactobacillus plantarum* and *L. reuteri* hydrolyze the toxin, reducing its activity. By reducing bioavailability and protecting the body, LAB and probiotics minimize the negative effect of mycotoxins on the organism by decreasing their absorption in the intestine [14–18]. In addition, LAB protect the liver of animals. Strains of

Lactobacillus rhamnosus and *L. brevis* reduce oxidative stress and liver damage caused by mycotoxins [19].

Materials and methods. More than 10 samples of compound feed were studied. The samples were collected from various feed-producing farms in the Tashkent region (Fig. 1). Mycological plating was performed according to GOST 10444.12-2013. Mold isolates were obtained from the compound feed cultures, and their taxonomic identification was carried out on the basis of phenotypic characteristics in accordance with Samson [8]. The antagonistic properties of lactic acid bacteria were determined by the agar block method. The isolated lactic acid bacteria were grown in Petri dishes on MRS agar medium for 2 days. The phytopathogenic micromycetes were grown on Czapek agar for 6 days in a thermostat at 28 °C. The experiments were carried out in triplicate, and the antifungal activity of the lactic acid bacteria was determined by the absence of a pathogen growth line [20].



Fig. 1. Some of the compound feed samples collected for microbiological studies.

Results. Lactic acid bacteria were screened for antagonistic activity against mycotoxigenic fungi. The lactic acid bacteria strains *Lactobacillus plantarum* M-1, *Pediococcus pentosaceus* A-1, *Pediococcus acidilactici*, *Enterococcus faecium* A-2 and *Weissella cibaria* from the collection of the Laboratory of Food and Feed Microbiotechnology were selected for screening against mycotoxigenic fungi. These bacterial strains showed antifungal activity against the phytopathogenic micromycetes *Alternaria alternata*, *Verticillium dahliae*, *Fusarium species*, *Aspergillus flavus* and *Aspergillus oryzae*. In the experiment, the strain *L. plantarum* M-1, isolated from plant samples, formed a zone of strong inhibition of the phytopathogenic strain *A. alternata* with a diameter of 35 mm, of *V. dahliae* — 33 mm, of *F. species* — 20 mm, of *A. flavus*

— 29.6 mm and of *A. oryzae* — 34 mm. The lactic acid bacterium *P. pentosaceus* A-1 formed a growth inhibition zone with a diameter of 25 mm against the phytopathogenic strains *A. alternata* and *V. dahliae*, 22 mm against the micromycete *F. species*, and an active inhibition zone of 26 mm against *A. flavus*. The strain *Pediococcus acidilactici* showed antifungal activity against *Penicillium*; the inhibition zone of the phytopathogen was 34 mm.

At the same time, no change was observed with respect to the micromycete *A. oryzae*. The strain *E. faecium* A-2 formed growth inhibition zones with a diameter of 20 to 30 mm against all tested phytopathogenic micromycetes (Fig. 2).

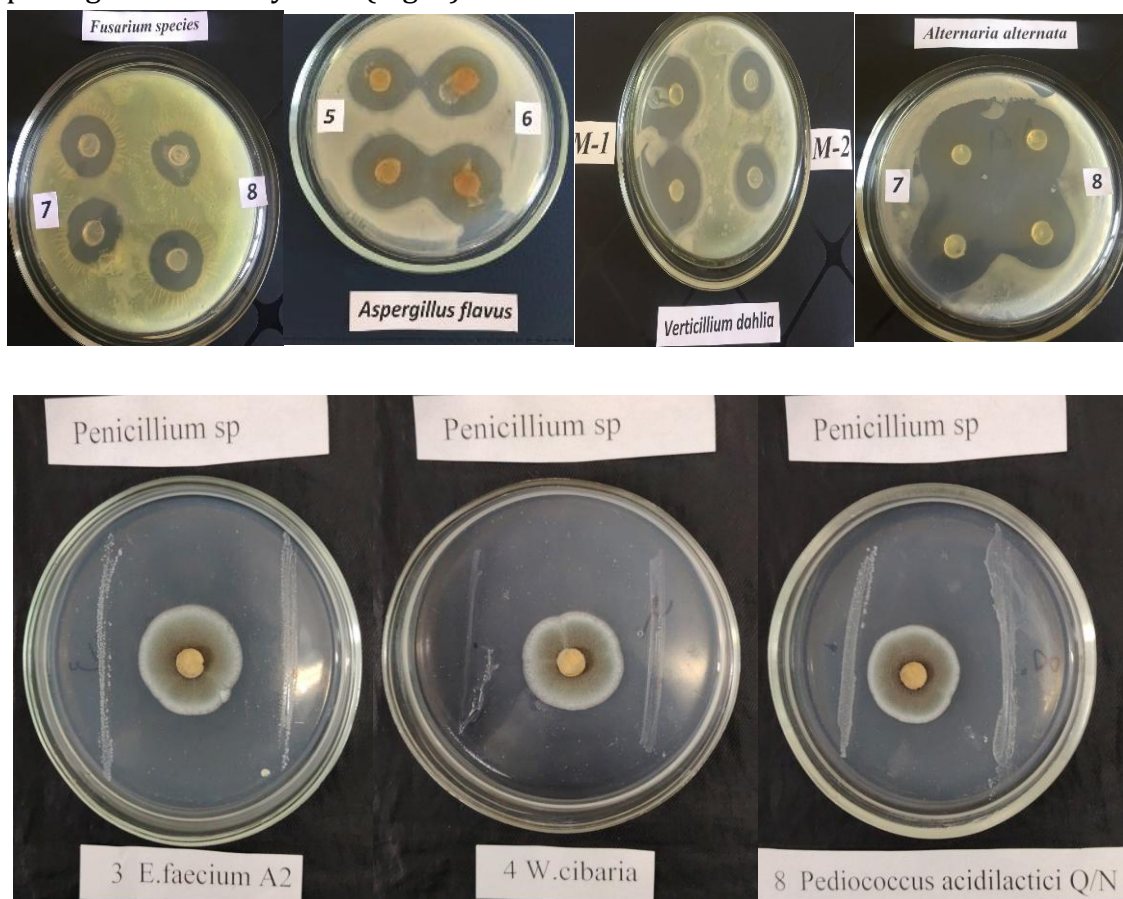


Fig. 2. Inhibition zones of lactic acid bacteria against phytopathogenic micromycetes.

Conclusions. Thus, molds are the dominant group of microflora contaminating compound feeds. In most of the studied mold-contaminated feed samples, *Aspergillus flavus* and *Penicillium species* dominated in both frequency and abundance. Strains with aflatoxin-producing activity were identified among them. The presence of toxigenic activity in molds isolated from compound feeds has been demonstrated in Uzbekistan for the first time. Probiotics have an immunosuppressive effect on aflatoxin AFB1, enhancing the response of the immune system. At present, the probiotic complexes Bacell-M, based on *Bacillus subtilis*, are used in animal husbandry for the detoxification of feeds, and LAB can be used as functional products to reduce the risk of aflatoxicosis in humans. The screening of lactic acid bacteria against mycotoxigenic fungi demonstrated their antagonistic activity. *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Enterococcus faecium* and *Weissella cibaria* are distinguished not only by their environmental safety but also by their specific antagonistic properties. Substances such as organic acids, 3-phenyllactic acid, bacteriocins and hydrogen

peroxide produced by these bacteria directly inhibit the growth of pathogenic fungi. Lactic acid bacteria and probiotics are a promising, safe tool for neutralizing mycotoxins, especially in combination with physicochemical methods. Their use makes it possible to reduce economic losses and improve the safety of the food chain.

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