



preventing mycotoxins by the utilization of organic compounds and probiotic microorganism in animal feed

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Abstract

Mycotoxins are secondary metabolites synthesized by fungi. Contamination of feed by mycotoxins poses a significant risk to public health. Contamination may transpire throughout the food chain, resulting in numerous diseases in humans and animals, as well as economic detriment. Numerous detoxification approaches encompass physical, chemical, and biological aspects. strategies, have been developed to eradicate mycotoxins in feed. The biological technique of mycotoxin detoxification by microorganisms is dependable, effective, cost-efficient, and user-friendly in comparison to physical and chemical methods. It is essential to ascertain the toxicity of the metabolites produced during mycotoxin biodegradation. These compounds may exhibit toxicity levels that are either lower or higher than that of the parent substance. Conversely, the processes behind the biological regulation of mycotoxins remain ambiguous. This article provides an overview of the most hazardous mycotoxins and the several microorganisms and natural compounds capable of mycotoxin detoxification.

Keywords: feed safty, mycotoxin, probiotic, biopreservation.

Introduction

Currently, food losses represent a significant global issue, particularly given the increasing world population and the reality that around one-third of all food produced for human use is either lost or waste.^(۱) In underdeveloped nations, post-harvest loss rates are significant, with ۳۰–۴۰% happening during the post-harvest and processing phases.

^(۲) In industrialized nations, comparable loss rates (۳۰%) are observed at the retail or consumer levels^(۱).

Diverse factors contribute to significant global feed loss, with microbial decomposition being a primary factor that impacts organoleptic quality, including appearance, texture, flavor, and scent. Fungi are a significant concern among spoilage microorganisms at all stages of the food chain due to their capacity to thrive in various and often extreme settings. In addition to adversely affecting feed quality, several fungal species, including *Aspergillus*, *Penicillium*, *Alternaria*, and *Fusarium*, possess the capacity to synthesize secondary metabolites that can be hazardous to people and animals, collectively referred to as mycotoxins. Furthermore, mycotoxins can last many food processing stages, hence posing food safety risks^(۳). Fungi, primarily in the form of airborne spores (either sexual or asexual), can colonize and proliferate at several points of a product's lifecycle, including during cultivation, post-harvest, processing, storage, and handling by producers, wholesalers, retailers, and consumers. The repercussions of pollution at every stage can evidently result in economic losses for both producers and consumers^(۱). Furthermore, producer losses may be exacerbated by a detrimental brand image resulting from consumer discontent.

In this setting, it is imperative to mitigate food losses by managing fungal contamination throughout all phases of food processing chains. Three primary stages can be delineated to categorize elements contributing to fungal contamination: (i) the environment, where water, soil, and air serve as natural habitats for fungi; (ii) raw materials—such as post-harvest crops, meats, and milk—where fungal presence is associated with food management practices during harvesting, collection, transportation, storage, and packing^(۴). and (iii) during the processing of feed. Fungal contamination in field crops is typically managed through the application of synthetic fungicides in conjunction with crop management strategies, including crop rotation, the utilization of resistant cultivars, and tillage^(۵). Fungicides are extensively utilized to safeguard post-harvest fruits and vegetables; however, alternative treatments such as ozone disinfection, chlorine application, acidified hydrogen peroxide, pH modification with sodium bicarbonate, surface sterilization via irradiation or thermal methods, and waxing with active coatings containing fungicidal agents and preservatives are also employed. Additionally, several packaging methods are employed to safeguard crops from mechanical damage, the most prevalent entry point for microbial illnesses^(۶). The possible detrimental environmental and health impacts of specific fungicides and preservatives have prompted a shift towards more natural alternatives. Regarding agriculture, excessive residual pesticide levels on harvested produce, regulatory measures, the development of fungal resistances, and environmental repercussions have prompted the advancement of biocontrol agents. This rapidly expanding sector, particularly for fruits, vegetables, and cereals, necessitates natural solutions to mitigate crop losses. A variety of products including bacteria, fungus, and yeasts are presently marketed globally^(۷). Preservatives are extensively utilized in raw materials and processed meals.

Overview of Major Mycotoxins:

Aflatoxins, ochratoxin A, zearalenone, deoxynivalenol, nivalenol, fumonisin B^۱ and B^۲, and patulin are the predominant mycotoxins that may contaminate food and feed along the food chain^(۸). Aflatoxins are secondary metabolites of fungi mostly generated by *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nominus*, and *Aspergillus niger*. Approximately ۱^۸ aflatoxins have been found, with aflatoxin B^۱ (AFB^۱) being the most prevalent. ^(۹) Aflatoxin B^۲ (AFB^۲), aflatoxin G^۱ (AFG^۱), aflatoxin G^۲ (AFG^۲), aflatoxin M^۱ (AFM^۱), and aflatoxin M^۲ (AFM^۲). Aflatoxins influence protein synthesis by binding to cellular DNA. Group B exhibits blue fluorescence, while Group G displays green fluorescence under ultraviolet light. Aflatoxin pollution primarily occurs in warm and humid areas^(۱۰).

Patulin (PAT) is a mycotoxin predominantly generated by species of *Penicillium*, *Byssoschlamys*, and *Aspergillus*. Patulin pollution can inflict significant harm on animals, including cancer, by impacting several organs such as the kidneys, liver, and intestines. It can contaminate food items, including fruits and vegetables, particularly apples and apple-derived products^(۱۱).

Ochratoxin A (OTA) is the predominant toxin found in grapes and grape-derived goods; however, it can also infect other foods, including coffee, spices, beer, and certain meat products. OTA is predominantly synthesized by *Aspergillus ochraceus* and *Penicillium verrucosum*. *Aspergillus carbonarius* and *Aspergillus niger* are capable of producing ochratoxin A, particularly in grapes and wines ^(۱۲). OTA exhibits remarkable stability at elevated temperatures. It exhibits neurotoxicological consequences.

Fumonisin B^۱ (FB^۱) is the predominant and most toxic among the over ۱^۵ known kinds of fumonisins. FB^۱ can induce many harmful consequences in animals, including neurotoxicity, hepatotoxicity, and nephrotoxicity. FB^۱ is a mycotoxin synthesized by *Fusarium* species, including *Fusarium verticilloides* and *Fusarium proliferatum*. It is present in numerous crops, predominantly in corn and maize-derived food or feed products^(۱۳).

Trichothecene mycotoxins constitute a category of sesquiterpenoid metabolites synthesized by *Fusarium* species. They typically contaminate grains and pose a risk to human and animal health. Approximately ۲۰۰ tetracyclic sesquiterpenoids have been recognized within the trichothecene category^(۱۴).

Deoxynivalenol (DON) has been identified as a contaminant in cereal crops including barley, wheat, and maize, along with their derivatives. It is predominantly generated by *Fusarium* species. DON may induce toxic and immunotoxic consequences in animal species. It is a powerful inhibitor of protein synthesis^(۱۵).

Zearalenone is a β -resorcylic acid lactone synthesized by various species of *Fusarium*, including *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium cerealis*, *Fusarium equiseti*, and *Fusarium semitectum*. This mycotoxin contaminates grains like maize and wheat, posing several risks to people and animals, including cytogenetic toxicity, reduced fertility, embryotoxicity, and immunotoxicity^(۱۶).

Bacteria

Numerous bacterial species possess the capability to breakdown mycotoxins, including lactic acid bacteria and various other species. *Tetragenococcus halophilus*, *Rhodococcus erythropolis*, and *Mycobacterium fluoranthenorans* have demonstrated the ability to degrade AFB₁; *Pediococcus parvulus* and *Lactobacillus acidophilus* are effective in the biocontrol of OTA, AFB₁, and AFM₁; *Bifidobacterium animalis* is beneficial for patulin management; *Pseudomonas otitidis* and *Bacillus velezensis* Strain ANSB-۱E can detoxify ZEN. The degradation process is influenced by various parameters, including incubation duration, medium, microorganism type, bacterial cell concentration, and pH level^(۱۷-۱۹).

Numerous microorganisms have been documented to possess the capability to breakdown many mycotoxins. AFB₁ and ZEN have been concurrently destroyed by a microbial consortium, TADC۷; *Rhodococcus pyridinivorans* strains (K۴۰۸ and AK۳۷) can simultaneously degrade AFB₁, T-۲, and ZEN, whereas certain lactic acid bacteria strains are capable of degrading several mycotoxins. *Pseudomonas fluorescens* strain ۳JW₁ can both digest AFB₁ and limit the synthesis of AFB₁ by *Aspergillus flavus*. It decreases the production of AFB₁ by *Aspergillus* by ۹۷,۸٪, ۹۹,۴٪, and ۹۵,۸٪ in the culture media, peanut medium, and peanut kernels, respectively^(۲۰, ۲۱).

Consequently, mycotoxin biodegradation is an efficacious approach, although it is contingent upon many elements. Rigorous investigations are required for each biocontrol strain to ascertain the ideal circumstances for its application.

Yeast

Yeasts can detoxify mycotoxins through biodegradation, bioadsorption, or the suppression of mycotoxin synthesis^(۲۲).

The biodegradation process can occur using an enzyme extracted from yeast or by utilizing the yeast itself. Hong Cao et al. demonstrated the breakdown activity of aflatoxin B₁ by an oxidase enzyme derived from the fungus *Armillariella tabescens*. The degradability of aflatoxin oxidase has been demonstrated by high-performance thin-layer chromatography (HPTLC). The primary mechanism was believed to be the breaking of the bis-furan ring within the aflatoxin molecule. *Meyerozyma guilliermondii* has demonstrated the capability to regulate patulin in pears. The capacity of *Meyerozyma guilliermondii* to degrade patulin in pear wounds escalates with an increased concentration of yeast cells. The ideal temperatures for wounds and entire fruits are ۲۰ °C and ۴ °C, respectively^(۲۳).

Conversely, yeast biocontrol may encompass bioadsorption techniques. Certain *Saccharomyces* strains may eliminate OTA contamination through adsorption; the removal efficiency can be improved from ۴۵٪ to ۹۰٪ by applying heat treatment to the microbe and adjusting the medium to a lower pH. In a separate instance, the incorporation of sugar at a temperature of ۲۰ °C during OTA reduction by *Saccharomyces cerevisiae* increased the OTA reduction rate in a semi-synthetic medium^(۲۴).

Ultimately, yeast-mediated mycotoxin biocontrol may involve the suppression of mycotoxin synthesis. Ponsone et al. investigated the efficacy of various yeast strains isolated from Argentinean vineyards in inhibiting the growth of the ochratoxigenic *Aspergillus* strain Nigri and assessed their impact on OTA. This study revealed the inherent presence of biocontrol agents in the environment to mitigate fungal and mycotoxin issues. The findings indicated that these yeast strains may effectively inhibit the development of *Aspergillus carbonarius* and *A. niger*, as well as OTA buildup, by at least ۵۰٪ under varying water activity (aw) and temperature conditions^(۲۵).

Enzymes

Certain enzymes extracted from microbes or fungi has the capability to breakdown one or more mycotoxins. The Ery ۴ laccase from *Pleurotus eryngii* is capable of concurrently degrading AFB₁, FB₁, OTA, ZEN, and T-۲. Certain enzymes may detoxify only a single mycotoxin; for instance, *Armillariella tabescens* has been shown to possess the capability to degrade AFB₁. The degradation method is contingent upon the enzyme classification and the specific mycotoxins involved. Enzymes can convert the parent substance into a new entity or entirely degrade it^(۲۶).

Zeinvald-Lorestani et al. investigated the efficacy of a laccase enzyme on AFB₁. Under optimum conditions, ۱۷٪ of the total AFB₁ was destroyed by laccase after a duration of two days. The prooxidative characteristics and mutagenicity of the degraded product were inferior to those of AFB₁^(۲۷).

Bacillus amyloliquefaciens ASAG¹ can detoxify OTA by 98.0% after 24 hours of incubation and 100% after 48 hours. The carboxypeptidase isolated from the bacteria may degrade OTA by 41% and 72%, respectively, when incubated with the supernatant and the pure protein of the carboxypeptidase. (28)

A separate investigation demonstrated the impact of carboxypeptidases on OTA. Commercial protease A, commercial pancreatin, and an enzyme extract derived from *Aspergillus niger* MUM have demonstrated the capacity to degrade OTA to Ot, achieving reductions of 87.3%, 43.4%, and 99.8%, respectively, under ideal circumstances of pH 7.0 and a temperature of 37 °C after 20 hours (29).

Conclusions

The growing societal need for minimally processed and more natural food products, while maintaining their quality, safety, and shelf-life, has prompted inquiries into alternatives to chemical preservatives. In this respect, bacteria, fungus, and their metabolites serve as natural alternatives of interest for application in food as bioprotective agents to combat fungal deterioration and to meet consumer preferences and regulatory requirements. From a practical perspective, the disparity between the quantity of research and the availability of microbial cultures signifies a necessity for further facilitation of their application in food products. A primary component pertains to the essential function of in situ research utilizing modified fungal targets during the screening or validation of antifungal activity. Furthermore, the safety assessment, organoleptic neutrality, and stability of activity of the bioprotective cultures must be tested before commercialization. From a cognitive perspective, although antifungal agents have been extensively researched and typically exhibit synergistic effects, there remains a deficiency in understanding the comprehensive landscape of the molecules involved and their mechanisms of action. The integration of relevant biochemical analytical tools and omics methodologies could facilitate the elucidation of antifungal action mechanisms, perhaps uncovering novel targets for antifungal efficacy. Ultimately, if the identification of natural antifungal agents is crucial, it should be regarded as a component of best practices and within the HACCP framework as a hurdle technology to mitigate fungal deterioration.

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