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Sunita Lanje

Ph.D. Scholar, ICAR-National
Institute of Animal Nutrition
and Physiology, Bengaluru,
Karnataka, India

Hrishikesh Tekade

Ph.D. Scholar, ICAR- Indian
Veterinary Research Institute,
Izatnagar, Uttar Pradesh, India

Iqbal Hussain

Ph.D. Scholar, ICAR-National
Institute of Animal Nutrition
and Physiology, Bengaluru,
Karnataka, India

Microbial biotransformation: An innovative strategy to mitigate mycotoxin in feed

Sunita Lanje¹, Hrishikesh Tekade², Iqbal Hussain³

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ABSTRACT

Mycotoxins are harmful secondary metabolites predominantly synthesized by fungal genera such as *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium*. With hundreds of identified compounds, major variants including aflatoxins, ochratoxins, zearalenone, trichothecenes, and fumonisins pose severe threats to human and animal health due to their carcinogenic, teratogenic, mutagenic, and immunosuppressive properties. Global agricultural contamination is pervasive, causing substantial annual losses in food and feed supplies. Conventional physical and chemical decontamination approaches often present limitations, such as potential nutrient losses, environmental concerns, and residual toxicities. Consequently, biological detoxification utilizing microbes and enzymes has emerged as a promising, eco-friendly, and safe alternative. Microorganisms and specialized enzymes reduce mycotoxin toxicity through mechanisms like adsorption and biodegradation, specifically targeting structural functional groups such as lactone rings and epoxide structures. Regionally, studies highlight a high prevalence of mycotoxins in agricultural and animal feed supply chains such as extensive contamination reported across Indian feed surveys emphasizing the critical need for advanced detection, prevention, and biological control strategies to ensure food and feed safety.

Keywords: Biotransformation, Biodegradation, Functional Group, Mycotoxin

Introduction

Mycotoxins are toxic secondary metabolites produced mainly by fungi belonging to the genera *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium*. More than 300–400 mycotoxins have been identified, among which aflatoxins (AFs), ochratoxins (OTs), zearalenone (ZEN), trichothecenes, and fumonisins (FUMs) are the most important. These compounds can pose serious risks to both animal and human health because of their carcinogenic, teratogenic, mutagenic, and immunosuppressive properties. According to the research of the Food and Agriculture Organization of the United Nations (FAO), global crop products exhibited a mycotoxin contamination rate that could reach as high as 25%, with approximately 2% resulting in complete loss of nutritional and economic properties (Eskola *et al.*, 2020) [1]. Contamination may occur at different stages, including crop production, harvesting, processing, storage, and preparation of food and feed materials (Selvaraj *et al.*, 2015) [2]. The presence of mycotoxins in food and animal feed has been reported worldwide, with developing countries often facing a particularly high risk due to favorable climatic conditions and inadequate storage practices (Lv *et al.*, 2016) [3]. Therefore, effective strategies for the detection, prevention, and detoxification of mycotoxins are essential for protecting food and feed safety, animal productivity, public health, and the economic sustainability of related industries.

Several methods have been developed to detoxify mycotoxins, including physical, chemical, and biological approaches. Chemical treatments use acids, alkalis, oxidizing agents, or aldehydes to alter mycotoxin structures; however, they may leave harmful residues in food and feed (Zhu *et al.*, 2016) [4]. Physical methods, such as using adsorbents, may also be less effective and can lead to the loss of important nutrients (Zhu *et al.*, 2017) [5]. Biological detoxification is considered a promising alternative because it is specific, safe, cost-effective, and environmentally friendly. It uses microorganisms or enzymes to convert mycotoxins into less toxic or non-toxic compounds under mild conditions (Ji *et al.*, 2016) [6]. Yeasts, moulds, bacteria, algae, and actinomycetes have been studied for their ability to degrade mycotoxins in feed (Hathout *et al.*, 2014) [7]. However, the safety of the resulting metabolites must be carefully assessed, as some may remain toxic (Adegoke *et al.*, 2023) [8].

Bacteria and fungi capable of degrading mycotoxins are mainly isolated from animal digestive systems, contaminated plant materials, and soil (McCormick, 2013) [9]. Microorganisms reduce mycotoxin levels through two main mechanisms: adsorption and biodegradation (Loi *et al.*, 2018) [10]. Yeasts and *Lactobacillus* species can bind mycotoxins, while other microorganisms produce enzymes that transform them into less

Corresponding Author:

Hrishikesh Tekade

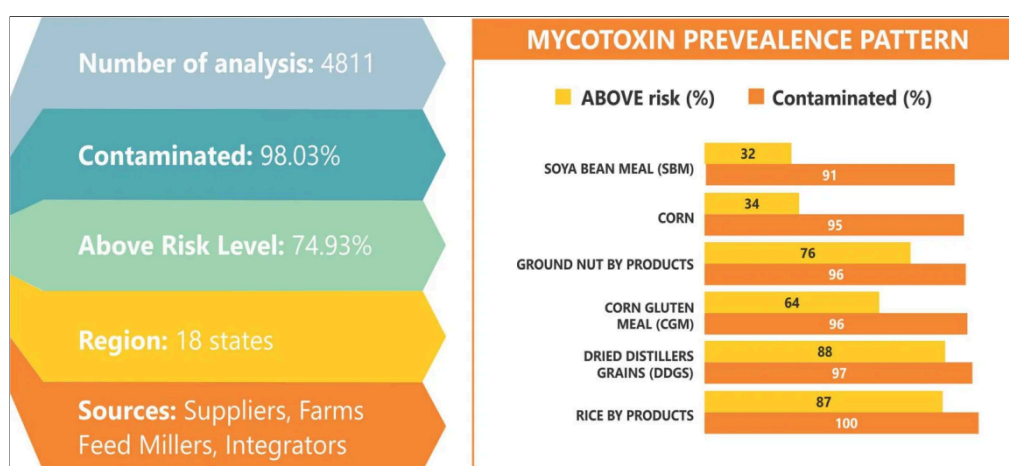
Ph.D. Scholar, ICAR- Indian
Veterinary Research
Institute, Izatnagar, Uttar
Pradesh, India

toxic compounds (Yao *et al.*, 2020) [11]. Enzymatic transformation is considered one of the most promising methods for mycotoxin detoxification. However, only a limited number of microbial enzymes involved in mycotoxin degradation have been identified and characterized (Loi *et al.*, 2017) [12]. Discovering and studying these enzymes is important for understanding the degradation process and developing effective detoxification technologies. Therefore, isolating new mycotoxin-degrading enzymes is essential for improving the safety of food and animal feed. According to Mesterházy *et al.*, 2020 [13] contamination with mycotoxins is responsible for the wastage of approximately 1.3 billion metric tons of food annually, equivalent to one-third of global food production.

Mycotoxin prevalence in India

Mycotoxin contamination is widely reported in Indian animal feeds, especially cattle and poultry feeds. Aflatoxins are among the most prevalent contaminants, followed by fumonisins, ochratoxin A, and

zearalenone. Surveys have detected aflatoxins in a considerable proportion of dairy concentrate feeds, and contamination with multiple mycotoxins is also common. According to a comprehensive multi-state survey by Cargill encompassing 4,811 feed and ingredient analyses across 18 states sourced from suppliers, farms, feed millers, and integrators mycotoxin contamination is pervasive in the animal feed supply chain. The survey revealed an overall contamination rate of 98.03%, with 74.93% of samples exceeding acceptable risk thresholds. Prevalence varies by ingredient type; protein and energy sources such as rice by-products and dried distillers grains (DDGS) exhibited alarmingly high contamination rates of 100% and 97% respectively, alongside substantial proportions testing above safety risk levels (87% and 88%). Similarly, corn and soybean meal (SBM) showed widespread contamination (95% and 91%), underscoring the continuous vulnerability of core feed ingredients to mycotoxicological challenges (Cargil., 2022) [14].

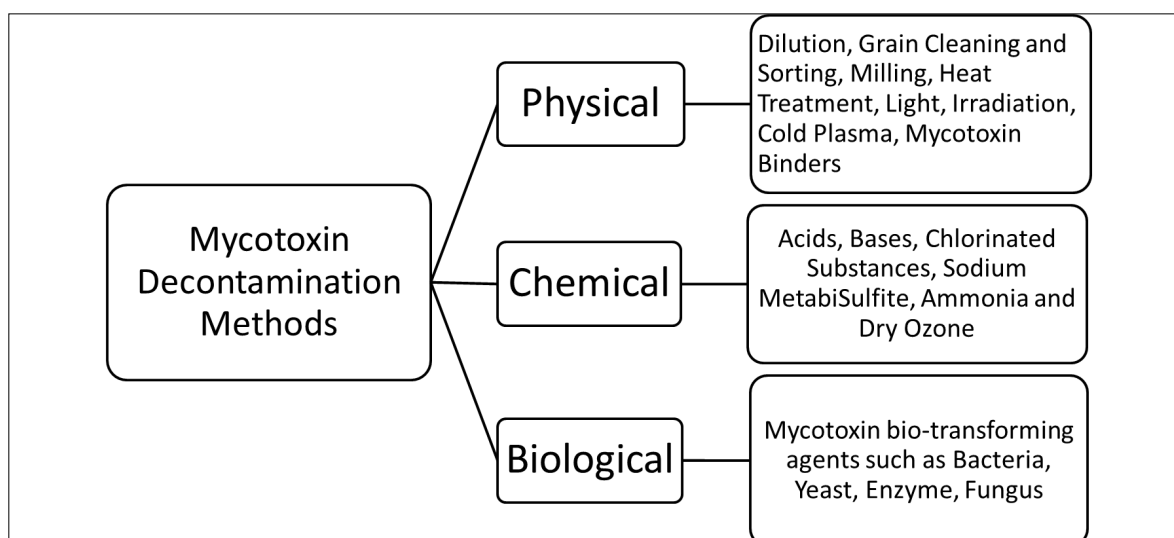


Characteristic of mycotoxin (Bennet *et al.*, 2003; Eskola *et al.*, 2020, Ndiaye *et al.*, 2022)

- Low molecular weight
- Heat - stable substances
- Unlike many bacterial toxins, non - antigenic; exposure does not induce a protective immune response

- Many are active at low dietary levels
- Specific target organs or tissues affected
- Toxic effects include immunosuppression, mutagenesis, teratogenesis and carcinogenesis
- Accumulation in tissues of food - producing animals or excretion in milk may result in human exposure

Mycotoxin decontamination methods



Contamination can be substantially lowered through physical techniques such as washing, sorting, dehulling, sieving, and thermal processes like boiling, roasting, microwave heating, infrared treatment, and extrusion which eliminate infected portions or break down toxins,

with extrusion showing particular success against ochratoxin A (Lee, 2020) [15]. Additionally, adsorption methods utilize binding agents like bentonite and cyclodextrins to capture and clear out mycotoxins such as aflatoxin M1, *Alternaria* toxins, and ochratoxin A, though

these procedures can sometimes compromise overall food quality (Lemli *et al.*, 2022) [16].

Chemical techniques—utilizing alkaline compounds, oxidizing agents, salts, and acids—are frequently employed to neutralize toxins in animal feed and food supplies (Varga *et al.*, 2005) [17]. These methods work by converting dangerous mycotoxins into safer derivatives. For example, lactic acid breaks down aflatoxin B1 (AF_{B1}) by hydrolyzing its lactone ring to create aflatoxin D1 (AF_{D1}), a much less toxic form (Jubeen *et al.*, 2020) [18]. Similarly, citric acid breaks down AF_{B1} under acidic conditions by forming a beta-keto acid intermediate, which then undergoes decarboxylation and lactone ring hydrolysis. This process also yields AF_{D1} , which is far less hazardous than the original toxin, displaying roughly 18 times lower toxicity and reduced mutagenic activity (Méndez-Albores *et al.*, 2005) [19]. Although chemical treatments work well, they create worries about environmental safety, residue buildup, and overall health protection. Meanwhile, using pesticides and fungicides stays heavily debated because they linger in biological systems and carry potential toxicological risks (Daou *et al.*, 2021) [20]. Due to the drawbacks of traditional approaches, researchers have been prompted to create greener, more advanced technologies for managing mycotoxins in the food supply.

Microbial biotransformation

Recently, biological decontamination techniques such as microbial degradation and adsorption—have gained preference over traditional chemical and physical methods (Agriopoulou *et al.*, 2020) [21]. These techniques offer a safer and more effective way to neutralize mycotoxins while safeguarding the nutritional integrity of feed and food products. Microbial adsorption involves utilizing microorganisms to bind or take up toxins for removal, whereas microbial degradation relies on active compounds secreted by microbes to alter the primary structures of mycotoxins. Consequently, these toxins are converted into significantly less hazardous or completely harmless derivatives (Guan *et al.*, 2021) [22].

Enzymatic degradation offers several distinct benefits over using live microorganisms, including simpler application, high repeatability, consistent performance, superior efficiency, and strict specificity (Ndiaye *et al.*, 2022) [23]. Additionally, this treatment can take place under mild conditions such as optimal pH and temperature ranges with minimal loss of dietary nutrients (Ndiaye *et al.*, 2022) [23]. Moreover, research indicates that biological enzymes can help mitigate tissue or bodily damage triggered by mycotoxins (Prasad *et al.*, 2023) [24]. Consequently, mycotoxin-degrading enzymes hold strong promise for future development, though their application requires a thorough understanding of their structures, degradation pathways, and resulting byproducts (Ndiaye *et al.*, 2022) [23].

Biological detoxification offers an eco-friendly method for reducing mycotoxin levels in animal feed and food by utilizing microbes and their enzymes to clear out, bind, or alter toxins into safer byproducts (Pandey *et al.*, 2023) [25]. This generally happens through adsorption—where toxins attach to microbial cell walls to lower their availability—and biodegradation, which relies on enzymatic conversion (Zhou *et al.*, 2017) [26]. Effective detoxifying microbes include species like *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Bacillus licheniformis*, and *Lactobacillus* spp., which utilize processes like reduction, oxidation, hydrolysis, and binding. Specialized enzymes such as oxidoreductases, peroxidases, laccases, esterases, and lactonases focus on specific structural parts of mycotoxins to transform them into less hazardous compounds. Furthermore, the genes driving these functions are frequently grouped into gene clusters, enabling smooth, efficient breakdown via pathways like lactone ring adjustments and amide bond hydrolysis (Liu *et al.*, 2024) [27].

Common functional groups of mycotoxins and their

possible transformation by microbial enzymes

Aflatoxins

Produced by specific fungi, aflatoxins (AFs) are dangerous secondary metabolites capable of causing severe short- and long-term health issues in humans and animals. While scientists have discovered more than 20 types of aflatoxins, feed supplies typically encounter just four naturally: B1, B2, G1, and G2. Their names are derived from how they glow either blue or green under ultraviolet light, as well as how they travel across thin-layer chromatography plates (Giray *et al.*, 2007) [28]. Among the various aflatoxins, AF_{B1} stands out as exceptionally dangerous and is classified as a Group 1 natural carcinogen (Adebo *et al.*, 2017) [29]. Meanwhile, aflatoxins M1 and M2 develop as hydroxylated byproducts when animals process aflatoxins B1 and B2, subsequently appearing in their milk and posing contamination risks to dairy items. The primary culprits behind aflatoxin production are the mold species *Aspergillus flavus* and *A. parasiticus*, which synthesize these compounds via the polyketide pathway (AboDallam *et al.*, 2020) [30].

Because the lactone ring of aflatoxin B1 (AF_{B1}) is readily hydrolyzed, it serves as an accessible target for breakdown. Gonzalez *et al.* (2019) cloned a 753 bp PCR product from *Bacillus subtilis* RC1B, *B. cereus* RC1C, and *B. mojavensis* RC3B capable of degrading AF_{B1} , confirming it as the *aiiA* gene that codes for N-acyl-homoserine lactonase. This discovery suggests that the lactone ring on AF_{B1} is the primary site attacked by the degrading enzyme. Research by Liu *et al.* (2021) demonstrated that laccase safeguards animals against toxicity by mitigating inflammation and oxidative stress triggered by AF_{B1} , lowering liver cell apoptosis, and minimizing pathological harm to both kidney and liver tissues. Furthermore, biological enzymes can effectively protect the body from AF_{B1} -induced damage.

B. Ochratoxins

Ochratoxins (OTs) are a group of mycotoxins frequently discovered in food and animal feed, synthesized primarily by *Penicillium* and *Aspergillus* fungi, such as *P. verrucosum*, *P. nordicum*, *A. ochraceus*, *A. carbonarius*, and *A. niger* (Kumar *et al.*, 2020) [31]. Structurally, they are coumarin derivatives that include ochratoxins A (OT_A), B (OT_B), and C (OT_C), with OT_A standing out as the most widespread and hazardous variant (Bayman *et al.*, 2006) [32]. Ochratoxin frameworks consist of a dihydroisocoumarin structure connected to a phenylalanine molecule through an amide bond. Because ochratoxin A (OT_A) shares a structural resemblance with phenylalanine—an essential amino acid—it can competitively block phenylalanine hydroxylase activity within the kidneys and liver, consequently halting related protein synthesis. The enzymes that can be used to degrade OT_A are mainly amidohydrolases, carboxypeptidases, and lipases.

OT_A can be biodegraded by hydrolyzing the amide bond that connects the L-b-phenylalanine molecule to the OT_a part of the molecule. Crude lipase products from *A. niger* have been reported to hydrolyze OT_A via its amide bond (Abrunhosa *et al.*, 2007) [33]. Moreover, their ability to degrade OT_A was found to be higher than that of CPA at pH 7.5 and 37 °C. Elhady *et al.* (2022) [34] found that the use of *B. subtilis* fermentation extract conferred a significant improvement on immune toxicity and nephrotoxicity in broilers. This protective effect can be attributed to the CP and other proteases in the fermentation extract of *B. subtilis*, which can hydrolyze OT_A into non-toxic OT_a .

C. Trichothecenes

Filamentous fungi including *Aspergillus*, *Penicillium*, *Fusarium*, *Spicellum*, and *Stachybotrys* naturally produce trichothecenes (TCNs), with *Fusarium* serving as the most frequent source of contamination in grains, vegetables, and animal feed (Polak-Sliwinska *et al.*, 2021) [35]. As tetracyclic sesquiterpenoid epoxides, TCNs comprise a massive family of over 200 toxins that share a core 12, 13-epoxytrichothec-9-ene (EPT) structure (Khaneghah *et al.*, 2020) [36]. Based on the specific substitution patterns on their EPT ring, they are

divided into four groups (types A, B, C, and D), with types A and B drawing the most concern due to their potent toxicity (Vander Fels-Klerx *et al.*, 2010) [37]. Common type A TCNs feature compounds like T-2 toxin (T-2), HT-2 toxin (HT-2), and neosolaniol (NEO), whereas type B TCNs are best represented by deoxynivalenol (DON) and its acetylated variants (McCormick *et al.*, 2011) [38]. Ultimately, their toxicity which disrupts the synthesis of proteins, RNA, and DNA relies directly on the presence of the 12, 13-epoxide ring, the 9-10 double bond, and acylated side groups (Khaneghah *et al.*, 2020) [36].

De-epoxidation of trichothecenes is mainly documented in anaerobic bacteria found in soil environments or the digestive tracts of ruminants and poultry. One such strict anaerobe, the gram-positive *Eubacterium* strain BBSH 797 (marketed as Biomin BBSH® 797), was isolated from rumen fluid and is known to reductively de-epoxidize the C12,13 epoxide ring in Type A and Type B trichothecenes like T-2 toxin and DON (Fuchs *et al.*, 2002) [39]. Additionally, researchers have identified and recombinantly expressed the epimerization enzymes from *Devosia mutans* 17-2-E-8 in *E. coli*, noting that the process begins with an oxidation step catalyzed by DepA—a Type I pyrroloquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH) specific to DON epimerization (Carere *et al.*, 2018a) [40].

D. Fumonisin

Fumonisin (FBs) are mycotoxins derived from polyketides, primarily synthesized by *Fusarium* molds that frequently contaminate corn and other crops (Li *et al.*, 2012) [41]. Structurally, FBs closely mimic long-chain sphingolipid bases like sphinganine and sphingosine key intermediates in sphingolipid metabolism. By inhibiting ceramide synthase and halting the synthesis of complex sphingolipids, they trigger diverse pathological issues in humans and animals (Silva *et al.*, 2010) [42]. Their toxic mechanism relies heavily on the presence of tricarballic acid (TCA) side groups and a free amino group; research shows that removing these chemical groups substantially lowers both plant and mammalian toxicity (Voss *et al.*, 2007) [43]. *Sphingopyxis* sp. strain MTA144 converts *HFB*₁ into 2-keto-*HFB*₁ via deamination, using a pyridoxal phosphate-dependent aminotransferase with pyruvate serving as an amino acceptor (Heinl *et al.*, 2011) [44]. Additionally, certain fumonisin-producing *Aspergillus niger* strains accumulate deaminated fumonisin B1 within their culture supernatants (Garnham *et al.*, 2020) [45]. This conversion is driven by a monoamine oxidase, which is also capable of deaminating fumonisin derivatives FB₂ and FB₃, showing the highest activity toward FB₂.

E. Zearalenone

Zearalenone (ZEA) is a nonsteroidal mycotoxin frequently found as a contaminant across various cereal crops (Rai *et al.*, 2018) [46]. As a secondary metabolite, it is produced by multiple *Fusarium* mold species that typically thrive on plants grown in temperate regions, including corn, maize, and other grains (Al-Jaal *et al.*, 2019) [47]. Because ZEA functions as a xenoestrogen with a chemical structure resembling natural estrogens, it can attach to estrogen receptors and trigger hormonal disruptions, potentially causing reproductive disorders in both humans and livestock (Kowalska *et al.*, 2016) [48]. The lactone ring within its molecular structure is essential for its toxicity; studies indicate that breaking this ring can dramatically decrease its harmful effects (Popiel *et al.*, 2014) [49].

Three primary classes of enzymes—peroxidase, lactase, and laccase—are capable of breaking down zearalenone (ZEN). ZEN degradation occurs through various pathways encompassing processes such as reduction, hydrolysis, and glycosylation. Currently, the principal mechanism driving enzyme-mediated ZEN breakdown involves using laccase or lactonase to disrupt ZEN's lactone ring structure, which may also be accompanied by the modification or oxidation of the C2/C4 hydroxyl groups and the C6' ketone group. Xu *et al.* (2020) [50] isolated and characterized a vital gene (*BAMF_RS30125*) from *Bacillus amyloliquefaciens* H6 that has the ability to break down zearalenone

(ZEN). Following heterologous expression and protein sequence analysis, they determined that *BAMF_RS30125* functions as a protease classified within the CoA thioesterase YBGC/FADM family. Designated as ZTE138, this protein is capable of cleaving ZEN's lactone ring by disrupting its lactone bond.

F. Deoxynivalenol

Deoxynivalenol (DON), frequently referred to as vomitoxin, is a trichothecene mycotoxin synthesized by *Fusarium* fungi and predominantly occurs in cereal grains like corn, barley, and wheat. Consuming even small amounts of DON can impair human and animal immune systems and compromise intestinal barrier integrity. Furthermore, DON triggers cytotoxicity through mechanisms that induce apoptosis, autophagy, oxidative stress, and the suppression of associated signaling pathways. Deoxynivalenol (DON) acts as a powerful inhibitor of protein synthesis by blocking the aminoacyl site on ribosomes, which obstructs aminoacyl-tRNA binding and halts peptidyl transferase activity. This ribosomal binding occurs via hydrogen bonding involving the C12 and C13 epoxide rings, alongside van der Waals forces acting on the C3 hydroxyl group and the C9–C10 double bonds. Consequently, the primary toxic functional groups in DON are identified as the C3 hydroxyl group and the epoxy ring located at the C12–C13 position (Yao *et al.*, 2020) [11].

Several bacterial strains have been identified that can alter the epoxide structure of deoxynivalenol (DON) to produce the metabolite DOM-1, including *Slackia* sp. D-G6, *Eggerthella* sp. DII-9, *Bacillus* sp. LS100, and *Eubacterium* sp. BBSH 797 (Sun *et al.*, 2023) [51]. In animals, DOM-1 is typically regarded as a non-toxic byproduct, indicating that de-epoxidation serves as an effective route for breaking down DON (Guerrero-Netro *et al.*, 2021) [52]. Abraham *et al.* (2022) [53] identified DepBRleg, a novel enzyme belonging to the AKR18 aldo-keto reductase family from *Rhizobium leguminosarum*. This enzyme can utilize both NADPH and NADH as coenzymes, though its catalytic efficiency is substantially lower—by a factor of 40—when using NADH compared to NADPH. Furthermore, the study highlighted the potential functions of specific amino acid residues—Gln-294, Arg-290, and Lys-217—in determining NADPH specificity.

Conclusion

Mycotoxin contamination remains a critical global challenge, threatening food and feed safety, animal productivity, and public health. Traditional physical and chemical detoxification methods are frequently limited by high costs, potential nutrient degradation, and environmental or residue concerns. As a result, biological detoxification utilizing microorganisms and specialized enzymes has emerged as a safer, highly specific, and eco-friendly alternative. By targeting vulnerable structural components such as lactone rings and epoxide groups through pathways like biodegradation and adsorption, biological agents effectively convert hazardous mycotoxins into non-toxic or less harmful derivatives. However, successfully integrating these advanced biocatalytic and microbial solutions into commercial agricultural systems requires a thorough understanding of enzymatic structures, specific degradation pathways, and the safety profiles of resulting metabolites. Continued research and discovery in mycotoxin-degrading enzymes will be vital for safeguarding future food security and industrial sustainability.

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