



## YEASTS AS BIOLOGICAL DETOXIFIERS OF MYCOTOXINS IN AGRICULTURAL PRODUCE – A Review

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**Abstract:** *Some filamentous fungi produce mycotoxins as their secondary metabolites. They are highly toxic compounds that are extremely harmful to agricultural produce such as grains, nuts, and fruits. Most mycotoxins pose serious threats to human and animal health. The increase in population worldwide and the enlargement of the major planting area of agricultural products every year, as also increase mycotoxin contamination of these products. The common techniques adopted to lower the limit to a safe level include chemical treatments (acids, bases), physical treatments (boiling, roasting), biological control (plants, animals. and microorganisms). To prevent the use of antifungal chemicals, biocontrol using microbes such as bacteria and fungi could be a feasible alternative. There is a need for biological control strategies using microorganisms such as yeasts. They are safe, non-toxic, without side effects, and maintain the nutritional quality of agricultural products. The cell walls of yeast comprise polysaccharides of glucose (glucans), glycosidic proteins, chitin, and mannans. The mode of action of detoxification by yeast is due to the attachment of mycotoxins to the components of its cell wall. The enzymatic degradation activity that occurs biologically includes glucosylation, acetylation, ring cleavage, hydrolysis, decarboxylation and deamination, caused by the enzymes produced from the yeasts. The use of yeast strains having high mycotoxin binding effects could be a necessary alternative for detoxification of mycotoxins in agricultural produce. This review aimed to summarize the detoxification of mycotoxins by yeasts in diverse dimensions: biodegradation, bioadsorption and the inhibition of mycotoxin production.*

**Keywords:** *Bioenzymatic degradation, Mycotoxins, Biological control, Grains*

### 1. Introduction

The secondary metabolites produced by filamentous fungi, known as mycotoxins mainly belong to the genera *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium*. There are about 100 mycotoxins known to contaminate farm products, such as cereals, nuts and fruits [1-3]. The various factors that affect production, contamination of foods and feeds by mycotoxins occur interdependently to affect fungal colonization and/or production of the mycotoxins. They include physical factors (moisture, relative humidity, temperature and mechanical damage), chemical factors (carbon dioxide, oxygen, composition of substrate, pesticide and fungicides), and biological factors (plant variety, stress, insects, spore load). The biological factors

have been further subcategorized into intrinsic factors, including fungal species, strain specificity, strain variation, and instability of toxigenic properties [4]. This is greatly affected by prevailing weather conditions such as optimum temperatures, increased humidity, and increased rainfall that facilitate fungal growth and mycotoxin production [5]. Its contamination can occur throughout the food value chain from pre-harvest through postharvest. It was reported that 25% of agricultural products may be contaminated by mycotoxins globally, resulting in approximately 2% total loss of nutritional and economic attributes, and causing diverse toxic effects on human and animal health even at low levels [6, 7]. Moreover, it was reported that mycotoxins are found in 60-80% of agricultural

products, this increase may likely be due to the improved detection methods and the impact of climate change. Inclusively, over 50% of agricultural produce contain more than one type of mycotoxin [6].

## 2. Major fungal mycotoxins in agricultural produce

The most essential agro-economic and public health types of mycotoxins that can contaminate agricultural produce meant for food and feed are: Aflatoxins, Ochratoxin A (OTA), Fumonisin (Immunotoxic); Zearalenone (ZEA) (Estrogenic and Reproductive Toxins); Trichothecenes (Neurotoxic). Deoxynivalenol (DON) and Nivalenol; Patulin [8].

There is an increase in susceptibility of different agricultural products, such as maize and groundnuts, to mycotoxin contamination. These products are major sources of human and animal exposure to risks associated with mycotoxins, such as aflatoxin and fumonisin [9]. These mycotoxins not only pose significant food safety concerns but also greatly lower their economic value, thus affecting the financial returns of farmers [10]. Aflatoxin is a group of mycotoxins that are difuranocoumarin derivatives produced via a polyketide pathway by several strains of *Aspergillus flavus*, *Aspergillus parasiticus* and the uncommon *Aspergillus nomius*, but other species, such as *A. pseudotamarii*, *A. parvisclerotigenus*, and *A. bombycis* of section *Flavi*; *A. ochraceoroseus* and *A. rambellii* from section *Ochraceorosei*; and *Emericella astellata* and *E. venezuelensis* from Nidulatan, have also been reported as aflatoxin producers [11]. At least 18 different types of aflatoxin have been reported, but the six main types are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1), and aflatoxin M2 (AFM2) [12]. Aflatoxin B1, B2, G1, and

G2, with aflatoxin B1 being the most toxic [5]. Aflatoxin M1 and M2 are commonly found in the milk of animals that have consumed contaminated feed [13]. The level of metabolism and the final products formed determine the differences in species' reaction to Aflatoxin. The toxicity, carcinogenicity, and mutagenicity efficacy of the different aflatoxin types varies in descending order from B1 > M1 > G1 > B2 > M2 > G2 [12, 14].

Aflatoxins have carcinogenic, haemorrhagic, mutagenic, tremorgenic, genotoxic, teratogenic, immunosuppressive, and growth-reducing effects in both humans and animals, where the liver is the main target organ [5]. There are several official methodologies to detect aflatoxin contamination in food samples; Enzyme-Linked Immunosorbent Assay (ELISA) being the most commonly used method; then High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectroscopy (LC-MS), and Thin Layer Chromatography (TLC) [15,16]. More Rapid methods for detecting aflatoxins are now available, such as Spectroscopic techniques for detecting quality and safety [17]; Hyperspectral imaging (HSI) technique for detection of fungal contamination in maize kernels [18]; Fourier transform-infrared (FT-IR), Near-infrared (NIR) and Raman spectroscopic techniques for evaluation of grain and grain-based products [19, 20].

Ochratoxin A (OTA) is mostly found in grapes and grape-derived products, but it can also occur in other food products such as coffee, spices, beer, and in some meat and meat products. OTA is mainly produced by fungi, *Aspergillus ochraceus* and *Penicillium verruscum* [21]. The kidney is the major target organ. *Aspergillus carbonarius* and *Aspergillus niger* can also produce OTA, especially in grapes and wines found mostly in European

countries. It has both neurotoxicological and nephrotoxic effects as well as the mammary functions [22, 23]. Thus, the maximum OTA levels in cereals and dried vine fruits are regulated by the EU since March, 2002 [24, 25].

Fumonisin (FUMs) are toxins produced by several *Fusarium* species, in which more than 15 types have been identified. Fusarium B1 is the most common and toxic, causing neurotoxicity, hepatotoxicity, and nephrotoxicity in animals [26, 27]. FB1 is a mycotoxin produced by *Fusarium species* such as *Fusarium verticillioides* and *Fusarium proliferatum*. It is found mostly in corn and corn-based food or feed. The toxicity of FUMs shows their ability to destroy sphingolipid metabolism by hindering the enzyme ceramide synthase, an enzyme that influences the acylation of sphinganine and sphingosine. It is well established that the FUMs cause equine leukoencephalomalacia and porcine pulmonary oedema, which are carcinogenic in experimental rats [28].

Zearalenone (ZEA) is a phenolic resorcylic acid lactone produced by several species of *Fusarium*, including *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium cerealis*, *Fusarium equiseti*, and *Fusarium semitectum* [29]. This mycotoxin infects cereals such as maize and wheat and can be hazardous to humans and animals, such as cytogenetic toxicity, reducing fertility, embryotoxicity, and immunotoxicity [30]. ZEA is a non-steroidal oestrogen and its major metabolites D-zearalenol and E-zearalenol reflect significant oestrogenic activity in humans and animals. Pigs are very sensitive to ZEA whereas poultry are very tolerant. Trichothecenes (TCT) are a group of sesquiterpenoid compounds produced by *Fusarium species*. They usually contaminate grains and pose a threat to human and animal well-being [31]. Around

200 tetracyclic sesquiterpenoids have been identified as part of the trichothecene group. Deoxynivalenol (DON) and nivalenol (NIV), and T-2 Toxin (T-2) are the more significant trichothecenes [32]. The major toxic effect of TCT mycotoxins occurs as primary inhibition of protein synthesis at the cellular level such as those cells lining the gastrointestinal tract, the skin, lymphoid and erythroid cells [28].

Patulin (PAT) is a mycotoxin produced mostly by *Penicillium*, *Byssoschlamys*, and *Aspergillus species* [33]. Patulin contamination can cause a lot of damage to animals, such as cancer, by affecting different organs, including the kidney, liver, and intestine. It can contaminate agricultural produce such as fruits and vegetables, especially apples and apple-based products [34]. Conventional techniques for decontamination mainly involved physical and chemical methods which result in challenges such as incomplete patulin degradation, high technical cost, and reduction in fruit quality.

### 3. Socio-economic impact of mycotoxin contamination

Mycotoxicoses in humans or animals are characterized as food or feed related, non-contagious, non-transferable, non-infectious, and non-traceable to microorganisms other than fungi. Once the contaminated food or feed is removed the clinical symptoms also decrease [28]. The effect caused by mycotoxicosis in relation to human wellbeing depends on various factors such as age, weight, gender/sex, type and amount of food consumed, infectious agents contacted, and the presence of other types of mycotoxins and bioactive substances. Exposure to small quantities of aflatoxins by oral, respiratory, or absorption by the skin can cause cancer, liver diseases, teratogenic and genetic mutations [35]. It was reported that AFs and

FBs are the most relevant mycotoxins, resulting in recognized adverse effects in fetuses and children. Getting in contact with AFs during embryo development is associated with fetal growth reduction, while being in contact with FUMs increases the risk of neural tube defects in newborn babies [36]. Babies are affected through breast milk (AFM1), corn infant formulas and baby foods containing mycotoxin-contaminated ingredients such as milk from animals, rice, oat and soybean protein [36]. Toxicogenic fungi-contaminated food may pose hazardous effects to the well-being which includes stunted growth, compromised immune function, vomiting, and gastroenteric and carcinogenic diseases at acute and chronic states [3]. Human and livestock health is adversely affected by the consumption of mycotoxin-contaminated food products, which affect the marketability of food commodities and raise food safety concerns [37]. It is estimated that more than five billion people are exposed to mycotoxins daily through unknown pathways and consume contaminated foods every day [1]. The economic impact of mycotoxins includes loss of human and animal life, increased health care and veterinary care costs, reduced livestock production, disposal of contaminated foods and feeds, and investment in research and applications to reduce the severity of mycotoxin level [38]. Mycotoxins are responsible for causing a wide range of detrimental effects in animals. The economic impact of mycotoxins due to decreased animal productivity, higher incidence of disease (caused by immunosuppression and disruption to vital organs), and reduced reproductive ability is greatly higher than the impact on animal mortality. Mycotoxins also disrupt the functions of various organs and tissues at reduced concentrations, including the gastrointestinal tract, kidney,

or liver tissue, and the neurological, reproductive, and immune systems [39]. There is variation in the feedstuffs used and between and within animals, the mycotoxicosis severity from feed is different in many animal species [40]. For example, non-ruminants are sensitive to trichothecenes, while poultry and ruminants are less sensitive to some trichothecenes [41]. Poultry is also largely affected by both T-2 and DON but is very resistant to the estrogenic effects of ZEA [42].

Many countries and international organizations, such as the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the European Union (EU) through the European Food Safety Authority (EFSA) have set up strict controls of maximum residue levels of mycotoxin in foodstuffs because of their toxicity and effects on human health [43].

Mycotoxin takes a long time to break down, due to their heat and acid resistance and can remain in crops for long periods, even after physical signs of fungal infection have been removed [7]. So far, many preventive strategies have been implemented in practice at both pre-harvest and post-harvest stages, resulting in varying degrees of accomplishment. Although, decontamination strategies can be introduced when toxigenic fungi infect crops and accumulate mycotoxins in them. The common techniques adapted to lower the limit to a safe level include chemical detoxification (acids, bases, ozone, ammonia), physical treatments (adsorption, heat processing, UV irradiation), and biological processing (microbial transformation, fermentation, enzymatic hydrolysis). In either case, the nature of a particular toxigenic- fungus and the pH of the medium, amongst other factors, contribute to the success of the technique used [44].

Beneficial microorganisms can control mycotoxins by hindering the growth of toxin-producing fungi at pre-harvest and post-harvest. This improves the host's resistance and leads to the production of natural metabolites that prevent mycotoxin formation [45]. A recent trend is the use of bioremediation which is a decontaminant and also has the advantage of adding value without any harmful effect, thus, yeasts are known as characters of bioremediation [46]. Bioremediation is a novel and eco-friendly way, to detoxify contaminated substrates by removing or breaking down harmful compounds using the beneficial effects of living forms such as plants, animals and micro-organisms.

#### **4. Biocontrol yeasts**

Biological control agents defend crops from disease damage in various ways, without having a direct antagonistic contact with the pathogen in plant tissues. They may promote induced systemic resistance, release of antimicrobial metabolites, and pathogen inhibition through non-target effects. Microbial interactions involve a struggle for nutrients and space, triggering an indirect mode of action, fungal pathogens' parasitic relationships, biofilm formation, antifungal compounds production, stimulation of plant defense mechanisms, including reactive oxygen species (ROS) production may all contribute to the antagonistic nature of yeast filamentous fungi. Yeasts are unicellular, eukaryotic microbes; they obtain their nutrition by secreting various hydrolytic enzymes (proteases, glycosidases, lipases) to digest organic matter. Yeast could be either spent yeast or live yeast, both can be used for mycotoxin detoxification, but the efficacy and mechanism may be different: Spent yeast, also called inactivated or dead yeast, can bind to mycotoxins through physical and chemical reaction (adsorption

process), while live yeast cannot only adsorb mycotoxins but also degrade or transform them through enzymatic reactions. Some yeast species, such as *Saccharomyces cerevisiae*, have been shown to possess enzymes that can break down specific mycotoxins. Generally, live yeast may be more effective at detoxifying mycotoxins due to their enzymatic activity. However, spent yeast can still provide some level of mycotoxin binding potential and then detoxify. The various factors that can influence the effectiveness of yeast-mycotoxin detoxification include: species and strain of yeast, type and concentration of mycotoxins, prevailing environmental conditions (e.g., relative humidity, temperature, pH), presence of other microorganisms or available nutrients [47]. Yeast can also absorb amino acids and monosaccharides across the cell wall. The cell wall of yeast comprises  $\beta$ -1,3-Glucans, glycoproteins, mannoproteins, chitin microfibrils, while the intracellular chemical components of yeast cells include amino acids and oligopeptides, carbohydrates, inorganic salts, monosodium glutamate, ribonucleic acids (RNA), enzymes, and cofactors [48]. Yeast and yeast cell wall-derived components have mycotoxin adsorption efficacy, thereby reducing the detrimental effects of mycotoxin exposure on animal health and productivity especially, increasing nutrient absorption and decreasing the accumulation of mycotoxins in various organs [49-52]. The yeast cell wall exhibits a bilayered structure, the inner layer  $\beta$ -1,3-glucan and chitin microfibrils, and the outer layer,  $\beta$ -1,6-glucan network with N-glycosylated mannoproteins [53]. Studies reveal that mycotoxin removal is through adsorption to cell wall constituents, including mannans and polysaccharide  $\beta$ -D-glucans [49]. Yeast employs diverse mycotoxin detoxification mechanisms: Biodegradation

(enzymatic degradation), Bioadsorption (Non-covalent physical binding), Inhibition of mycotoxin biosynthesis [54].

#### 4.1. Biodegradation of Mycotoxins by Yeasts

The use of yeast or the enzyme obtained from yeast can be involved in the biodegradation method.

Prado et al. [55] reported that yeasts belonging to the genus *Saccharomycopsis* (*S. schoenii* and *S. crataegensis*) were used as biocontrol agents. It was observed that these yeasts were able to reduce aflatoxin B1 and G1 produced by *A. parasiticus* in peanuts. These results indicate that biocontrol with selected microbes could limit the spore dispersion and also decrease the mycotoxin production by phytopathogens in stored grains. Prado [56] examined *S. cerevisiae* YEF 186 against *A. parasiticus* in two peanut cultivars (IAC Runner and IAC Caiap' o) with two incubation periods (7 and 14 days) and two inoculation methods (yeast inoculated simultaneously or three hours before the pathogen). The authors revealed that the best reduction of aflatoxin B1 (74.4%) was obtained when the yeast was inoculated to the pathogen after 7 days. They concluded that this reduction was probably due to aflatoxin attachment to the cell wall of the yeast or aflatoxin degradation by yeast. In a study, the fungus *Armillariella tabescens* produces an oxidase enzyme that is involved in the degradation activity of aflatoxin B1 using high-performance thin-layer chromatography (HP-TLC) [57]. The cleavage of the bis-furan ring of the aflatoxin molecule is the major mode of action. The yeast *Meyerozyma guilliermondii* has been shown to have the ability to control patulin in pear. As the concentration of the cells of yeast increases, the patulin degradation efficacy of *Meyerozyma guilliermondii* in pear wounds also increases. The ideal temperatures for

the study are 20 °C and 4 °C in wounds, likewise, in whole fruits [58].

Recently, the molecular inhibitory mechanism of *M. guilliermondii* against *P. expansum* was explored when the protein expression profile and transcriptomic changes were studied. It was reported that the majority of proteins and genes, differentially expressed were involved in the synthesis of secondary metabolites, ATP, cellular basal metabolism, immune response to the environment, genetic information processing, and metabolism. There are many enzymes responsible for the synthesis of ATP; ATPase is considered the most important enzyme in ATP synthesis. It was anticipated that ATPase not only provided energy for the basic metabolism of *P. expansum*, but also was the major carrier for the life processes of *P. expansum*. The ATP activity of *P. expansum* treated with *M. guilliermondii* decreased; thus, the energy supply was reduced and the growth of *P. expansum* was inhibited [59]. The intracellular enzymes of *M. guilliermondii*, a short-chain dehydrogenase (SDR) was involved in the process of degradation [60]. The degradation efficacy of *M. guilliermondii* towards patulin would be a new approach for the patulin detoxification [60].

The yeast *Saccharomyces cerevisiae* undergoes degradation of zearalenone (ZEN) possibly involving enzymes such as enolase, NADH-cytochrome b5 reductase in a laboratory setting; and PRX1p. A non-conventional yeast *Rhodospiridium paludigenum*, can decrease the patulin constituents through an inducible, degrading enzyme located on its cell wall, producing a less harmful desoxypatulinic acid [61]. Studies revealed that there is decrease in aflatoxin production observed with *Saccharomyces boulardii* (72.8%) and *Saccharomyces cerevisiae* (65.8%) when applied singly during grains storage, while

there were synergistic effects in probiotic combinations, the highest aflatoxin reduction was obtained with *S. boulardii* + *L. delbrueckii* (96.1%), after which *S. boulardii* + *S. cerevisiae* (71.1 %), and *L. delbrueckii* + *S. cerevisiae* (66.7%,). All the probiotic strains used in this study retained viability in high numbers on the grains over 300 days, thus, revealing an innovative treatment for preventing aflatoxin contamination in peanut grains [62].

#### 4.2. Mycotoxin Absorption by Yeasts

Yeasts such as *Saccharomyces cerevisiae*, and yeast-based products can serve as mycotoxin binding agents, which can be incorporated into animal feed and human diets to bind and mitigate mycotoxin contamination. The mode of action of detoxification by yeast is due to the attachment of mycotoxins to cell-wall components. Mannan is one of the components of the yeast cell wall which plays a major role in mycotoxin binding. In research, using broiler chicks fed with naturally mycotoxin-contaminated feed (aflatoxin, ochratoxin, zearalenone and T-2 toxin) resulting in growth depression, the capacity of esterified glucomannan in antagonizing the toxic effects was investigated. Results show a significant reduction in growth suppression [54].

Additionally, *Saccharomyces cerevisiae* strains possess mycotoxin-binding potentials, capturing ochratoxin A and zearalenone. The removal of OTA by *Saccharomyces* strains was done by an adsorption mechanism. The mechanism depends on the OTA molecule's ionic attributes, the yeast membrane position, and the concentration of the biomass. The zearalenone binds to  $\beta$ -d-glucans on the yeast cell wall. Also, *S. cerevisiae* residues in beer have been shown to demonstrate mycotoxin-binding potential [63]. The highest removal rate was observed in zearalenone, 75–77% of the toxin was

effectively bound onto the cell wall. In another study by Petrucci *et al.* [64], the capacity of three strains of *S. cerevisiae* and *Saccharomyces boulardii* to capture Ochratoxin A (OTA) was investigated under conditions simulating gastrointestinal pathway, currently, the yeast *Sporidiobolus pararoseus*, which possess mycotoxin binding ability, was produced on a large-scale successfully targeting in animal feed additives applications [65].

#### 4.3. Inhibition of mycotoxin production

Studies indicate that specific yeast strains can inhibit the biosynthesis of ochratoxin A (OTA), a hazardous mycotoxin. Notably, strains of *Pichia anomala* and *Saccharomyces cerevisiae* were able to substantially reduce OTA toxin produced by the fungus *Penicillium verrucosum*. Additionally, inhibition of mycotoxinogenic moulds by yeast *Debaryomyces hansenii*, which was used to control OTA produced by two toxinogenic strains of *Penicillium nordicum* obtained from dry to cured meat. The yeast inhibits the capacity of moulds to produce spores and decreases the OTA constituents; the resultant effect also depends on the inoculation time and water activity [66]. Moreover, the positive effects of *Kluyveromyces* spp. demonstrated biocontrol activity against *Aspergillus flavus*, suppressing mycelial growth of mold and disease symptoms on maize [67]. Research reveals that *Saccharomyces cerevisiae* suppresses the growth of total mold count, specifically *Aspergillus ochraceus* and *Aspergillus niger*, and reduces ochratoxin A (OTA) invasion in coffee [68].

Microorganisms including bacteria, fungi especially yeasts, as well as their isolated enzymes, have been used for the biodegradation of mycotoxins. These microorganisms serve as a source of enzymes that can be used to decontaminate

agricultural commodities or used as feed additives. More and more degradation enzymes of mycotoxins have been purified and identified from microorganisms [69, 70]. They can metabolize, and also to detoxify mycotoxins into stable, less toxic, or completely nontoxic products [71]. For instance, yeasts can efficiently convert patulin into nontoxic or low-toxic substances through biodegradation. Alternatively, it can use physical adsorption, which has the advantages of safety, high efficiency, and environmental friendliness. Despite the inherent complexity of the production environment, relying solely on yeast as a control agent proves to be inherently unstable and difficult to implement on a large scale way. Integration control, enhancement of yeast resilience, improvement of yeast cell wall adsorption capacity, and research on additional patulin-degrading enzymes will facilitate the practical application of this approach [72].

## 5. Conclusions and Future Perspectives

Agricultural products affected by mycotoxin contamination cause a serious threat to both animal production and human well-being, thus resulting in large worldwide economic losses annually. To reduce or eliminate the level of mycotoxins in farm produce to a safe consumption level, common techniques used include chemical, physical, and biological treatments. Currently, some pathogenic strains have developed resistance against synthetic fungicides; therefore, researchers' concern about environmental and food safety. When detoxification occurs, food products' organoleptic properties and nutritional quality should not be altered, and products resulting from toxic degradation should not be produced. The uses of antagonistic yeasts are now common due to their advantageous attributes in food and

environmental safety, and also in controlling postharvest diseases in agricultural produce. The application of yeast strains as biological detoxifiers of mycotoxins in agricultural produce using mycotoxin detoxification mechanisms such as biodegradation, bioadsorption, and inhibition of mycotoxin biosynthesis offers a safe and valuable alternative for mycotoxin detoxification in food products. This also relies on the stability of the yeast-mycotoxin bond during the gastrointestinal transit for optimal detoxification.

In summary, detoxification of mycotoxins using yeast strains provides a reliable method for the management of mycotoxins in foods and feeds, and provides basic information for the risk assessment of mycotoxins for food and feed safety. More investigation is needed in this area to harness the detoxification potential of yeasts in mycotoxigenic foods, and to clone and express the genes responsible for yeast-mycotoxin detoxification

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