Review Article

Biological Decontamination Processes and Methods of Mycotoxins using Lactic Acid Bacteria and Enzymes: Present and Future

Sowmya K.L¹ , Ramalingappa B²

¹Davangere University, Research Scholar, Department of Studies in Microbiology, Davangere-577007, Karnataka. ¹Davangere University, Senior Professor, Department of Studies in Microbiology, Davangere-577007, Karnataka.

(Received: 14-09-2024 Revised: 19-10-2024 Accepted: 10-11-2024)

Corresponding Author: **Ramalingappa B**. Email: ramalingappa.88@gmail.com

ABSTRACT

Introduction and Aim: Mycotoxins are toxic by-products of fungi that can appear in bakery foods including bread, cake, and biscuits, among others. Mycotoxins generated by filamentous fungus (moulds) have a severe influence on the sustainability of food sources and cause significant economic loss. The aim is to highlight the critical need for biological detoxification methods, particularly utilizing lactic acid bacteria (LAB) and enzymes, to effectively degrade mycotoxins into less harmful substances. This research emphasizes the potential of LAB in addressing mycotoxin contamination in food.

Materials and Methods: The study employed various methods for the extraction and analysis of mycotoxins, including solid-liquid extraction (SLE) and solid phase extraction (SPE) using immuneaffinity columns. Detection techniques utilized were High Performance Liquid Chromatography (HPLC), mass spectrometry (MS), and tandem mass spectrometry (MS/MS) for multiclass determination of mycotoxins. Additionally, the effectiveness of lactic acid bacteria (LAB) strains in degrading specific mycotoxins was assessed through in vitro experiments under controlled conditions.

Results: Specific lactic acid bacteria (LAB) strains demonstrated significant degradation capabilities for various mycotoxins, achieving degradation rates of up to 90%. For instance, certain strains were effective in breaking down ochratoxin A and aflatoxin B1, with degradation percentages varying between 60% to 100% depending on the strain and conditions.

Conclusion: Mycotoxins are unavoidable contaminants in food, posing serious health risks, and emphasizes the importance of biological decontamination methods using lactic acid bacteria (LAB) and enzymes.

Keywords: Fungi, Mycotoxins, Analysis, Lactic acid bacteria, Enzymes, biological decontamination.

INTRODUCTION

Mycotoxins are byproducts of the secondary metabolism of filamentous fungi, and their names come from the combination of the ancient Greek terms mykes and toxon, which respectively mean "mould" and "poisonous arrow" (1). According to Zhou *et al.,* (2) and Wang *et al.,* (3) fungus create hazardous compounds called mycotoxins. They are harmful to human health and are typically present in food products provided by supermarket chains and grocery stores as natural contaminants. By making food unfit for ingestion, they have an adverse influence on human health (4). The three mould genera *Aspergillus, Fusarium,* and *Penicillium* play a major role in the production of mycotoxins. The most hazardous mycotoxins are known as aflatoxins, which are mostly generated by *A.flavus* and *A.parasiticus* (5). While aflatoxins B1, B2, G1, and G2, fumonisins B1, B2, and B3, ochratoxin A, deoxynivalenol, zearalenone and patulin are the most frequent mycotoxins discovered in bakery food products (6) (Table 1). Because mycotoxins have varied chemical structures and can have a range of hazardous effects on both animals and peoples, they are one of the most important and concerning problems relating to food safety. According to Oueslati *et al*., (7) mycotoxins can be hazardous in four different ways: acute, chronic, mutagenic, and teratogenic. Deterioration of liver or renal function, which in severe cases may result in death, is the acute mycotoxin poisoning impact that is most frequently cited (8). Significant amounts of dietary toxins often cause acute consequences, therefore fatal instances are typically limited to livestock or less developed regions of the world where control resources are scarce. Concern for the population's long-term health originates from chronic consequences. Since many toxins are found in food, we eat every day in little amounts. According to Sforza *et al*., (9) certain mycotoxins are genotoxic, carcinogenic, and may harm the kidneys, liver, or immune system. The best way to lower mycotoxin levels in food is by decontamination, which involves removing these toxins from the raw material before it is eaten. For the decontamination of food and feed, several physical, chemical, and biological techniques are employed (10) (Figure 1). To degrade or eliminate fungal toxins beyond affecting raw materials quality, other techniques are also combined. In feed and food industry, mycotoxin content is removed or reduced via chemical processes such as oxidation, alkalization, reduction, ammoniation, and acidification (8). Numerous substances, including calcium hydroxide monoethylamine, sodium hydroxide, chlorine, ammonia, sodium hydroxide and ozone, have the ability to lower the mycotoxin content in food. Mycotoxins can be partially destroyed chemically via processes like oxidation and alkalinization, but accomplishing so also destroys some of the important nutrients. Mycotoxins can be partially removed physically using processes such mopping, grinding, colouration, illumination, afloat, rinsing and removing flawed grains (1).

However, the use of hundreds of microorganisms, such as fungus, yeast, and bacteria, was suggested as a highly promising method for biologically removing mycotoxins (11).

Biological detoxification is the process of breaking down fungal toxins using microbeproduced enzymes and their metabolites. Lactic acid bacteria are the main bacteria that degrade fungal toxins, and Mycotoxins are bio transformed into less hazardous or harmless molecules during biological detoxification.

Biological detoxification has the potential to use hundreds or even thousands of suitable microorganisms. The aim of present research focused to highlight the critical need for biological detoxification, including lactic acid bacteria detoxification and to detoxify mycotoxins utilising microorganisms and enzymes (8).

Figure 1: Schematic representation of mycotoxin detoxification methods.

2 Occurrence of major mycotoxin contamination

2.1 Aflatoxins:

A set of harmful, structurally similar secondary metabolites known as aflatoxins (Figure 2) are mostly generated by *Aspergillus species* found in foods, such as *A.flavus* and *A.parasiticus*. Aflatoxin exists in a variety of forms, including B1, B2, G1, G2, M1 and M2 (12). The most frequent type of these mycotoxins detected in foods is B1. According to Bennett *et al*., (13) *Aspergillus flavus* may create aflatoxins B1 (AFB1) and B2 (AFB2), whereas AFB1, AFB2, AFG1 and AFG2 produced by *Aspergillus parasiticus*. The liver is the principal organ impacted by aflatoxins, which also have hepatotoxic, teratogenic, mutagenic, and immunosuppressive effects. Aflatoxins have been associated to both chronic carcinogenicity and acute toxicity in case of animals and humans too. In accord with Boevre *et al.,* (14) aflatoxinproducing fungus may also grow on milk, almonds, ground nuts, walnuts, peanuts and pistachio nuts as well as cereals like rice, maize, sorghum, barley, and oats. Due to its great toxicity when compared to other aflatoxin kinds, the fungus toxin Aflatoxin B1 has been the subject of the most investigation. According to reports, the synergy with the targeted fungi gene expression is the process by which aflatoxin B1 is degraded.

(www.chemspider.com).

Because of the major concerns about AF contamination in food and feed and their negative consequences on human health and the economy, AFs have been subject to intense FDA surveillance since 1969. Simply AFs are subject to specified FDA action limits among all mycotoxins; the others are simply governed by advisory levels (13). Aflatoxin exposure has been deemed carcinogenic to humans by the World Health Organisation (WHO) and the International Agency for Research on Cancer (IARC) (15).

2.2 Ochratoxin A:

Ochratoxins (Figure 3) are mycotoxins produced by *Penicillium* sp and *Aspergillus* sp of fungus, and it has shown to cause cancer growth in both people and animals (16). The most dangerous toxin in this category, ochratoxin A, it has been implicated in numerous medical issues,along with nephrotoxic, hepatotoxic, teratogenic, and carcinogenic disorders (17). Ochratoxins A can be found in meat products, dried vine fruit and spices, beer, wine, cocoa, coffee, cereals, beers, and wines (18). Ochratoxin A is typically found in solid meals, making biological detoxification especially difficult. In numerous foods, lactic acid bacterial strains and their metabolites demonstrated synergetic and antagonistic degrading pursuit. Among all sources of ochratoxin A, coffee and wine are known to be the main sources of ochratoxin A consumption (19). The increase in hydrophobicity, according to the researchers, is what allowed the warm disable lactic acid bacterial cells to absorb ochratoxin A to such a degree (20). In liquid environment, the detoxifying capacity of *P. parvulus* against ochratoxin A was established by Abrunhosa *et al*., (21). 90% of ochratoxin A was broken down in 19 hours under ideal circumstances (at 30 °C/7 days). This was accomplished by the conversion of OTA into OT. The size of the inoculum and temperature for incubation had a significant impact on the

Figure 3: Chemical structure of Ochratoxin A (www.chemspider.com).

2.3 Fumonisins:

Fumonisins, is a kind of non-fluorescence mycotoxin, it was identified in 1988. Fumonisins are hydrophilic mycotoxins that cannot entirely dissolve in organic solvents, in contrast to the majority of other mycotoxins (13). *F. proliferatum* also produces fumonisins. Fumonisin B1, which was discovered in 1988, is the most significant type of the all fumonisins (28) that have now been discovered and are divided into A, B, C and P categories (22). Plants are home to the bulk of the fumonisins family, or fumonisin B1 (FB1). FB1 is typically present in maize kernels (23). According to Sweeney *et al*. (24) fumonisins can found in wheat, asparagus spears, barley, sorghum, sorghum, soybeans, medicinal herbs, figs and black tea.

Figure 4: Chemical structure of Fumonisins (www.chemspider.com).

2.4 Zearalenone:

Zearalenone (Figure 5) earlier named as F2 toxin, is an estrogenic fungal toxin synthesised by *Fusarium* species involves *F.crookwellense, F.cerealis, F.culmorum, F.equiseti,* and *F.graminearum*, mainly occurring contamination with wheat, barley and maize fields and is frequently found in sorghum, rye and maize (16). The favoured substrates for zearalenone synthesis are wheat and rice, while oat and barley generate relatively little of the toxin. High humidity and cool temperatures are ideal for zearalenone manufacturing. DON and ZEA contamination usually coexist, while aflatoxins are less common. According to Yazar *et al*., (25) at normal temperature, ZEA is stable and largely removed at maximum temperatures.

Figure 5: Chemical structure of Zearalenone (www.chemspider.com).

2.5 Patulin:

Patulin (Figure 6) is a polyketide and watersoluble fungal toxin that was first identified in 1943. On fruits and vegetables, it is generated by specific *Penicillium* and *Aspergillus* species, with *P.expansum* is the major fungus for its synthesis (13). Other fruits like peaches, grapes and pears have been susceptible to contamination of patulin, albeit it mostly affects apple juice, apple products

and also apple (26). Because patulin can penetrate body tissues to prevent synthesis of protein, it lowers content of glycogen in kidney, intestinal tissues and liver, patulin residues can pose unique safety risks. Initially investigated as a possible antibiotic, patulin has now been found to have hazardous effects on humans, including nausea, vomiting, ulceration, and haemorrhage (27). Although the International Agency for Research on Cancer (IARC) have not sufficient data for this theory [28]. In both animals and peoples, cancer caused by patulin. Studies on patulin biodegradation by LAB strains are extremely a few. 80% of patulin is degraded by *Bifidobacterium animalis.*

Figure 6: Chemical structure of Patulin [\(www.chemspider.com\)](http://www.chemspider.com/).

3. Techniques involved in the detection of food mycotoxins

The presence of mycotoxins in food is a major global concern for food safety. A few common approaches, such as sampling, homogenization, extraction, clean-up, and ultimately detection of mycotoxins in food by qualitative and quantitative analysis, are frequently used to evaluate the amounts of mycotoxins in food samples (29).

In food, fungal toxins detection involves numerous scientific methods, including, ELISA, capillary electrophoresis (CE), gas chromatography (GC), QPCR and thin layer chromatography (TLC), have been proposed. But, the most common technique is HPLC (High Performance Liquid Chromatography) and mass spectrometry (MS) analysis. Tandem mass spectrometry (MS/MS) and ultrahigh performance liquid chromatography (UHPLC) have become very popular in recent years, especially in the detection of residues in the presence of other contaminants and for the multiclass determination of mycotoxins (8).

Figure 7: General steps involved in the mycotoxins analysis in food.

LC-MS and HPLC method are very suitable technique for the detection of mycotoxins. Because food matrices are so complex, an extraction and clean-up purification stage is frequently necessary prior to analysis. Solid-liquid extraction (SLE) is the most widely used technique, followed by solid phase extraction (SPE), which makes use of immune-affinity columns (IACs) containing particular antibodies to the target analyte. The most common sample treatments are covered in multiple publications that give an overview of the several methods recommended for identifying mycotoxins in food (30).

4. Biological Detoxification

Biological detoxification refers to the employment of microorganism enzymes and their metabolites to destroy mycotoxins. Hundreds or thousands of relevant microbes and metabolites are used, that makes biological detoxification a potential option. Microorganisms that are utilised for detoxification must satisfy specific requirements, such as being non-pathogenic and safe to employ (31). Numerous microbes have been proposed as food detoxifying agents. The most frequent microorganisms employed to detoxify food are fungi, yeast, and bacteria (32-33), the potential of lactic acid bacteria, *C.rubrum Rhizopus* sp*, C.lipolytica, A.niger, M.ambiguous T.viride* and a number of other microbes for detoxification. According to Nichea *et al*., (34) assessment of several *Aspergillus* species, these fungi have the enzymes necessary to break down and transform the aflatoxins (B1 to B2 and B3) from food items. However, according to various research, some bacteria may be used to detoxify mycotoxins from food (12). *Flavobacterium aurantiacum* was first bacteria utilized to breakdown B1 aflatoxin B1 in feed, and it has been shown that the pursuit was correlated with the enzymes (35). *P.aeruginosa* N17-1 was able to significantly destroy a number of aflatoxins, involves aflatoxin B1, B2, and M1 in nutritional porridge, as revealed by Sangare *et al*., (36). Additionally, it has been shown that some microbes use fungal toxins as a carbon source. From soil *Agrobacterium-Rhizobium* were isolated that were change deoxynivalenol into the minor harmful metabolite 3-keto-4 deoxynivalenol (37). Bondy *et al*., (38) Deoxynivalenol was decomposed by the bacteria Devosia mutans 17-2-E-8, and the primary metabolite was 3-keto-deoxynivalenol, which had a lower toxicity than the mycotoxin.

5. Detoxification of mycotoxins by LAB (Lactic Acid Bacteria)

Because they naturally develop in the human gut and are highly safe to use in food, LAB is one of the main microorganisms utilised for the degradation of mycotoxins (34). This enables them to perform effectively for the removal of mycotoxins. Proteins hydrolyze by many proteolytic enzymes are produced by LAB. These enzymes include cell wall bound proteinase, which breaks down proteins into peptide transporters, polypeptides, which carry peptides and intracellular peptidases into cell, which convert peptides into amino acids (11). Proteolytic enzymes of lactic acid bacteria are crucial in detoxification process in food products (21). Two crucial pathways for mycotoxin detoxification from food are produced by lactic acid bacteria. Using active LAB or use LAB-produced enzymes. Many bioactive metabolites are created by LAB, and they can stop the growth of fungus as well as the contamination of food or the generation of mycotoxins (fungal toxins) (34). Several studies reported (El-Nezami *et al*., 1998, Peltonen *et al*., 2001, Sezer *et al*., 2013, Mendoza *et al*., 2009, Slizewska *et al*., 2011, Huang *et al*., 2017, Fuchs *et al*., 2008, Luz *et al*., 2018, Franco *et al*., 2011, Rogowska *et al*., 2019) (39-48) mycotoxins in foods can be degraded (detoxification) up to 80- 90% by using LAB (Figure 8 and Figure 9).

Figure 8: LAB reported degradation of Aflatoxin B1 in food and feeds.

Figure 9: LAB reported degradation of different mycotoxins in food and feeds.

6. Mechanisms of degradation of mycotoxins by lactic acid bacteria strains

Adsorption of mycotoxins by the cell wall of LAB strains, has been proposed as an alternative technique for removing mycotoxins from certain foods. Polysaccharides, protein, and peptidoglycans were found in the cell walls of LAB strains and were connected to this action (49- 50). Wang *et al*., (28) report that in cultured medium, the binding activity of certain LAB led to a reduction in patulin. Due of the number of binding sites, the binding quest was aided by choosing strains with a high particular area of surface and cellular volume. According to the researchers, protein and polysaccharides have a major role in the adsorption of patulin. According to different research, the incubation temperature, the complication and food pH, the quantity of LAB cells, the strain of LAB, the beginning concentration of mycotoxins, and the number of LAB cells all had an effect on the binding of fungal toxins by lactic acid bacterial cells. The survival of the cells was not required, according to

Dalie *et al*., (51) since the aflatoxin B1 linked to a certain monoclonal antibody in cell wall. In order to lessen mycotoxins in food, LAB strains create metabolites including phenolic compounds, fatty acids, acids and low molecular weight bioactive peptides. These metabolites may reduce toxicity of the fungal toxins by binding with them (34). Since the processes of fungal toxin breakdown and elimination by lactic acid bacterial cells and metabolites are still have not fully understood, a number of suggestions have been made up, including the involvement of proteolytic enzymes in mycotoxin degradation and interaction of definite metabolites with the fungal toxins. Toxin adsorption by lactic acid bacterial cells, degradation of toxins by enzymes of lactic acid bacteria, and toxin interaction with LAB metabolites were three plausible mechanisms, according to the results of prior research **(Figure 10).**

Figure 10: Mechanisms of degradation of mycotoxins by lactic acid bacteria strains [8].

6.1. Degradation of Aflatoxin by LAB:

Aflatoxin B1 is reportedly broken down by contact with the targeted fungus and gene expression. According to research by Gomaa *et al*., (52), there is a correlation between the decline in the Omt-A gene (60–70%), which is required for the biosynthesis of aflatoxin B1 by *A.flavus* and *A.parasiticus*, and the decline in aflatoxin B1 levels. This study confirmed that the utilisation of that particular strain for applications of food was constrained by the maximum cell density is essential for decontamination. Due to the ability to bind bacterial cell's to aflatoxin B1, the strains *L. rhamnosus* and *L. amylovorus* were able to eliminate more than 50% of it (43). In another investigation, yoghurt with a high degree of aflatoxin M1 breakdown was created by combining a single strain (*L. plantrium*) with yoghurt strains (*S.thermophilus* and *L.bulgaricus*). According to earlier studies on aflatoxins, a number of LAB isolates can reduce the amount of aflatoxin in a range of solid, liquid and semiliquid food systems. Therefore, the biological detoxifying pursuit of lactic acid bacteria is a favourable method for many food applications (53).

6.2 Degradation of Ochratoxin by LAB:

Lactic acid bacterial strains and their metabolites manifest synergetic and antagonistic degrading activities in a variety of diets. The *L. acidophilus* VM 20 strain dramatically (by 95%) decreased the levels of ochratoxin A in a liquid media as a result of its antagonistic activity. To further demonstrate the detoxifying abilities, Hepatoma cell line (HepG2) was utilised. The new strain digested more than 50% of ochratoxin A (45). The LAB binding activity was discovered to be influenced by lactic acid bacterial cell density, ochratoxin A concentration, pH level, and LAB cell survival. The levels of ochratoxin A in liquid medium were reduced by 50% in *L. acidophilus* CH-5, *L. rhamnosus* GG*, L. sanfranciscensis*, *L. brevis and L. plantarum* BS; nevertheless, the lactic acid bacterial cells with ochratoxin A were discovered to be revocable (20-21). The detoxifying capacity of the lactic acid bacterial strains *L. brevis*, *L. sanfranciscensis* and *L. plantarum* showed 50% decrease in ochratoxin A after a 30-minute incubation in 1 M phosphate buffer by An in vitro investigation (sodium acetate 0.615%, EDTA 0.1%, MgCl2 0.254%, raffinose 29.72%, pH 6.2). According to the research studies, the increase in hydrophobicity is what made the thermic disable lactic acid bacterial cells absorb ochratoxin A at prominent level (20). Ines *et al*., (54) discovered that in liquid environment, detoxifying pursuit of *P.parvulus* towards ochratoxin A, about 90% of ochratoxin A was broken down in 19 hours, incubated at 30 °C for 7 day. This was achieved by the transformation of OTA into OT. The incubation temperature and inoculum size both significantly affected the degradation activity. A new research shown the superiority of mixing many strains with *S. cerevisiae* in a collegial system to boost the pursuit of ochratoxin A breakdown (55).

6.3 Degradation of Patulin by LAB:

Studies on patulin biodegradation by LAB strains are quite few. 80% of patulin is degraded by *Bifidobacterium animalis* VM, and the activity was found to be correlated with both the media patulin content and LAB cell density. By using human hepatoma cell line (HepG2), in the presence of the chosen lactic acid bacteria and it demonstrated an enhanced division rate and the detoxifying activity was further validated (21 & 45). The heat-inactivated L. brevis 20,023 exhibited an intense ability to bind patulin to the cell membranes of the selected lactic acid bacteria in a liquid media. The inclusion of functional groups, such as polysaccharides and proteins, was observed to significantly enhance patulin adsorption into lactic acid bacterial cells (31). In a liquid solution at pH 4 and an incubation temperature of 37 °C, the percentage of patulin metabolised by the LAB strains *Bifidobacterium bifidum* 6071 and *L. rhamnosus* 6149 was 52.9% for viable cells (56). Patulin may be extensively decomposed at low pH values, which may be the primary reason in lactic acid bacteria degradation activity. New research examined the chemical breakdown of patulin in the presence of ascorbic acid apple juice. In apple juice incorporated with 0.25% (w/v) ascorbic acid, patulin reduced by 60%, and biodegradation resulted in the generation of less-toxic metabolites (57).

7. Methods of mycotoxin detoxification by enzymes from microorganisms

Most promising techniques is enzyme detoxification for reducing mycotoxins since it doesn't have certain major limitations associated with chemical procedures, such as nutritional loss, raw materials with chemical contamination, prolonged, and high prices (11). Numerous microorganisms, such as bacteria, moulds, and yeasts, participate in the detoxification of fungal toxins by enzymes (58). Mycotoxins are frequently converted by microbial enzymes into less harmful or innocuous metabolites, destruction, or inactivation (59). Most bacteria employ degradation as a process during daily activity to transform certain compounds into less dangerous or even non-toxic byproducts. The crucial active molecules released by bacteria during the degrading process are called enzymes (60).

In order to detoxify the most well-known mycotoxins, such as AF, ZEN, PAT, TCT, and OTA, a significant amount of scientific research has concentrated on enzymatic degradation (61- 63). Numerous enzymes from fungus, bacteria and yeast have been found and examined for their ability to alter or transform mycotoxins in various ways (64). Numerous *Aspergillus* species enzymes are associated in aflatoxin degradation. Additionally, it was shown that an isolate of *A.niger* from feed samples may eradicate AFB1 (65). Other fungi have reportedly been found to secrete oxidative enzymes such laccase and manganese peroxidase, which may help in the detoxification of aflatoxins (66). It has been found that many *Aspergillus* sp. enzymes are necessary for the degradation of aflatoxin. *Aspergillus niger* isolate from samples of feed have been shown to destroy AFB1 (67). After heating, 94.7% of AFB1 in *Bacillus velezensis* DY3108 supernatant could be converted into less dangerous metabolites. Recent research has shown that *Bacillus pumilus* may kill 88% of AFB1 (68). It's noteworthy to notice that *B.pumilus* ES-21, convert ZEN of 95.7% into 1-(3,5-dihydroxyphenyl)-6 hydroxyl undecen-1-one)69]. Overall, lactonase (70-71), peroxidase (72) and laccase have been identified as the three types of enzymes that break down ZEN (61). The primary benefit of lactonase Zhd101, which was found in *S. cerevisiae* and *L. reuteri*, was that it was highly effective at degrading ZEN. Lactonase has a high capacity for degradation, not highly thermostable and it inhibits (73). Zhd518 was found by Wang *et al*., (74) as a lactonohydrolase enzyme with potent ZEN-degrading activity. The same authors claim that Zhd518 is a viable alternative for ZEN detoxification due to its strong specific action against ZEN and its derivatives. For the first time, a *Gliocladium roseum* lactonase that is capable of efficiently digesting ZENG was found and called ZENG by Zhang *et al*., (75). At a pH of 7.0, this recombinant enzyme has exceptional detoxifying activity towards ZEN and its derivatives, zearalenol (-ZOL), and -zearalanol (-ZAL).

Table 2: Percentage of mycotoxin degradation by enzymes and their microorganisms.

	Mycotoxin Microorganism	Name of the	$%$ of	Referenc
		enzymes	degradatio	
OTA		Aspergillus nigeCarboxypeptida:99%		Abrunhos
	MUM 03.58	e		et al., 200
OTA	Bacillus	Carboxypeptida:72%		Chang
	amyloliquefaciele			al., 2015
	s ASAG1			
AFB1	Phanerochaete	Manganese	86%	Wang
	sordida	peroxidase		al., 2011
AFB1	Trametes	Laccase		67%Lorestania
	versicolor			et al., 201
AFB1	Pleurotus eryngiLaccase		86%	Loi et al
				2018
ZEN	Pleurotus eryng Laccase		100%	Loi et al
				2018
ZEN	Gliocladium	ZENG	60%	Zhang
	roseum			al., 2020
ZEN	lignocellulose-Manganese		34%	Tang et al
	degrading fungi Peroxidase			2013

Figure 11: Percentage of mycotoxin degradation by enzymes and their microorganisms.

Many mycotoxins feature connections, such as lactone rings, amides, or ester linkages, that can be degraded by certain enzymes. The hydrolytic process needs aqueous conditions, but no cosubstrates or coenzymes are necessary. It was presumably made feasible by the hydrolytic reaction's simplicity that pure hydrolases against fumonisin and ZEN used to feed additives were commercially viable (76-77). Some of the biotransformation reactions of mycotoxins by a few microbial enzymes are shown in Table 2 & Figure 11.

8. Applications of LAB in foods

Due to their capacity to breakdown mycotoxins, LAB strains are among the most significant alternatives for the detoxifying activity in meals. LAB's potential in food applications may be attributed to characteristics including its generally recognised as safe (GRAS) status, cheap production costs, and variety of food applications (56). Additionally, LAB strains might lessen the financial dropping correlated with food goods by increasing the storage capacity of those products and decreasing production of fungal toxins.

For manufacture of baked foods like sourdough and fermented grains, LAB are essential. Numerous lactic acid bacterial strain like *L. plantarum*, were showed lower the amount of aflatoxin B1 in bread (78). In contaminated apple juice, the quantity of patulin was effectively decreased by 80% when LAB was employed to break it down without harming the juice's quality. The applications of Lactic acid bacteria are satisfactory to digest fungal toxins in a scope of foods because of its potent enzymatic system and rapid adaptability to different substrates. Recently Lactic acid bacteria has been proposed as a dependable technique for eliminating or degrading mycotoxins from foods (79).

LAB strains may be employed as a co-culture in foods, such as in the hurdle technique for removing mycotoxins from dairy products (80). Additionally, it was noted that using whey permeate cheese fermented with lactic acid bacteria and restrain a number of metabolites was a successful method for lowering the fungal toxin from *Fusarium sp.* in wheat grains malting used to make baked goods and beverages (81). LAB fermentation can drastically lower the amount of mycotoxin in food. Additionally, to lessen the loss brought on by mycotoxin contamination, fermented food products might be converted into other processed food items.

CONCLUSION

Mycotoxins are unpredictable and inevitable pollutants in food products all over the world. Numerous fungi create mycotoxins, which are the primary cause of a major health concern to consumers, and these mycotoxins are found in food products. The best way to lower mycotoxin levels in food is by decontamination, which involves removing these toxins from the raw material before it is eaten. For the decontamination of food and feed, a variety of physical, chemical, and biological techniques are employed. Biological detoxification is a highly recommended method with the potential use of numerous microbes. LAB is the main microorganism for the degradation of fungal toxins and the biotransformation of mycotoxins into smaller or harmless molecules is referred to as detoxification. It has been shown that certain LAB strains may degrade certain mycotoxins in food by adhering to their cell walls or by decomposing them with their enzymes. As a result, greater research into the processes of mycotoxin destruction is required. Future research should concentrate on combining certain lactic acid bacterial strains with distinct detoxification mechanisms in order to optimise the effectiveness of mycotoxin breakdown. In addition, it's important to identify and choose the best microbes and enzymes for effective detoxification as well as to investigate the enzymatic properties and catalytic roles of enzymes in food.

CONFLICT OF INTEREST

The authors declare that there is no Conflict of **Interest.**

FUNDING

There is no funding to report.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

REFERENCE

1. Dimosthenis Kizis, Aikaterini-Eleni Vichou, Pantelis, I., Natskoulis. Recent Advances in Mycotoxin Analysis and Detection of Mycotoxigenic Fungi in Grapes and Derived Products. *Sustainability.* 2021;13:1-26.

- 2. Shu, X., Wang, Y., Zhou, Q., Li, M., Hu, H., Ma, Y., Chen, X., Ni, J., Zhao, W., Huang, S., Wu, L. Biological degradation of aflatoxin B 1 by cell-free extracts of Bacillus velezensis DY3108 with broad PH stability and excellent thermostability. *Toxins.* 2018;10:1-15.
- 3. Wang, J., Ogata, M., Hirai, H., Kawagishi, H. Detoxification of aflatoxin B1 by manganese peroxidase from the white-rot fungus *Phanerochaete sordida* YK-624. *FEMS Microbiol Lett.* 2011;314:164-169.
- 4. Ming-Tzai Chen., Yuan-Hsin Hsu., Tzu-Sui Wang., Shi-Wern Chien. Mycotoxin Monitoring for commercial foodstuffs in Taiwan. *Journal of Food and Drug Administration.* 2016;24:147-156.
- 5. Wogan, G.N., Pong, R.S. Aflatoxins. *Ann N Y Acad Sci.*1970;174(2):623-35.
- 6. Streit, E., Naehrer, K., Rodrigues, I., Schatzmayr, G. Mycotoxin occurrence in feed and feed raw materials worldwide: long-term analysis with special focus on Europe and Asia*. J Sci Food Agric.* 2013; 93(12):2892-99.
- 7. Oueslati, S., Romero-Gonzalez, R., Lasram, S., Frenich, A.G., Vidal, J.L.M. Multi-mycotoxin determination in cereals and derived products marketed in Tunisia using ultra-high performance liquid chromatography coupled to triple quadrupole mass spectrometry. *Food Chem Toxicol.* 2012;50(7):2376-81.
- 8. Belal, J., Muhialdin, Nazamid saari, Anis Shobirin Meor Hussin. Review on the Biological Detoxification of Mycotoxins Using Lactic Acid Bacteria to Enhance the Sustainability of Foods Supply. *Molecules.* 2020;25:1-12.
- 9. Stefano Sforza, Chiara Dall'asta, Rosangela Marchell. Recent Advances in Mycotoxin Determination in Food and Feed by Hyphenated Chromatographic Techniques/Mass Spectrometry. *Mass Spectrometry Reviews.* 2006;25:54-76.
- 10. Yang, X., Zhang, Q., Chen, Z.Y., Liu, H., Li, P. Investigation of *Pseudomonas fluorescens* strain 3JW1 on preventing and reducing aflatoxin contaminations in peanuts. *PLoS ONE.* 2017;12:e0178810.
- 11. Wu, Q., Jezkova, A., Yuan, Z., Pavlikova, L., Dohnal, V., Kuca, K. Biological degradation of aflatoxins. *Drug Metab. Rev.* 2009;41:1-7.
- 12. Sato, I., Ito, M., Ishizaka, M., Ikunaga, Y., Sato, Y., Yoshida, S., Tsushima, S. Thirteen novel deoxynivalenol-degrading bacteria are classified within two genera with distinct degradation mechanisms. *FEMS Microbiol.* 2012;327:110-117.
- 13. Bennett, J.W., Klich, M. Mycotoxins. *Clin. Microbiol. Rev.* 2003; 16:497-516.
- 14. Boevre, M., Mavungu, J.D., Landshchoot, S., Audenaert, K., Eeckhout, M., Maene, P. Natural occurrence of mycotoxins and their masked forms in food and feed products. *World Mycotoxin J.* 2012; 5:207- 219.
- 15. Kensler, T.W., Roebuck, B.D., Wogan, G.N., Groopman, J.D. Aflatoxin: A 50-year odyssey of mechanistic and translational toxicology. *Toxicol. Sci.* 2011; 20: S28-S48.
- 16. Hussein, H.S., Brasel, J.M. Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology*. 2001; 167:101-134.
- 17. Abdel-Wahhab, M.A., Abdel-Galil, M.M., El-Lithey, M. Melatonin counteracts oxidative stress in rats fed an ochratoxin A contaminated diet. *J. Pineal Res.* 2005;38:130-135.
- 18. Wanigasuriya, K.P., Peiris, H., Ileperuma, N., Peiris-John, R.J., Wickremasinghe, R. Could ochratoxin A in food commodities be the cause of chronic kidney disease in Sri Lanka? *Trans. R. Soc. Trop. Med. Hyg.* 2008;102:726-728.
- 19. Ostry, V., Malir, F., Toman, J., Grosse, Y. Mycotoxins as human carcinogens-the IARC Monographs classification. *Mycotoxin Res.* 2017;33:65-73.
- 20. Piotrowska. The adsorption of ochratoxin A by *Lactobacillus species. Toxins.* 2014; 6:2826-2839.
- 21. Abrunhosa, L., Paterson, R.R., Venancio, A. Biodegradation of ochratoxin A for food and feed decontamination. *Toxins.* 2010;2:1078- 1099.
- 22. Rheeder, J.P., Marasas, W.F., Vismer, H.F. Production of fumonisin analogs by Fusarium species. *Appl. Environ. Microbiol.* 2002;68:2101-2105.
- 23. Reddy, K.R.N., Nurdijati, S.B., Salleh, B. An overview of plant-derived products on control of mycotoxigenic fungi and mycotoxins. *Asian J. Plant Sci.* 2010; 9:126-133.
- 24. Sweeney, M.J., Dobson, D.W. Mycotoxin production by *Aspergillus, Fusarium and Penicillium species*. *Int. J. Food Microbiol.* 1998;43:141-158.
- 25. Yazar, S., Omurtag, G.Z. Fumonisins, trichothecenes and zearalenone in cereals. *Int. J. Mol. Sci.* 2008;9:2062-2090.
- 26. Harrison, M. A., Presence and stability of patulin in apple products: *A review. J. Food Saf.* 1989;9:147-153.
- 27. Puel, O., Galtier, P., Oswald, I.P. Biosynthesis and toxicological effects of patulin. *Toxins.* 2010;2:613-631.
- 28. Wang, L., Wang, Z., Yuan, Y., Cai, R., Niu, C., Yue, T. Identification of key factors involved in the biosorption of patulin by inactivated lactic acid bacteria (LAB) cells. *PloS ONE.* 2015; 10:0143431.
- 29. Moretti, A., Logrieco, A.F., Susca, A. Mycotoxins: An underhand food problem. *Methods Mol. Biol.* 2017; 1542:3- 12.
- 30. Pundir, R. K., Jain, P. Qualitative and Quantitative analysis of microflora of Indian bakery products. *Journal of Agricultural Technology:* 2011;7(3):751-762.
- 31. Wang, J., Xie, Y. Review on microbial degradation of zearalenone and aflatoxins. *Grain Oil Sci. Technol.* 2020;3:117-125.
- 32. Varga, J., Toth, B. Novel strategies to control mycotoxins in feeds: A review. *Acta Vet. Hung.* 2005-53:189–203.
- 33. Karlovsky, P., Suman, M., Berthiller, F., De, M.J., Eisenbrand, G., Perrin, I., Oswald, I.P., Speijers, G. Impact of food processing and detoxifIcation treatments on mycotoxin contamination. *Mycotoxin Res.* 2016-32:179– 205.
- 34. Nichea, M., Palacios, S., Chiacchiera, S., Sulyok, M., Krska, R., Chulze, S., Ramirez,

M. Presence of multiple mycotoxins and other fungal metabolites in native grasses from a wetland ecosystem in Argentina intended for grazing cattle. *Toxins.* 2015-7:3309–3329.

- 35. Smiley, R.D., Draughon, F.A. Preliminary evidence that degradation of aflatoxin B1 by *Flavobacterium aurantiacum* is enzymatic. *J. Food Prot.* 2000;63:415-418.
- 36. Khaneghah, A.M., Farhadi, A., Nematollahi, A., Vasseghian, Y., Fakhri, Y. A systematic review and meta-analysis to investigate the concentration and prevalence of trichothecenes in the cereal-based food. *Trends Food Sci. Technol.* 2020;102:193-202.
- 37. Shima, J., Takase, S., Takahashi, Y., Iwai, Y., Fujimoto, H., Yamazaki, M., Ochi, K. Novel detoxification of the trichothecene mycotoxin deoxynivalenol by a soil bacterium isolated by enrichment culture. *Appl. Environ. Microbiol.* 1997;63:3825-3830.
- 38. He, J.W., Bondy, G.S., Zhou, T., Caldwell, D., Boland, G.J., Scott, P.M. Toxicology of 3-epideoxynivalenol, a deoxynivalenoltransformation product by Devosia mutans 17–2-E-8. *Food Chem. Toxicol.* 2015;84:250- 259.
- 39. El-Nezami, H., Kankaanpaa, P., Salminen, S., Ahokas, J. Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B1. *Food Chem. Toxicol.* 1998;36:321-326.
- 40. Peltonen, K., El-Nezami, H., Haskard, C., Ahokas, J., Salminen, S. Aflatoxin B1 binding by dairy strains of lactic acid bacteria and bifidobacteria. *J. Dairy Sci.* 2001;84:2152- 2156.
- 41. Sezer, C., Guven. A., Oral, N.B., Vatansever, L. Detoxification of aflatoxin B1 by bacteriocins and bacteriocinogenic lactic acid bacteria. *Turk. J. Vet. Anim. Sci.* 2013;37:594- 601.
- 42. Hernandez-Mendoza, A., Garcia, H.S., Steele, J.L. Screening of *Lactobacillus casei* strains for their ability to bind aflatoxin B1. *Food Chem Toxicol.* 2009; 47:1064-1068.
- 43. Slizewska, K., Smulikowska, S. Detoxification of aflatoxin B1 and change in microflora pattern by probiotic in vitro

fermentation of broiler feed. *J. Anim. Feed Sci.* 2011;20:300-309.

- 44. Huang, L., Duan, C., Zhao, Y., Gao, L., Niu, C., Xu, J., Li, S. Reduction of aflatoxin B1 toxicity by *Lactobacillus plantarum* C88: A potential probiotic strain isolated from Chinese traditional fermented food "tofu". *PLOS ONE.* 2017; 12:1-6.
- 45. Fuchs, S., Sontag, G., Stidl, R., Ehrlich, V., Kundi, M., Knasmuller, S. Detoxification of patulin and ochratoxin A, two abundant mycotoxins, by lactic acid bacteria. *Food Chem. Toxicol.* 2008; 46:1398-1407.
- 46. Luz, C., Ferrer, J., Manes, J., Meca, G. Toxicity reduction of ochratoxin A by lactic acid bacteria. *Food Chem. Toxicol.* 2018;112:60-66.
- 47. Franco, T.S., Garcia, S., Hirooka, E.Y., Ono, Y.S., Dos Santos, J.S. Lactic acid bacteria in the inhibition of *Fusarium graminearum* and deoxynivalenol detoxification. *J. Appl. Microbiol.* 2011; 111:739-748.
- 48. Rogowska, A., Pomastowski, P., Walczak, J., Railean-Plugaru, V., Rudnicka, J., Buszewski B. Investigation of Zearalenone Adsorption and Biotransformation by Microorganisms Cultured under Cellular Stress Conditions. *Toxins.* 2019;11 :463-465.
- 49. Chapot-Chartier, M.P., Kulakauskas, S. Cell wall structure and function in lactic acid bacteria. Microb. *Cell Fact.* 2014;13: S9.
- 50. Zeinvand-Lorestania, H., Sabzevari, O., Setayesh, N., Amini, M., Faramarzib, A., Ahmadabadi, A.N., Faramarzi, A.M. Comparative study of in vitro prooxidative properties and genotoxicity induced by aflatoxin B1 and its laccase-mediated detoxification products. *Chemosphere.* 2015;135:1-6.
- 51. Ben Taheur, F., Kouidhi, B., Al Qurashi, Y.M.A., Ben Salah-Abbes, J., Chaieb, K. Review: biotechnology of mycotoxins detoxification using microorganisms and enzymes. *Toxicon.* 2019;160:12-22.
- 52. Gomaa, E.Z., Abdelall, M.F., El-Mahdy, O.M. Detoxification of aflatoxin B1 by antifungal compounds from *Lactobacillus brevis* and *Lactobacillus paracasei*, isolated

from dairy products. *Probiotics Antimicrob. Proteins.* 2018;10:201-209.

- 53. Elsanhoty, R.M., Salam, S.A., Ramadan, M.F., Badr, F.H. Detoxification of aflatoxin M1 in yoghurt using probiotics and lactic acid bacteria. *Food Control.* 2014;43:129-134.
- 54. Abrunhosa, L., Ines, A., Rodrigues, A.I., Guimaraes, A., Pereira, V.L., Parpot, P, Venancio, A. Biodegradation of ochratoxin A by *Pediococcus parvulus* isolated from Douro wines. *Int. J. Food Microbio.* 2014;188:45- 52.
- 55. Markowiak, P., Slizewska, K., Nowak, A., Chlebicz, A., Zbikowski, A., Pawlowski, K., Szeleszczuk, P. Probiotic microorganisms detoxify ochratoxin A in both a chicken liver cell line and chickens. *J. Sci. Food Agric.* 2019; 99:4309-4318.
- 56. Hatab, S., Yue, T., Mohamad, O. Removal of patulin from apple juice using inactivated lactic acid bacteria. *J. Appl. Microbiol.* 2012;112:892-899.
- 57. Assaf, J.C., Nahle, S., Chokr, A., Louka, N., Atoui, A., El Khoury, A. Assorted methods for decontamination of aflatoxin M1 in Milk using microbial adsorbents. *Toxins.* 2019;11:304
- 58. Hathout, A.S., Aly, S.E. Biological detoxifcation of mycotoxins: A review. *Ann Microbiol.* 2014;64:905-919.
- 59. Lyagin, I., Efremenko, E. Enzymes for detoxifcation of various Mycotoxins: origins and mechanisms of catalytic action. *Molecules.* 2019;24:2363.
- 60. Guan, Y., Chen, J., Nepovimova, E., Long, M., Wu, W., Kuca, K. Aflatoxin detoxification using microorganisms and enzymes. *Toxins.* 2021;13:1-17.
- 61. Loi, M., Fanelli, F., Cimmarusti, M.T., Mirabelli, V., Haidukowski, M., Logrieco AF, Caliandro R, Mule G. In vitro single and combined mycotoxins degradation by Ery4 laccase from *Pleurotus eryngii* and redox mediators. *Food Control.* 2018; 90:401-406.
- 62. Zhang, Z., Xu, W., Wu, H., Zhang, W., Mu, W. Identification of a potent enzyme for the detoxification of Zearalenone. *J Agric Food Chem.* 2020;68:376-383.
- 63. Tang, Y., Xiao, J., Chen, Y., Yu, Y., Xiao, X., Yu, Y., Wu, H. Secretory expression and characterization of a novel peroxiredoxin for zearalenone detoxification in Saccharomyces cerevisiae. *Microbiol Res.* 2013;168:6-11.
- 64. Taheur, F.B., Fedhila, K., Chaieb, K., Kouidhi, B., Bakhrouf, A., Abrunhosa, L. Adsorption of aflatoxin B1, zearalenone and ochratoxin A by microorganisms isolated from kefir grains. *Int J Food Microbiol.* 2017;251:1-7.
- 65. Zhang, W., Xue, B., Li, M., Mu, Y., Chen, Z., Li, J., Shan, A. Screening a strain of *Aspergillus niger* and optimization of fermentation conditions for degradation of aflatoxin B1. *Toxins.* 2014;6:3157-3172.
- 66. Alberts, J.F., Gelderblom, W.C.A., Botha, A., van Zyl, W.H. Degradation of aflatoxin B1 by fungal laccase enzymes. *Int J Food Microbiol.* 2009;135:47-52.
- 67. Paris, M.P.K., Schweiger, W., Hametner, C., Stuckler, R., Muehlbauer, G.J., Varga, E. Zearalenone-16-o-glucoside: a new masked mycotoxin. *J. Agric. Food Chem.* 2014;62:1181-1189.
- 68. Sangi, F., Abbas, M., Miman. Identification of new aflatoxin B1-degrading Bacteria from Iran. *Iran J Toxicol.* 2018;12:39-44.
- 69. Wu, L., Wang, W., Yao, K. Effects of dietary arginine and glutamine on alleviating the impairment induced by deoxynivalenol stress and immune relevant cytokines in growing pigs. *Plos One.* 2013;8:69502.
- 70. Wang, J.Q., Yang, F., Yang, P.L., Liu, J., Lv, Z.H. Microbial reduction of zearalenone by a new isolated *Lysinibacillus sp.* ZJ-2016-1. *World Mycotoxin J.* 2018;11:571-578.
- 71. Assaf, C.E.H., De Clercq, N., Van Poucke, C., Vlaemynck, G., Van Coillie, E., Van Pamel, E. Effects of ascorbic acid on patulin in aqueous solution and in cloudy apple juice. *Mycotoxin Res.* 2019; 35:341-351.
- 72. Corassin, C.H., Bovo, F., Rosim, R.E., Oliveira, C.A.F.F. Efficiency of *Saccharomyces cerevisiae* and lactic acid bacteria strains to bind aflatoxin M1 in UHT skim milk. *Food Control.* 2013; 31:80-83.
- 73. Hahn, I., Kunz-Vekiru, E., Twaruzek, M., Grajewski, J., Krska, R., Berthiller, F. Aerobic and anaerobic in vitro testing of feed additives claiming to detoxify deoxynivalenol and zearalenone. *Food Addit Contam - Part A Chem Anal Control Expo Risk Assess.* 2015;32:922-933.
- 74. Wang, L., Wu, J., Liu, Z., Shi, Y., Liu, J., Xu, X., Hao, S., Mu, P., Deng, F., Deng, Y. Aflatoxin B1 degradation and detoxification by *Escherichia coli* CG1061 isolated from chicken cecum. *Front Pharmacol.* 2019;9:1-9.
- 75. Khan, G.I. Eco-friendly bacterial biodegradation of mycotoxins. *Pure Appl. Biol.* 2020;9:1708-1717.
- 76. Heinl, S., Hartinger, D., Thamhesl, M., Schatzmayr, G., Moll, W.D., Grabherr, R. An aminotransferase from bacterium ATCC 55552 deaminates hydrolyzed fumonisin B1. *Biodegradation.* 2011; 22:25-30.
- 77. Gruber-Dorninger, C., Faas, J., Doupovec, B., Aleschko, M., Stoiber, C., Hobartner-Gußl. A. Metabolism of Zearalenone in the rumen of dairy cows with and without application of a Zearalenone-degrading enzyme. *Toxins.* 2021;13:1-18.
- 78. Saladino, F., Luz, C., Manyes, L., Fernandez-Franzon, M., Meca, G. In vitro antifungal activity of lactic acid bacteria against mycotoxigenic fungi and their application in loaf bread shelf-life improvement. *Food Control.* 2016; 67:273-277.
- 79. Ananthi, V., Subramanian, R.S., Arun, A. Detoxification of Aflatoxin B1 using Lactic acid bacteria. *Int. J. Biosci. Technol.* 2016;9:40.
- 80. Khaneghah, A.M., Chaves, R.D., Akbarirad, H. Detoxification of aflatoxin M1 (AFM1) in dairy base beverages (acidophilus milk) by using different types of lactic acid bacteriamini review. *Curr. Nutr. Food Sci.* 2017;13:78-81.
- 81. Juodeikiene, G., Bartkiene, E., Cernauskas, D., Cizeikiene, D., Zadeike, D., Lele, V., Bartkevics, V. Antifungal activity of lactic acid bacteria and their application for *Fusarium* mycotoxin reduction in malting wheat grains. *LWT*. 2018;89:307-314.