

Review Article

Eco-friendly bacterial biodegradation of mycotoxins

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Abstract

Mycotoxins are the secondary metabolites of some fungal species, produced during harsh conditions. It contaminates food and feed resulting in some serious illness of animals and humans. The combining effect of different mycotoxin has been reported to produce an additional threat to human health. It is reported that some chemicals such as fusaproliferin, beauvericin, and fusarin enhance the activity of mycotoxin. The route of transmission of these fungi is either air or insects, while a delay in harvesting may also increase the chance of contaminations in different food and feeds. The most common mycotoxin in feed is Aflatoxin, Fumonisin, Ochratoxin, Zearalenone and Deoxynivalenol. The biosynthesis of all these mycotoxins is highly influenced by the source of carbon and modulating differentiation of light. The aflatoxin is produced when the formation of fatty acid is interrupted. Many approaches have been developed for controlling mycotoxin have used physical, chemical and biological methods. Physical and chemical degradation of mycotoxins is thought to be unsuitable due to its effect on nutrition composition and the production of some comparatively more toxic compounds. Biodegradation is one of the safest methods for controlling mycotoxin. Biodegradation with environment friendly microorganisms is the focus of the current review. *Rhodococcus*, *Bacillus subtilis*, *Bacillus licheniformis*, Actinobacteria, and rumen fluid bacteria are the important bacterial species used for biodegradation of different types of mycotoxins.

Keywords: Biocontrol; Biotransformation; Decontamination; Detoxification

Introduction

Feed and their ingredients may be contaminated with some species of fungal secondary metabolites which is term as mycotoxin. The term mycotoxin was coined in the year 1955 for the first time when the

animal disease caused by fungi was described [1]. The presence of these fungal metabolites causes serious morbidity and mortality in humans and livestock [1]. These fungal toxins are highly toxic to animals. The risk of contamination remains high during the

storage process and causes great economic losses in agriculture and livestock [2]. The chance of contamination is increased in tropical and subtropical areas of the world [3]. The possible minimum limit of mycotoxin in milk is administrated by “food and drug administration” that the action level of aflatoxin is 0.5ppm for humans while 20ppb for other types of mycotoxin in different food and feed for both human and animal [1].

Feeds and their ingredients got affected by fungi through airborne transmission or by an insect in the field. Some tough conditions such as drought, temperature, pH variation, insect infestation, delayed harvesting and flood increase the level of contamination. During the period of free cultivation, there is some factor, which supports the growth of fungi and mycotoxin production, such as warm and humid conditions at the storage place, insufficient dryness [1].

The mycotoxins are low molecular weight compounds produced by saprophytic fungi such as *Fusarium*, *Aspergillus* and *Penicillium* as secondary metabolites. For the first time, the word mycotoxin has been known in 1800 by describing ergot alkaloids which are caused by T2 toxin during world war II in Russia. In 1960 mycotoxin was declared as an important toxin, when it was identified as the causative agent of Turkey X disease in England. This turkey X disease caused the death of thousands of people who consumed mycotoxin contaminated peanuts [4]. *Aspergillus flavus* aflatoxin was responsible for this disease. The ingestion of mycotoxin especially aflatoxin can cause liver and kidney damage and induce mutation. They have carcinogenic and immunosuppressive effects and also cause various chronic and acute teratogenicity, genotoxic and estrogenic in nature [5]. Mycotoxins are stable during food processing and contaminate the final food products. According to the international agency for

Cancer Research 2002, Aflatoxin B1 is categorized as group 1 carcinogenic toxin [6]. A number of people got infected from mycotoxin each year. An outbreak of hepatitis due to Aflatoxicosis in October 1974 in India claimed more than 100 deaths, which was linked to the consumption of contaminated maize, contain mycotoxin [7]. Another outbreak of mycotoxicosis that occurred in India in the year 1985 resulted in 175 deaths due to *Aspergillus* and *Fusarium associated* Deoxynivalenol and T2 toxin contamination of wheat [8]. The world largest outbreak of mycotoxicosis occurred in 2004 in Kenya in which 125 people died due to the consumption of Aflatoxin contaminated maize resulted in liver failure [9].

Different methods have been developed for the decontamination of mycotoxin, among them, some are not feasible due to its high cost and practical application for detoxification of mycotoxin. It is reported that some bacterial and fungal species have the ability to degrade mycotoxin through their enzymatic activity. In this review, the biological control of Aflatoxin, Fumonisin, Ochratoxin will be discussed in detail and alternative environment friendly systems for decontamination of mycotoxin will be focused [7]. Several research projects have been developed to mitigate the risk of mycotoxin through risk characterization, intervention, and prevention of mycotoxin from critical control point to storage and transportation of feed and food products. Although these strategies cannot completely remove or control the presence or production of mycotoxin, some of them can reduce it to an acceptable level [10].

The production of mycotoxins is usually due to the condensation reaction under some tough biological, chemical and physical conditions. The fatty acid is the primary metabolites used by fungi for the source of energy. Aflatoxin is formed as a result of

interruption in the reduction of the ketone group of fatty acids. Usually, the mycotoxin is produced during the late stage of the exponential phase or at the beginning of the stationary phase [11]. The combined effect of different mycotoxins has been reported to produce an additional threat to human health. It is well reported that some chemicals such as fusaproliferin, beauvericin, and fusarin enhance the activity of mycotoxin [12].

Mycotoxins' presence in food and feed is a global issue and has been reported repeatedly by the researchers. The impact of these mycotoxins on human and animals' health has been noted throughout history. Ergotism was the most common mycotoxicosis caused by the infection of *Claviceps purpurea* in rye crops in Europe in the tenth century. During world war II in Siberia, the cultivation was delayed which result in contamination with trichothecenes which are produced by *Fusarium spp.* [10]. It grabbed the attention of the researches in 1960 when turkey's disease in the UK caused the death of thousands of people. Aflatoxin was are known to be responsible for this disease, which caused such great mass destruction in united kingdom (UK) [13]. Worldwide surveyed in 2009 and 2010 by Ines Rodrigues and Karin Naehrer, 2012 reported the presence of different mycotoxin in feed ingredients. Highest rate of mycotoxin were found in south Asia, pakistan, india and bangladesh [14].

Detoxification of mycotoxin through chemical methods involves chemical reaction such as ammonition [15], peroxidation, ozonation, alkaline hydrolysis and the usage of bisulfates are reported against mycotoxins But understanding the toxicity of end products and its effect on health is always worth considering [10].

Human beings and animals are more influenced by the synergistic effect of two mycotoxins. The combined effect of more than one mycotoxins are not considered

worldwide but a single mycotoxin can also affect the health of human being and animal [10]. The major mycotoxins are Aflatoxins (AFs), Ochratoxin A (OTA), Zearalenone (ZEA), Fumonisin (FUM), Trichothecenes (TCTs) and especially Deoxynivalenol (DON) have been identified in more published data. The combination of 127 mycotoxins is described in the literature while most observed ones are AFs+FUM, DON+ZEA, AFs+OTA, and FUM+ZEA. Although few research projects have been done on the combination of more than one mycotoxin contamination in feed, however, the main combine effect has been noticed [16]. In account to determine the decontamination of mycotoxin through Bacteria and Fungi, It is important to analyze the structure of mycotoxin and identify their important functional groups [10].

Mycotoxin has been associated with many diseases. It causes several chronic and acute infections in human beings (Table 1) [17]. Etiology of a few diseases has been established while more cases of mycotoxicosis are expected to be identified in the future [18]. In addition to infection and allergy, fungi can produce mycotoxins and organic chemicals that are responsible for various toxicological effects as shown in (Fig. 1) [19].

Biosynthesis of mycotoxin

The production of secondary metabolites and differentiation of modulation is caused due to response to light by fungi. The effect of carbon sources on the biosynthesis of Aflatoxin, Ochratoxin and many other types of mycotoxins has also been studied for decades and gives conflicting results [23]. Buchi et al in 1967 reported the mechanism of biosynthesis of mycotoxin, Aflatoxin, in *Aspergillus Flavus*. This mechanism comprises of 23 enzymatic reactions, controlled by a group of 25 recognized coded genes about 70-kb in size on the DNA located on chromosome III. Mycotoxin biosynthesis

is influenced by certain nutritional variables including carbon and nitrogen as well as

external factors such as pH, light, oxidative stress and temperature [19].

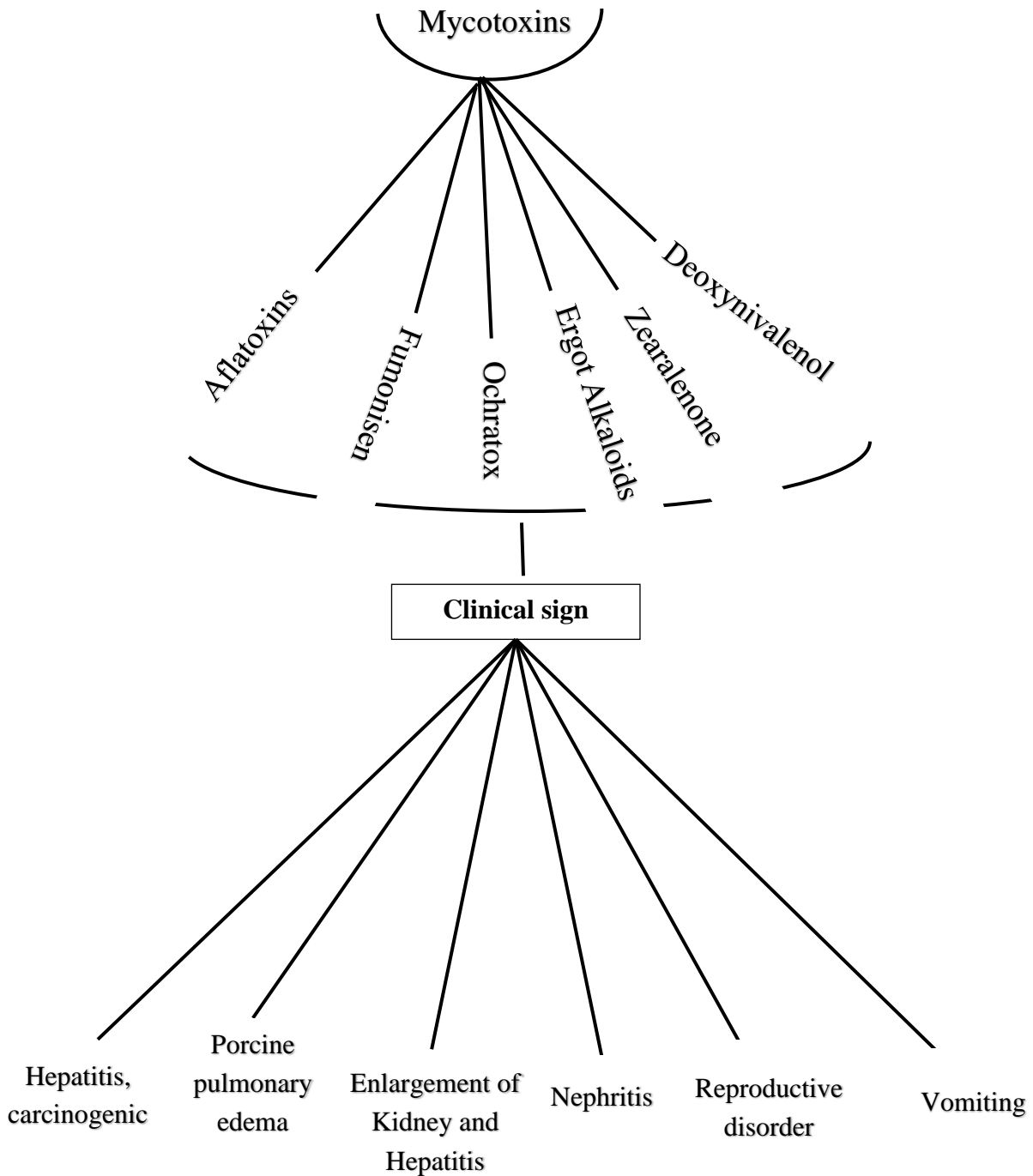


Figure 1. Flow chart of mycotoxin and its clinical complications

Table 1. Types of mycotoxin, effect and sources

Mycotoxins	Clinical Signs	Organ/System affected	References
Aflatoxins(B1,B2,M1,M2)	Hepatitis, poor response to vaccination, unspecific infections, carcinogenic, increased susceptibility to disease.	Liver, kidney, immune system	[6, 18, 20, 21]
Zearalenone	Hyperestrogenism, Reproductive disorders	Reproductive tract, mainly female	[6, 20, [21]
Deoxynivalenol	Feed refusal, vomiting.	Central nervous system, GUT epithelium, liver, immune system	[6, 20, [21]
T-2 Toxin	Oral and epithelial lesion, loss of appetite	GUT epithelium, liver, immune	[6, 20, [21]
Ochratoxins(A,B,C)	Nephritis, enlargement of kidney and hepatitis	Liver, kidney, immune system, inhibit RNA,DNA and protein synthesis in kidney	[6, 20-22]
Fumonisen	Porcine pulmonary edema(PPE) equine leukoencephalomalacia	Lungs and heart(pig), central nervous system(horse),liver, immune system	[6, 20, [21]
Ergot Alkaloids	Effecting blood supply	Central nervous system	[6, 20, [21]

Aflatoxin

When an interruption in fatty acid formation occurred, Aflatoxin is formed. Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2 are the four main Aflatoxins produced. These are derivatives of furanocoumarin with the nomenclature B and G derived from the colors of blue and green fluorescent produced on thin-layer chromatography plates under UV light. Different species of *Aspergillus* follow two different pathways, in one pathway, the sterigmatocystin formed as is the intermediate pathways While another route goes directly to the production of Aflatoxins (Fig. 2) [25]. The output of aflatoxin is typically the highest in acidic media and continues to decrease as the medium's pH increases [24].

Fumonisin

Fumonisin biosynthesis begins with polyketide condensation with alanine and polyketide dimethylated. These are followed by two propane-tricarboxylic acids being oxygenated, carbonyl reduced and esterified.

Fumonisin structure is linear, based on the chain of hydroxylated hydrocarbons. These are only fumonisin properties that distinguish them from other mycotoxins [26]. The genes that code for fumonisin production are situated at one locus of the genome of *F.verticilliodes*. The whole gene cluster involved in producing fumonisin is completely absent in *Fusarium graminearum*, but enzymes of fumonisin biosynthesis are present. There are four genes that are needed to esterify tricarballic acid while at the opposite end, a protein carrier and two other proteins that are considered for self-protection and sphingolipid metabolism. The mutation can change the structure and function of fumonisin in these genes [27]. In particular, fumonisin consists of 22 aminophenol carbon with two side groups of tricarballic acid (TCA), where two structural groups are essential in their mechanism of toxicity. Among the two functional groups of (TCA) the first side chain is primary amino group at C2 which cannot be changed, competitively prevents ceramide synthase,

thus preventing ceramide biosynthesis and sphingolipid metabolism in this free primary amino group of fumonisin-like compounds is a prerequisite for ceramide synthase inhibition, as N-acetylation of FB1 reduced or removed toxicity effects in rat liver slices and jimsonweeds. The toxicity effects of the

TCA side groups tend to differ. On the one hand, both phytotoxicity and mammalian cytotoxicity were found to be lacking in these side groups. And the corresponding backbones of aminophenol (AP1, AP2) were only 30–40% or 10% as active as the parent toxins [28].

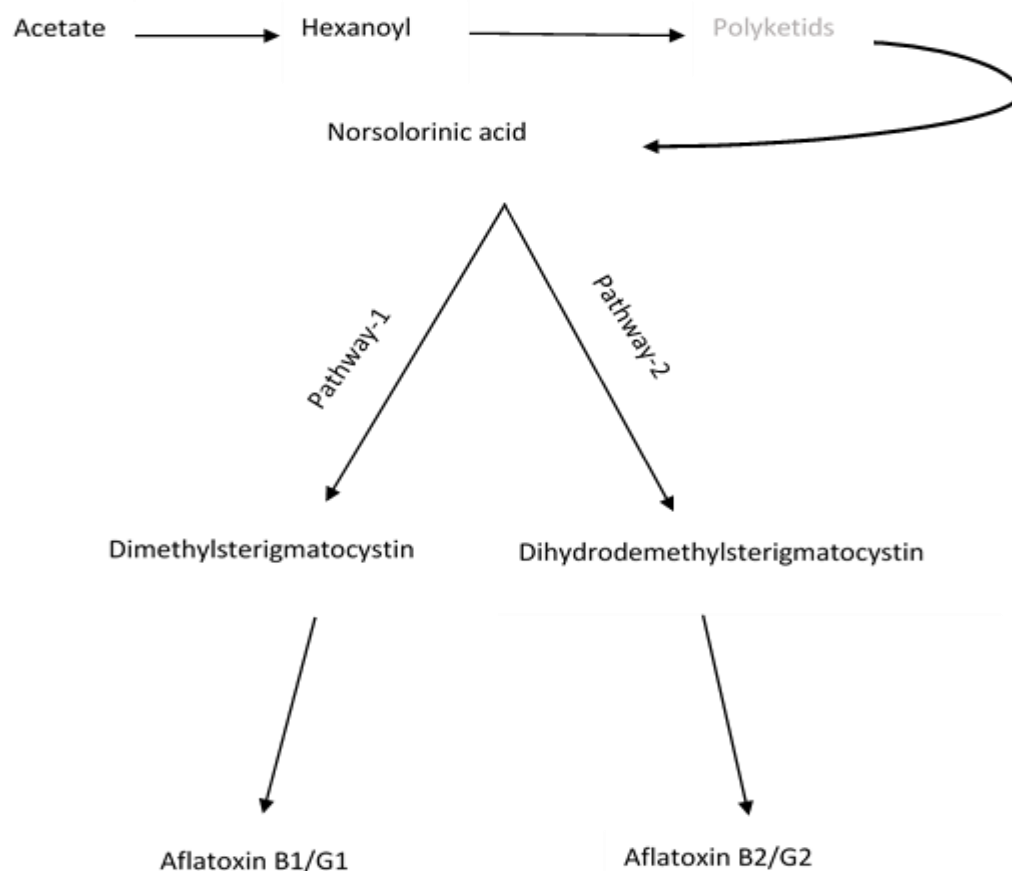


Figure 2. Biosynthesis of Aflatoxin (Adopted from Sweeney (2002) [25])

Trichothecenes

These mycotoxins can be produced by many fungal species but most commonly isolated from *Fusarium*. The special feature of this mycotoxin is that it inhibits protein synthesis in humans and other animals. These toxins are present worldwide in foods such as wheat, rice, and maize [26]. The biosynthesis pathway begins with trichodiene synthesis from farnesyl pyrophosphate cycling. The

one and only enzymes extracted and isolated from the species of fungi [25].

Ochratoxins

For the first time, the name given to this toxin is due to its production and isolation from *Aspergillus ochraceus* species. It has been noted that these are the most toxic among all mycotoxins and abundantly reported. This mycotoxin is produced when the biosynthesis of phenylalanine is interrupted, polyketides convert to mullein instead of phenylalanine

[29]. Several studies have shown that there is no ketoenol tautomerism but only a non-functional dual carbon bond of the mellein pentaketide chain available in this mycotoxin. Ochratoxin is chlorinated by a special enzyme “chloroperoxidase”. This enzyme facilitates there action of phospho ochratoxin with phenylalanine which results in ethyl esterification [30].

Microorganism involved in biodegradation of mycotoxin

Decontamination strategies are technologically diverse and based on physical, chemical, or biochemical principles to reduce mycotoxins in food and feed commodities. Many physical procedures are designed to remove highly contaminated fractions from bulk material by sorting washing, heating, irradiation, or combination approaches. The use of inorganic or organic mycotoxin binders is another physical removal strategy. While these adsorbent binders have some promising characteristics, some may have adverse nutritional effects

due to the binding of vitamins and minerals [28].

In milk, vegetable oil, corn, peanut, peanut butter, and peanut milk, the ability of the microorganism (Table 2) to remove Aflatoxins from food was demonstrated. More recently, Hao and Bracket used this microorganism to detoxify peanut milk. These researchers proposed that the biological detoxification of these or other foods and feeds could be of potential safe [31].

There are two sites on aflatoxins which shows the toxic activity, furofuran and lactone ring. The toxicity of aflatoxin is mitigate when the structure of these two sites changed. The complete mechanism of changing these sites through microorganism is not known. Although the tragetting of coumarin in Aflatoxin in biodegradation has been reported. The targeting of coumarin, the mutagenic property of aflatoxin is reduced [32].

Table 2. Bacterial species involved in biodegradation of mycotoxins

Bacterial species	Mycotoxin degraded	References
Rhodococcus	Aflatoxin A, aflatoxin B1	[33]
	Zearalenone	[34]
	Ochratoxin	
	Deoxynivalenol	
Acinetobacter	Fumonisin	[35]
	Ochratoxin	[36]
	Aflatoxin A, aflatoxin B1	[37]
Bacillus subtilis	Zearalenone	[38]
	Ochratoxin	[39]
	Deoxynivalenol	[40]
Rumen fluid bacteria	Fumonisin	[41]
	Aflatoxin	[40]
	Zearalenone	
	Ochratoxin	
Deoxynivalenol		

Economic importance

The United Nation’s Food and Agriculture Organization (FAO) estimated that around

25% of the world's agricultural products are contaminated with mycotoxins, leading to significant economic losses due to their

impact on human health, trade and animal productivity [42]. Out of 300 food and feed mycotoxins, only twenty mycotoxins are responsible for these losses (World Health Organization, 2010). In recent years, the rise in mycotoxin-related incidents may be due to the increasing number of extreme weather events and climate change. A recent report on fungi producing aflatoxin in Hungary also supports this hypothesis. Therefore the focus on developing advanced technologies accordingly, to detoxify feeds and food which is contaminated with these 20 species of *Aspergillus*, *Fusarium*, and *Penicillium* [34].

Conclusion

A mycotoxin is one of the important factors affecting the production of poultry products. It has the ability to produce some serious complications in human and animal health such as hepatitis, kidney infection, meningitis and cause abortion. Mycotoxin in poultry can stunt the growth of chicken which results in low meat and egg production. Many methods have been developed for the control of mycotoxins but the majority of it is not universally applicable due to their side effect one way or another. Chemical control or chemical biodegradation can effectively reduce the mycotoxin but the end product of chemical detoxification may produce some more toxic substances harmful to living things. Among different strategies, one of the most important approaches to control mycotoxin is biodegradation of mycotoxin with the help of Eco friendly microorganism. Some environment-friendly bacteria such as *Rhodococcus*, *Actinobacteria*, *Bacillus subtilis* and *Bacillus licheniformis* have been reported for successful biodegradation of different mycotoxins. Biodegradation is a comparatively safe method to control or reduce the number of mycotoxin contaminations. To control the production of mycotoxin other than biodegradation the use of fungi-free seeds and its storage at dry place

to avoid fungal growth can be a good strategy.

Authors' contributions

Conceived and designed the experiments: A Akbar, M Shafee, Performed the experiments: G Ishaq, Analyzed the data: H Sadia & G Ishaq, Contributed materials/ analysis/ tools: A Razaq, M Anwar & FU Rehman, Wrote the paper: G Ishaq & A Akbar.

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