Mycotoxins: A Review of Toxicity, Metabolism and Biological Approaches to Counteract the Production in Food

Nidhi Didwania
Deptt. of Biotechnology, FET,
Manav Rachna International University,
Faridabad, Haryana, India

P.C. Trivedi
Deen Dayal Upadhyay University,
Gorakhpur, UP, India

Abstract: *Mycotoxins are secondary metabolites produced by toxic species of fungi. These can cause ill effects in humans and animals. More than 250 mycotoxins have been detected, but relatively few are considered to be important to animal health. This review has its focus on the biochemistry and metabolism of mycotoxins in food and feed associated with risks to humans and livestock. This review is meant to be informative not only for health-conscious consumers but also for experts in the field, to pave the way for future research. This would also fill the existing gaps in our knowledge with regard to mycotoxins and food safety.*

I. INTRODUCTION

Mycotoxins are secondary metabolites of fungi. The poisoning by mycotoxin is referred to as **mycotoxicoses**. Because of their potent toxic nature and fairly common occurrence under natural conditions, mycotoxins have attracted world-wide attention in the recent years.

The major fungal genera producing mycotoxins include *Aspergillus, Fusarium and Penicillium* that invade crops at the field level and may grow on foods during storage under favorable conditions (temperature, moisture, water activity, relative humidity).

II. OCCURRENCE IN FOODS

Mycotoxins have been reported to be carcinogenic, tremorogenic, haemorrhagic, teratogenic, and dermatitis to a wide range of organisms and to cause hepatic carcinoma in human (Refai, 1988; van Egmond, 1989, Wary, 1981). More than a hundred species of filamentous fungi are known to produce mycotoxins and to cause toxic responses under naturally occurring conditions. Mycotoxins can enter the human and animal food chains by direct contamination when the food gets contaminated by toxic fungi while growing or after harvest, or indirect contamination, for example in milk from cows fed with contaminated food (Carlile *et al*., 2001). Mycotoxins contaminate up to 25% of the world's food supply. More than 300 mycotoxins are known, of which about 20 are serious contaminants of crops used in human foods and animal feeds. Mycotoxin contamination of foods and feeds depends highly on environmental conditions that lead to mould growth and toxin production (Van Egmond, 1989).

III. BIOSYNTHESIS AND METABOLISM

a. Biosynthesis and Metabolism of Aflatoxins:

A. flavus, *Aspergillus nomius* and *Aspergillus parasiticus* are the only fungal species known to produce aflatoxins (Cotty et al., 1994). However, as many as 20 different aspergilli, including *Aspergillus nidulans*, and species of *Bipolaris*, *Cbaetomiztm*, *Farrowia* and *Monocillizrm*, produce sterigmatocystin (ST), a highly toxic intermediate in the AFB1 biosynthetic pathway (Barnes et al., 1994). The initial step in generation of the polyketide backbone of AFB1 is proposed to involve polymerization of acetate and nine malonate units (with a loss of CO2) by a polyketide synthetase (PKS) in a manner analogous to fatty acid biosynthesis (Bhatnagar et al., 1992). An alternative and perhaps more plausible hypothesis involves the synthesis of a 6-carbon hexanoate starter unit by a fatty-acid synthase (FAS), which is then extended by a PKS (without further keto reduction) to generate a 20-carbon decaketide, noranthrone (Townsend et al., 1991). In either scheme,

noranthrone is then oxidized to the anthraquinone norsolorinic acid (NA) by a hypothesized oxidase (Bhatnagar et al., 1992).

The system responsible for the biotransformation of AFB1 basically has five mechanisms, represented by reactions of reduction, hydration, epoxidation, hydroxylation and ortho-demethylation. The aflatoxicol is produced by reduction of AFB1 by an NADPH-dependent cytoplasmic enzyme present in the soluble fraction of the liver. The toxicity of aflatoxicol is apparently much smaller than AFB1, but the conversion is reversible and the aflatoxicol can serve as a reservoir toxicity of AFB1 in the intracellular space, it can be converted in this mycotoxin by microsomal dehydrogenase. The aflatoxicol can also be metabolized to AFM1 and AFH1 (Biehl and Buck, 1987).

b. Biosynthesis and Metabolism of Fumonisins

The study of biosynthesis of fumonisins began in the past decade, which were cloned a cluster of genes called as cluster gene FUM, this cluster has, approximately, 42-Kb in length (Proctor et al., 2003). Fumonisin polyketide synthase (FUM1) was the first gene to be cloned and is the principal for the fumonisin biosynthesis. Since then, others cluster of genes required for fumonisin biosynthesis was identified, although some with function is not yet known (Desjardins and Proctor, 2007). According to Seo et al. (2001), the fumonisins biosynthesis began with the linear molecule synthesis of 20 carbons called polyketide. Second to Lorenzzetti et al. (2006), during the biosynthesis of fumonisins is not clear whether they are derived from a fatty acid or polyketide one, because both these compounds are derived from acetate.

The structure of fumonisin B1 is very similar to that of the free sphingoid base sphinganine. The sphingolipids plays important roles in membrane and lipoprotein structure, cell-to-cell communication, interaction between cells and extracellular matrix and regulation of growth factor receptors and as second messengers for a wide range of factors, including the tumor necrosis factor and interleukin-1 (Soriano et al., 2005). Fumonisin-induced disruption of sphingolipid metabolism is important in the cascade of events leading to altered cell growth, differentiation and injury observed both in vivo and in vitro (fumonisin disruption of ceramide). The fumonisins are competitive inhibitors with respect to both substrates (that is, sphinganine and fatty acyl coenzyme A) of sphinganine (sphingosine) N-acetyltransferase (ceramide synthase). (Soriano et al., 2005).

c. Biosynthesis and Metabolism of Trichothecenes

Trichothecenes biosynthesis begins with the cyclisation of the isoprenoid farnesyl pyrophosphate (FPP) to hydrocarbon trichodiene by the enzyme trichodiene synthase (Hohn and Van Middles worth, 1986)). The subsequent pathway involves a number of oxygenations, isomerisations, cyclisations and esterifications leading from trichodiene to diacetoxyscirpenol, T-2 toxin and 3-acetyl deoxynivalenol. All of the intermediates except those involved in the earlier steps of the non-macrocyclic biosynthetic pathway have been confirmed by feeding studies (Desjardins et al., 1993). In contrast, themacrocyclic biosynthetic pathway is much less understood; only the end products and late intermediates of the pathway have been isolated and characterized (Jarvis et al., 1991).

Compared with some of the other mycotoxins such as aflatoxin, the trichothecenes do not appear to require metabolic activation to exert their biological activity (Busby and Wogan, 1981). After direct dermal application or oral ingestion, the trichothecenes mycotoxins can cause rapid irritation to the skin or intestinal mucosa. In cell-free systems or single cells in culture, these mycotoxins cause a rapid inhibition of protein synthesis and polyribosomal disaggregation (Busby and Wogan, 1981). Thus, we can postulate that the trichothecenes mycotoxins have molecular capability of direct reaction with cellular components. Despite this direct effect, it is possible to measure the toxic kinetics and the metabolism of the trichothecenes mycotoxins. The lipophilic nature of these toxins suggests that they are easily absorbed through skin, gut, and pulmonary mucosa (Wannemacher and Wiener, 1997).

IV. CONTROL OF MYCOTOXIN IN FOODS

The best way of controlling mycotoxin contamination is by prevention and can be accomplished by reducing fungal infection in growing crops through the adoption of suitable cultural practices, by rapid drying or by the use of suitable preservatives (Sinha, 1993). Physical, chemical and biological methods have been investigated in order to prevent the growth of mycotoxin producing fungi, eliminate or reduce the toxin levels, degrade or detoxify the toxins in foods and feeds. Mycotoxins can be eliminated or detoxified by physical, chemical or biological techniques. Many chemicals including numerous acids, alkalis, aldehydes, oxidizing agents and several gases have been tested for their ability to degrade or inactivate aflatoxin and many other

mycotoxins (Samarajeenwa *et al*., 1990; Thanaboripat, 2002).

Natural plant extracts may provide an alternative way to protect foods or feeds from fungal contamination (Yin and Cheng, 1998). While dealing with grain protection, fumigation is the preferred method for applying substances into the bulks in order to control the biotic factors which damage the grains (Paster *et al*., 1995).

a. Aflatoxins

Aflatoxins are potent toxic secondary metabolites produced mainly by *Aspergillus flavus, A. parasiticus* and rarely *A. nomius*. Several bacterial species, such as *Bacillus, Lactobacilli, Pseudomonas, Ralstonia and Burkholderia spp*., have shown the ability to inhibit fungal growth and production of aflatoxins by *Aspergillus spp*. under laboratory conditions. For example, Palumbo et al. (2006) reported that a number of *Bacillus, Pseudomonas, Ralstonia* and *Burkholderia* strains could completely inhibit *A. flavus* growth. Munimbazi and Bullerman (1998) reported that more than 98% inhibition in aflatoxin production by *A. parasiticus* was caused by *B. pumilus*. El-Nezami et al. (1998) reported the ability of lactic acid bacteria to remove AFB1 from artificially contaminated liquid media. The removal was strain dependent and very fast, with two strains of *L. rhamnosus* removing about 80% of the toxin at the beginning of the incubation time period.

b. Fumonisins

Fungi belonging to the genus *Fusarium* are associated with the production of fumonisins. Among the fumonisins, fumonisin B1 (FB1) in particular is of international, agro economic, and food safety concern. For example, high doses of FB1-infested corn feed have been shown to cause pulmonary edema in swine, while lower doses lead to hepatic disease. Stiles and Bullerman (2002) studied the effect of *L. rhamnosus* on growth and mycotoxin production by *Fusarium* species, including *F. proliferatum, F. verticillioides* and *F. graminearum*. The results showed that production of FB1 was reduced up to 63.2%, FB2 up to 43.4% and deoxynivalenol and zearalenone up to 92% and 87.5%, respectively. *L. rhamnosus* was evaluated for its potential to remove or degrade zearalenone and á-zearalenol and both viable and non-viable cells were able to remove about 50% of the toxin from solution, indicating that binding rather than metabolism was the mechanism in action. When *L. rhamnosus* was exposed to both toxins at the same time, its ability to remove zearalenone and á-zearalenol from solution was significantly reduced; indicating that these toxins may share the same binding site on the bacterial cell . Niderkorn et al. (2006) screened lactic and propionic acid bacteria for their ability to remove deoxynivalenol and fumonisin from solution and they found that it was strain specific, with propionic acid bacteria being less efficient than LAB. *L. rhamnosus* removed up to 55% of deoxynivalenol, while *Leuconostoc mesenteroides* removed about 82% of FB1 and *L. lactis* removed 100% of FB2. *B. amyloliquefaciens* and Micro bacterium oleovorans isolates were shown to effectively reduce *F. verticillioides* propagules and fumonisin content in maize kernels at harvest when applied as seed coatings.

c. Ochratoxins

Ochratoxin A (OTA) produced by *Aspergillus* and *Penicillium spp.* is a natural contaminant in cereals and beverages. According to the available literature grape and wine are considered as the second major source of OTA intake after cereals. Thus, it is understandable that considerable research has been done concerning detoxification of these two food groups. Fuchs et al. (2008) screened 30 different LAB strains for their ability to remove OTA from solution, and they reported 95% removal of OTA in liquid media by *L. acidophilus*. Bejaouii et al. (2006) studied the degradation of OTA by 40 isolates of *Aspergillus* section Nigri species isolated from French grapes. They reported significant reduction of OTA in liquid medium.

d. Patulin

Patulin contamination of fruit-based foods and beverages is an important food safety issue due to the high consumption of these commodities throughout the world. Based on the experimental results available, it has been concluded that patulin produced by *Penicillium* spp. is genotoxic, although no adequate evidence of carcinogenity in experimental animals exists. Apple and apple products are main sources of patulin contamination. However, patulin has also been found in other fruits, such as pears, peaches, apricots, grapes and cheese. Extensive research has been carried out to study the effect of yeasts on biodegradation of patulin since 1990's. Stinson et al. (1978) observed decrease of patulin content in fermented apple juice contaminated with yeast during alcoholic fermentation. Recently, Moss and Long (2002) reported that commercial yeast *S. cerevisiae* transformed patulin into ascladiol. As reported by Moss (1998), acute toxicity of ascladiol amounted to only one-fourth of the strength of patulin.

Richelli et al. (2007) found the ability of *Gluconobacter oxydans* to degrade patulin more than 96% after twelve-hour treatment, due to change of chemical structure and the degradation product (ascladiol) of this mycotoxin. The genus *Gluconobacter*, whose taxonomy is at present under worldwide review, is made up of five different species, which have no health risk, and that are commonly used in food manufacturing. According to available literature, this method is much better understood compared with other decontamination methods.

V. PREVENTION AND FUTURE OUTLOOK

Prevention of fungal contamination and thereby toxin production can be achieved either during pre harvest stages by good crop husbandry and appropriate cultural practices and the use of a HACCP (Hazard analysis and critical control points) plan, as well as during postharvest stages by the application of proper drying, storage, and transport procedures. Application of fungicides at field levels might reduce mold growth resulting in the reduction of production of mycotoxins. Further, studies have to be conducted to look for a better and environmentally friendly alternative at the field level rather than relying on chemicals.

REFERENCES

[1]. Barnes SE, Dola TP, Bennett JW, Bhatnagar D (1994). Synthesis of sterigmatocystin on a chemically defined medium by species of *Aspergillus* and *Chaetomium*. Mycopathol. 125:173-178.

[2]. Bejaoui H, Mathieu F, TH ailladier P, Lebrihi A. (2006). Biodegradation of ochratoxin-A by *Aspergillus* section Nigrispecies isolated from French grapes: a potential means of ochratoxin-A decontamination in grape juices and musts. FEMS Microbiol Lett.:255:203-206.

[3]. Bhatnagar D, Ehrlich KC, Cleveland TE (1992).Oxidation-reduction reactions in biosynthesis of secondary metabolites. In: Handbook of applied mycology: mycotoxins in ecological systems.

[4]. Biehl ML, Buck WB (1987). Chemical contaminants: their metabolism and their residues. J. Food Nutr., 50: 1058-1073.

[5]. Busby WF, Wogan GN (1981). Trichothecenes. In: Mycotoxins and N-Nitroso Compounds: Environmental Risks. Shank RC (Es.). CRC Press, Florida, U.S.A., pp. 29-41.

[6]. Carlile, M. J., Watkinson, S.C. and Gooday, G.W. (2001). The Fungi. 2nd ed.Academic Press, San Diego

[7]. Cotty, P.J. (1988).Simple fluorescence method for rapid estimation of aflatoxin levels in a solid culture medium. Appl. Environm. Microbiol. 54: 274-276.

[8]. Desjardins AE, Hohn TM, McCormick SP (1993). Trichothecene biosynthesis in *Fusarium* species: chemistry, genetics and significance. Microbiol. Rev., 57: 595-604.

[9]. Desjardins AE, Proctor RH (2007). Molecular biology of *Fusarium* mycotoxins. Int. J. Food Microbiol. 119: 47-50.

[10]. El & Nezami H, Polychronaki N, Salminen S, Mykkänen H. (2002). Binding rather than metabolism may explain the interaction of two food & grade *Lactobacillus* strains with zearalenone and its derivative á & zearalenol. Appl Environ Microbiol.68:3545&3549.

[11]. Fuchs S, Sontag G, Stidl R, Ehrlich V, Kundi M, Knasmuller S. (2008). Detoxification of patulin and ochratoxin A, two abundant mycotoxins, by lactic acid bacteria. Food Chem Toxicol.:46:1398-1407.

[12]. Hohn TM, Van Middlesworth F (1986). Purification and characterization of the sesquiterpene cyclase trichodiene synthase from *Fusarium sporotrichioides*. Arch. Biochem. Biophys., 251: 756-761.

[13]. Jarvis BB, Mokhtan-Rejali N, Schenkl E, Barros C S, Matzenbacher NI (1991). Trichothecene mycotoxins from Brazilian Baccharis species. Phytochem., 30: 789-797.

[14]. Lorenzzetti RS, Vilas-boas IA, Garcia JE (2006). Genetic aspects of biosynthesis of fumonisins. Sci. Biol. Health, 8: 63-70.

[15]. Moss MO. Recent studies of mycotoxins. (1998) J Appl Microbiol Sympos Suppl.:84:62S-76S.

[16]. Moss MO, Long MT. (2002). Fate of patulin in the presence of the yeast *Saccharomyces cerevisiae*. Food Addit Contam.:19:387-399.

[17]. Munimbazi C, Bullerman LB. (1998). Inhibition of aflatoxin production of *Aspergillus parasiticus* NRRL 2999 by *Bacillus pumilus* Mycopathologia. 140: 163&169.

[18]. Niderkorn V, Boudra H, Morgavi DP. (2006). Binding of Fusarium mycotoxins by fermentative bacteria in vitro. J Appl Microbiol.:101:849&856.

[19]. Palumbo JD, Baker JL, Mahoney NE. (2006). Isolation of bacterial antagonists of A*spergillus flavus* from almonds. Microb Ecol.; 52(1):45–52.

[20]. Paster, N., Menasherov, M., Ravid, U. and Juven, B. (1995). Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. J. Food Prot. 58: 81-85

[21]. Proctor RH, Brown DW, Plattner RD, Desjardins AE (2003). Co-expression of 15 contiguous genes delineates a fumonisin biosynthetic gene cluster in *Gibberella moniliformis*. Fungal Genet. Biol., 38: 237-249.

[22]. Refai, M. K.(1988). Aflatoxins and Aflatoxicosis. J. Egypt. Vet. Med. Ass . 48(1): 1-19.

[23]. Richelli A, Baruzzi F, Solfrizzo M, Morea M, Fanizzi FP. (2007). Biotransformation of patulin by *Gluconobacter oxydans*. Appl Environ Microbiol.:73:785-792.

[24]. Seo JA, Proctor RH, Plattner MR (2001). Characterization of four clustered and co regulated genes associated with

fumonisin biosynthesis in *Fusarium verticilioides*. Fungal Genet. Biol., 34: 155-165.

[25]. Sinha, K.K. (1993). Mycotoxins. ASEAN Food J. 8: 87-93. Smith, J.E. and Moss, M.O. 1985. Mycotoxins. Formation, Analysis and Significance. John Wiley & Sons, Chichester.

[26]. Soriano JM, González L, Catalá AL (2005). Mechanism of action of sphingolipids and their metabolites in the toxicity of fumonisin B1. Prog. Lipid Res., 44: 345-356.

[27]. Stiles J, Bullerman LB. (2002). Inhibition of Fusarium species and mycotoxin production by *Bacillus pumilus* NEB1 and *Lactobacillus rhamnosus* VT1. Proceedings of 13th International Reinhardsbrunn Symposium. In: Modern Fungicides and Antifungal Compounds III Agro Concept GmbH. Dehne HW, Gisi U, Kuck KH, Russell PE, Bonn LH (Eds), Germany, May 14&18,

[28]. Stinson EE, Osman SF, Huhtanen CN, Bills DD. (1978). Disappearance of patulin during alcoholic fermentation of apple juice. Appl Environ Microbiol.: 36:620-662.

[29]. Thanaboripat, D., Im-erb, A. and Ruangrattanametee, V. (2002). Effect of Ling Zhi mushroom on aflatoxin production of *Aspergillus parasiticus*. In Yang Qian (ed.), Biological Control and Bio-technology. Heilongjiang Science and Technology Press, Harbin, pp.22-30.

[30]. Townsend CA, McGuire SM, Brobst SW, Graybill TL, Pal K, Barry CE (1991). Examination of tetrahydro- and dihydrobisfuran formation in aflatoxin biosynthesis: from whole cells to purified enzymes. In: Secondary-Metabolite Biosynthesis and Metabolism. Petroski RJ, McCormick SP (Eds.). Plenum Press, New York, U.S.A., pp. 141-154.

[31]. Van Egmond HP. (1989). Current situation on regulations for mycotoxins: overview of tolerances and status of standard methods of sampling and analysis. Food Addit Contam 6:139–88.

[32]. Wannemacher RW and Wiener S (1997). Trichothecene mycotoxins. In: Medical Aspects of Chemical and Biological Warfare.Sidell FR, Takafugi ET, Franz DR (Eds.). TMM Publications, Washington, U.S.A., pp. 655-676.

[33]. Wary, B. B. (1981). Aflatoxin, hepatitis, B. virus and hepatocellular carcinoma. New England J. Med. 305 (14): 833-843.

[34] Yin, Mei-Chen and Cheng, Wen-Shen. (1998). Inhibitory of *Aspergillus niger* and *Aspergillus flavus* by some herbs and spices. J. Food Prot. 61: 123-125. ❐