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Aflatoxin B_1 Occurrence, Biosynthesis and its Degradation

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Mycotoxins comprises of various fungal toxins, many of which have been implicated as carcinogen to man and animals. There are four major classes of mycotoxins namely Aflatoxins, Zearalenone, Ochratoxins, and Fumonisins. Among them Aflatoxins are the most toxic and carcinogenic compounds. Aflatoxin B_1 (AFB_1) has been defined as a potent carcinogen by the International agency for research on cancer (IARC) and is the most deadly toxin among the other aflatoxins present. AFB_1 is produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus.* These toxins are commonly found in developing countries such as Africa, India and Southeast Asia. The level of contamination is found high in cereals, dried fruits and peanuts that are used as food and feed. Even small quantity leads to cumulative effect and poses threat to human and animal health. This leads to the use of new technologies for testing hazardous foods. Physical, Chemical and Biological methods have be adopted for degradation of AFB_1, but large-scale, practical and cost-effective methods for detoxifying AFB_1 containing feedstuffs are currently not available. This review deals with the potential hazards of AFB_1 in concern with prevention strategy for fungal contamination.

Key words: Aflatoxin B_1, *Aspergillus flavus,* Mycotoxins.

Mycotoxins are the secondary metabolites produced by important saprophytic and spoilage fungi that are associated with severe toxic effects to vertebrates. Approximately 400 compounds are recognized as mycotoxins of which only few are addressed by food legislation. Most of the existing analytical methods focus on identification of these toxins, i.e., Trichothecenes, Aflatoxins, Zearalenone, Ochratoxin A, Fumonisins and Patulinn (Harrison *et al.,* 1990). In 1960, ten thousand young turkeys on poultry farms in England died due to "Turkey X disease". This is because of the consumption of peanut meal and it was found that this peanut meal was highly toxic to poultry and ducklings. The nature of the toxin clearly suggested that it might be from fungal origin. Later, the toxin producing fungus was identified as *Aspergillus.* Aflatoxin is a secondary metabolite of many saprophytic fungi. Foods and feeds are susceptible to invasion by *Aspergillus* species and results in the production of aflatoxins during pre-harvesting, processing, transportation and storage in warm climates. The contamination with aflatoxin in various agricultural and food products causes severe financial loss to the agriculture industries. There are at least 16 characterized structurally related aflatoxins, but the four major aflatoxins are AFB_1, AFB_2, AFG_1, AFG_2 and two more additional metabolic products, M_1 and M_2. Aflatoxin B_1 and B_2 emits blue fluorescence under UV-light, where as the Aflatoxin G_1 and G_2 gives yellow-green fluorescence under UV-light. *Aspergillus flavus* produces AFB_1 and AFB_2 while *Aspergillus parasiticus* produces AFB_1, AFG_1, AFB_2, and AFG_2. Some other species that produce aflatoxins are *Aspergillus pseudotamarii, Aspergillus bombycis,*

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Aspergillus ochraceous, Aspergillus nominus (Kurtzman *et al.,* 1987; Cotty *et al.,* 1999; Peterson *et al.,* 2001). More than 20 *Aspergillus sp.* produces sterigmatocystin as final product, which is the penultimate precursor of aflatoxin (Bennett *et al.,* 1983).

Chemistry of the aflatoxins

Aflatoxins are di-furano coumarins with AFB_2 and AFG_2 as individual derivatives of AFB_1 and AFG_1. The biosynthesis pathway of aflatoxins has been postulated as followed: norsolorinic acid → averufin → versiconalacetate → versicolorin-A → sterigmatocystin → AFB_1 → AFG_1. The toxicity of the aflatoxins decreases from AFB_1 → AFG_1 → AFB_2 → AFG_2, which is an indicator that the double bond at the 8, 9-position of the terminal furan ring is a key factor for the toxicity of the toxin (Figure. 1). Aflatoxins are heat stable compounds and normally do not easily degrade during common food or feed processing. However, several approaches for detoxification have been proposed, ranging from microbial, physical and chemical or even radiation (Chitrangada, D and Mishra, H.N., 2000). Aflatoxin leads to contamination of various agricultural commodities and cause various diseases, such as aflatoxicosis, in livestock, domestic animals and humans throughout the world. They cause liver damage, decreases milk and egg production, embryo toxicity in animals, when consumed in low dietary concentrations (Bennett J. W *et al.,* 1983 and Bhatnagar *et al.,* 1999).

Occurrence of aflatoxins in food and feed

The growth of aflatoxin producing Aspergillus species depends on substrate and environmental factors, such as water activity, temperature, pH and microbial competition. As a result, *A. flavus* and *A. parasiticus* are considered as xerophilic since they can grow at low water activities (aw 0.75-0.8). Both these fungi can grow in a temperature range from 12ºC to 48ºC, the best conditions for aflatoxin growth is around 25ºC. The produced aflatoxins can be found in a diverse range of products either in the field of pre-harvesting, storage or postharvest. However, higher level of aflatoxin contamination is mainly associated with post-harvest growth of Aspergillus moulds in poorly stored commodities. Aflatoxin concentrations in the mg/kg range have been detected (Bhat, R.V., 1988). The food items that have been reported to contain aflatoxins are cereals such as corn, barley and oats, dried fruits such as figs, nuts and oilseeds such as peanuts and cotton seeds as well as spices such as pepper, paprika or chillis. However, corn and peanuts are the most commonly contaminated food items worldwide. There are several reviews on the occurrence of aflatoxins (Peltonen *et al.,* 2008; Shu Guan *et al.,* 2008; Teniola *et al.,* 2005; Rustom, I.Y.S., 1997; Croy, R.G et al 1978 and Robertson *et al.,* 1970) which clearly show that the occurrence of aflatoxins in food and feed is still a relevant issue in food safety.

Formation of DNA adduct

The aflatoxins are among the most potent genotoxic agents among the existing mycotoxins known. AFB_1 induces chromosomal aberrations, micronuclei, sister chromatid exchange, unscheduled DNA synthesis, chromosomal strand breaks, and forms adducts in human cells (Busby, W.F *et al.,* 1985; Foster, P.L *et al.,* 1983 and Essigmann, J.M *et al.,* 1977). Aflatoxin containing an unsaturated terminal furan ring, which binds covalently with the DNA and leads to the formation of epoxide. The specific role of cytochrome P450 is, it metabolises aflatoxin to 8,9-exo-epoxide and 8,9-endo-epoxide respectively. The reaction of AFB_1–8,9-exo-epoxide with DNA strongly induces adduct formation. AFB_1–8,9-exo-epoxide appears to be the only product of AFB_1 involved in reaction with DNA in the N_7 guanine position. The imidazole portion of the formed AFB_1–N_7-Guanine adduct gives rise to a ring opened formamidopyrimidine (AFB_1–FAPY) and the other minor N_7-guanyl adducts can happen through enzymatic oxidation of AFP_1 and AFM_1 (Croy, R.G et al 1978 and Johnson, W.W and Guengerich, F.P., 1977). The exception is AFQ_1, which is comparatively poor substrate for epoxidation to occur and presenting very low potency for DNA binding (Croy, R.G and Wogan, G.N., 1981; Yu, M.W *et al.,* 1997 and Theo, K. B *et al.,* 2000).

Aflatoxin degradation

There are several methods adopted to degrade aflatoxin and each of them has its own limitations. This led the workers to explore novel and effective methods of detoxification.

Physical methods

Physical method includes cleaning, mechanical sorting, separation and heat treatment, ultrasonic treatment, and irradiation. During the

cleaning process dust, husks, hair, and shallow particles are separated from the contaminated food. Mechanical sorting and separation process cleans food material free of aflatoxin contaminated grains, while washing procedures includes using water or sodium carbonate solution. Thermal processing such as boiling, microwave heating and irradiation were applied for inactivation of the toxin or to reduce its content in foods and feeds. These methods have some effect on aflatoxin reduction in peanut meal (Coomes *et al.,* 1966), but this lone approach may be insufficient because aflatoxin are heat resistant within the range of conservative food processing temperatures (80-121°C). Use of gamma rays and ultraviolet rays would appear to be unproductive in toxin inactivation (Feuell, 1966) while soft x-rays and electron irradiation at higher doses could possibly affect not only the contaminated food, but the non-contaminated food also and make them unfit for consumption. (Frank and Grunewald, 1970). When it comes to the extraction process, solvent extraction procedure for removal of aflatoxin from food sample was extensively carried out by a research group at the Southern Regional Research Center in New Orleans. Whereas variety of solvents such as methanol, ethanol, acetone, chloroform, benzene, and aqueous isopropanol have been intensively used for the removal or elimination of aflatoxin from cottonseed and peanut meals (Gardner *et al.,* 1968;

Table1.

Micro organism	Removal of AFB_1 (%)	References
Bacteria		
Flavobacterium aurantiacum NRRL B-184	22-40%	Ciegler *et al.,* 1966
Rhodococcus erythropolis	17%	Teniola *et al.,* 2005
Lactobacillus rhamnosus	80%	Peltanone *et al.,* 1998
Enterococcus faecium M74	19.3 - 30.5%	Ali Topcu *et al.,* 2010
Enterococcus faecium EF031	23.4 - 37.5%	Ali Topcu *et al.,* 2010
Nocardia corynebacterioides DSM 12676	60%	Teniola *et al.,* 2005
Mycobacterium fluoranthenivorans sp. nov. DSM 44556T	>90%	Teniola *et al.,* 2005
Nocardia corynebacterioides DSM 20151	<90%	Teniola *et al.,* 2005
Lactobacillus paracasei LOCK 0920	55%	Slizewska and Smulikowska, 2011
Lactobacillus brevis LOCK 0944	55%	Slizewska and Smulikowska, 2011
Lactobacillus plantarum LOCK 0945	55%	Slizewska and Smulikowska, 2011
Tetrahymena pyriformis	58%	Dorothera *et al.,* 1967
Myxococcus fulvus ANSM068	80.70%	Shu Guan *et al.,* 2010
Stenotrophomonas maltophilia	78-84%	Shu Guan *et al.,* 2008
Fungi		
Peniophora sp. SCC0152	40.45%	Alberts *et al.,* 2009
Peniophora ostreatus St2-3	35.90%	Alberts *et al.,* 2009
Penicillium griseofulvum	52.40%	Hussein *et al.,* 2007
Penicillium urticae	35.40%	Hussein *et al.,* 2007
Paevilomyces lilacinus	53.90%	Hussein *et al.,* 2007
Trichoderma viride	67.20%	Hussein *et al.,* 2007
Candida utilis	38.20%	Hussein *et al.,* 2007
Sachromyces cerevisiae	51.70%	Hussein *et al.,* 2007
Pleurotus ostreatus	-	Motomura *et al.,* 2003
Trametes versicolor	40 - 90%	Zjalic *et al.,* 2006
Yeast		
Saccharomyces cerevisiae	40-70%	Shetty *et al.,* 2006
Trichoderma strains	65-85%	Shantha *et al.,*1999
Armillariella tabescens	-	Liu *et al.,* 1988
Trichosporon mycotoxinivorans	-	Molnar *et al.,* 2004
Saccharomyces cerevisiae LOCK 0140	55%	Slizewska and Smulikowska, 2011

Rayner and Dollear, 1968; Rayner *et al.,* 1970). The drawbacks of these process is high cost, removing of toxin and disposing the contaminated materials, percentage of removal of contaminated material from uncontaminated material is low (Yi annikouris *et al.,* 2002)

Chemical method

The structure of AFB_1 contains furo-furan ring, which is responsible for the toxicity and double bond in the furan ring determines its potency. AFB_1 is usually detoxified to a less toxic compound by removal of the double bond from furan ring and opening of the lactone ring. The treatment of aflatoxin with strong acids such as HCl (pH 2) reduced AFB_1 levels to 19.3% within 24 hrs (Doyle *et al.,* 1982). It destroyed the biological activity of AFB_1 and AFG_1 to the hemiacetal forms AFB_2a and AFG_2a respectively (Heathcote and

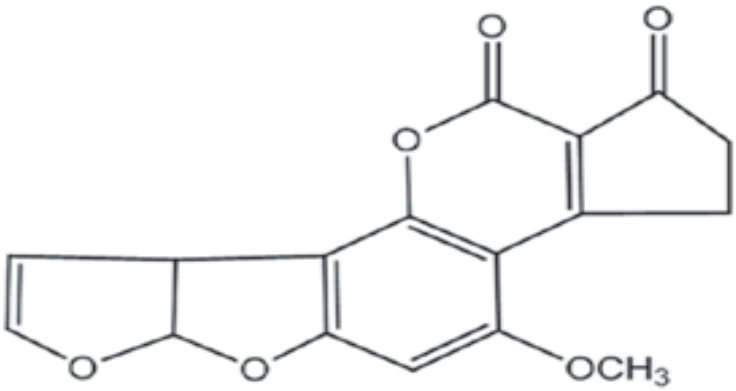

Fig. 1. Aflatoxin B_1 (AFB_1)

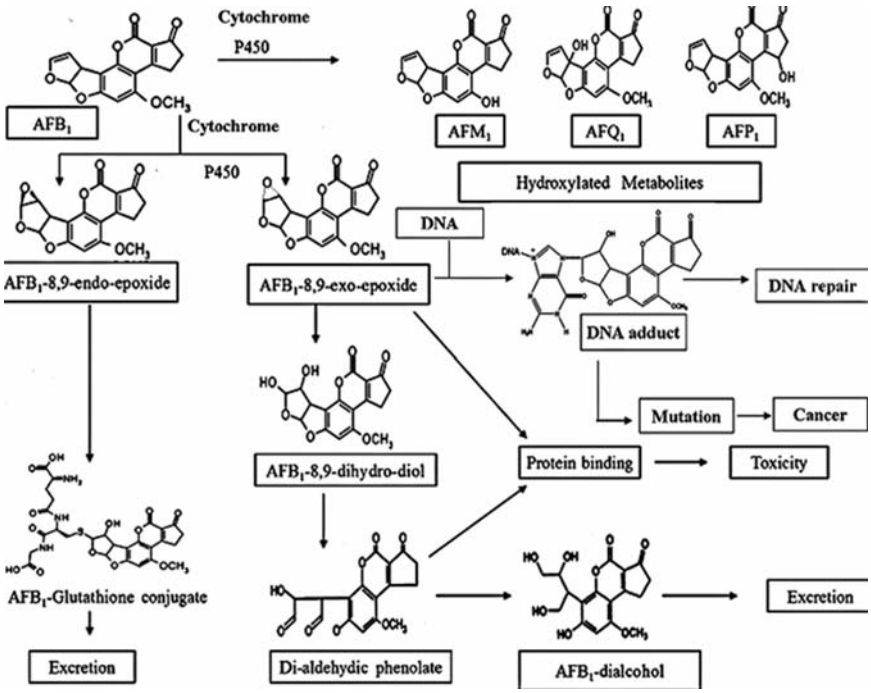

Fig. 2. Overview of biotransformation pathway of AflatoxinB_1 (AFB_1) (Modified from Eaton *et al*, 1994)

Hibbert, 1978). On the other hand, treatment of aflatoxin with basic chemicals such as, ammonium hydrochloride or gaseous ammonia (NH_3) effectively detoxifies aflatoxin, in agricultural commodities and animal feeds. The detoxification of aflatoxins in maize was more than 75% (Burgos-Hernandez *et al.,* 2002). Treatment of contaminated peanut and cottonseed with ozone successfully degraded AFB_1 and AFG_1 than AFB_2 and AFG_2 this is mainly due to the terminal double bond in the dihydrofuran ring which is more susceptible to Ozone attack. AFB_2 and AFG_2 lack this double bond. On Ozone treatment AFB_1 and AFG_1 levels were reduced to 77% and 80% respectively in peanuts. However, ozonization reduced the nutritive value of treated meal as determined by duckling and rat feeding trials (McKenzie *et al.,* 1997). The use of aforementioned chemicals in combination with physical treatments such as thermal processing can be used for the detoxification of food products contaminated with mycotoxins, which increased the efficacy of mycotoxins degradation but this technique requires expensive equipments and leads to loss of nutrition in product.

Biological detoxification

An alternative approach to remove the toxic and carcinogenic potential of mycotoxins is the biological detoxification. Biological detoxification method involves the use of enzymes for the degradation and detoxification of aflatoxin modifying of toxins that led to less toxic products. Studies in this area were significantly increased with the current advances in the field of microbiology, molecular biology and genetic engineering. Earlier work by Ciegler *et al.,* (1966) identified *Flavobacterium aurantiacum* NRRL B-184, which is capable of removing AFB_1 irreversibly from a variety of food products such as milk, oil, peanut butter, peanuts and maize without leaving toxic by-products. AFB_1 was degraded by *Flavobacterium aurantiacum* in the existence of seryl and sulfhydryl group inhibitors trace metal ions and divalent cations and chelators (D'Souza, D.H and Brackett, R.E., 2000^a, 2000^b, 1998). Acid producing bacteria's such as *Lactobacillus plantarum* and Lactobacillus acidophilus were found to detoxify aflatoxin in maize (Linderfelser and Ceigler, 1970). Lactic acid bacteria and probiotic bacteria were reported to adhere to AFB_1 and remove it (Peltonen *et al.,* 2008). *Tetrahymena pyriformis* at a dose rate of 22×10^6 cells detoxified AFB_1, by converting it into its hydroxyl products to an extent of 5 % in 24 hrs and 67% in 48 hours (Robertson *et al.,* 1970). Biological detoxification of AFB_1 by several fungal species was also reported. The drawback of AFB_1 detoxification by fungi was their long time incubation which was more than 120 hrs. AFB_1 degradation by bacteria, fungi and yeast are reported in the (Table.1).

CONCLUSION

AFB_1 is one of the potent toxins that challenges human with its various adverse effects. Among the various sources of this aflatoxin, Aspergillus sp. is the most important. AFB_1 is one of the threats for mankind considering its carcinogenic, mutagenic and teratogenic effects. Prevalence of AFB_1 in the contaminated food may lead to serious complications. Hence the degradation of this toxin from the environment was achieved by several methods. Detoxification by biological approach is found to be more convincing than physical and chemical methods. Research on exploring novel biological pathways to detoxify aflatoxin is being carried out and we expect an ecofriendly and cost effective degradation method.

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