Aflatoxin B<sub>1</sub> 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<sub>1</sub> (AFB<sub>1</sub>) 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<sub>1</sub> 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 been adopted for degradation of AFB<sub>1</sub>, but large-scale, practical and cost-effective methods for detoxifying AFB<sub>1</sub> containing feedstuffs are currently not available. This review deals with the potential hazards of AFB<sub>1</sub> in concern with prevention strategy for fungal contamination.

Key words: Aflatoxin B<sub>1</sub>, 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<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub> and two more additional metabolic products, M<sub>1</sub> and M<sub>2</sub>. Aflatoxin B<sub>1</sub> and B<sub>2</sub> emits blue fluorescence under UV-light, where as the Aflatoxin G<sub>1</sub> and G<sub>2</sub> gives yellow-green fluorescence under UV-light. Aspergillus flavus produces AFB<sub>1</sub> and AFB<sub>2</sub>, while Aspergillus parasiticus produces AFB<sub>1</sub>, AFG<sub>1</sub>, AFB<sub>2</sub> and AFG<sub>2</sub>. Some other species that produce aflatoxins are Aspergillus pseudotamarii, Aspergillus bombycis,
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 AFB₁ and AFG₁ as individual derivatives of AFB₁ and AFG₁. The biosynthesis pathway of aflatoxins has been postulated as followed: norsolorinic acid → averufin → versicoloracetate → versicolorin-A → sterigmatocystin → AFB₁ → AFG₁. The toxicity of the aflatoxins decreases from AFB₁ → AFG₁ → AFB₂ → AFG₂, 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 chemical or even radiation (Chitrangada, D and Mishra, H.N., 2000). Aflatoxin leads to chemical or even radiation (Chitrangada, D and Mishra, H.N., 2000). Aflatoxin leads to chemical or even radiation (Chitrangada, D and Mishra, H.N., 2000). Aflatoxin leads to chemical or even radiation (Chitrangada, D and Mishra, H.N., 2000).

Formation of DNA adduct

The aflatoxins are among the most potent genotoxic agents among the existing mycotoxins known. AFB₁ 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-epoxide and 8,9-endo-epoxide respectively. The reaction of AFB₁→8,9-epoxide with DNA strongly induces adduct formation. AFB₁→8,9-endo-epoxide appears to be the only product of AFB₁ involved in reaction with DNA in the N₇ guanine position. The imidazole portion of the formed AFB₁→N₇-Guanine adduct gives rise to a ring opened formamidopyrimidine (AFB₁–FAPY) and the other minor N₇-guanyl adducts can happen through enzymatic oxidation of AFB₁ and AFM₁ (Croy, R.G et al 1978 and Johnson, W.W and Guengerich, F.P., 1977). The exception is AFQ₁, 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;

### Table 1

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Removal of AFB₁ (%)</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
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<tr>
<td><em>Flavobacterium aurantiacum</em> NRRL B-184</td>
<td>22-40%</td>
<td>Ciegler et al., 1966</td>
</tr>
<tr>
<td><em>Rhodococcus erythropolis</em></td>
<td>17%</td>
<td>Teniola et al., 2005</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em></td>
<td>80%</td>
<td>Peltanone et al., 1998</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em> M74</td>
<td>19.3 - 30.5%</td>
<td>Ali Topcu et al., 2010</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em> EF031</td>
<td>23.4 - 37.5%</td>
<td>Ali Topcu et al., 2010</td>
</tr>
<tr>
<td><em>Nocardia corynebacterioides</em> DSM 12676</td>
<td>60%</td>
<td>Teniola et al., 2005</td>
</tr>
<tr>
<td><em>Mycobacterium fluoranthemivorans</em> sp. nov. DSM 44556T</td>
<td>&gt;90%</td>
<td>Teniola et al., 2005</td>
</tr>
<tr>
<td><em>Nocardia corynebacterioides</em> DSM 20151</td>
<td>&lt;90%</td>
<td>Teniola et al., 2005</td>
</tr>
<tr>
<td><em>Lactobacillus paracasei</em> LOCK 0920</td>
<td>55%</td>
<td>Slizewska and Smulikowska, 2011</td>
</tr>
<tr>
<td><em>Lactobacillus brevis</em> LOCK 0944</td>
<td>55%</td>
<td>Slizewska and Smulikowska, 2011</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em> LOCK 0945</td>
<td>55%</td>
<td>Slizewska and Smulikowska, 2011</td>
</tr>
<tr>
<td><em>Tetrahymena pyriformis</em></td>
<td>58%</td>
<td>Dorothera et al., 1967</td>
</tr>
<tr>
<td><em>Myxococcus fulvus</em> ANSM068</td>
<td>80.70%</td>
<td>Shu Guan et al., 2010</td>
</tr>
<tr>
<td><em>Sienetromonas malthophilia</em></td>
<td>78-84%</td>
<td>Shu Guan et al., 2008</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Peniophora sp.</em> SCC0152</td>
<td>40.45%</td>
<td>Alberts et al., 2009</td>
</tr>
<tr>
<td><em>Peniophora ostreatus</em> St2-3</td>
<td>35.90%</td>
<td>Alberts et al., 2009</td>
</tr>
<tr>
<td><em>Penicillium griseofulvum</em></td>
<td>52.40%</td>
<td>Hussein et al., 2007</td>
</tr>
<tr>
<td><em>Penicillium urticae</em></td>
<td>35.40%</td>
<td>Hussein et al., 2007</td>
</tr>
<tr>
<td><em>Paevilomyces lilacinus</em></td>
<td>53.90%</td>
<td>Hussein et al., 2007</td>
</tr>
<tr>
<td><em>Trichoderma viride</em></td>
<td>67.20%</td>
<td>Hussein et al., 2007</td>
</tr>
<tr>
<td><em>Candida utilis</em></td>
<td>38.20%</td>
<td>Hussein et al., 2007</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>51.70%</td>
<td>Motomura et al., 2003</td>
</tr>
<tr>
<td><em>Pleurotus ostreatus</em></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Trametes versicolor</em></td>
<td>40 - 90%</td>
<td>Zjalic et al., 2006</td>
</tr>
<tr>
<td><strong>Yeast</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>40-70%</td>
<td>Shetty et al., 2006</td>
</tr>
<tr>
<td><em>Trichoderma</em> strains</td>
<td>65-85%</td>
<td>Shantha et al., 1999</td>
</tr>
<tr>
<td><em>Armillariella tabescens</em></td>
<td>-</td>
<td>Liu et al., 1988</td>
</tr>
<tr>
<td><em>Trichosporon mycotoxinivorans</em></td>
<td>-</td>
<td>Molnar et al., 2004</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> LOCK 0140</td>
<td>55%</td>
<td>Slizewska and Smulikowska, 2011</td>
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</table>
Rayner and Dollear, 1968; Rayner et al., 1970). The drawbacks of these processes include high cost, removing the contaminated materials, and disposing of the contaminated material. The percentage of removal of contaminated material from uncontaminated material is low (Yi annikouris et al., 2002).

**Chemical method**

The structure of AFB₁ contains furan-furan ring, which is responsible for the toxicity and double bond in the furan ring determines its potency. AFB₁ is usually detoxified to a less toxic compound by removal of the double bond from the furan ring and opening of the lactone ring. The treatment of aflatoxin with strong acids such as HCl (pH 2) reduced AFB₁ levels to 19.3% within 24 hrs (Doyle et al., 1982). It destroyed the biological activity of AFB₁ and AFG₁ to the hemiacetal forms AFB₂a and AFG₂a respectively (Heathcote and...
Hibbert, 1978). On the other hand, treatment of aflatoxin with basic chemicals such as, ammonium hydrochloride or gaseous ammonia (NH₃) 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 AFB₁ and AFG₁ than AFB₂ and AFG₂; this is mainly due to the terminal double bond in the dihydrofuran ring which is more susceptible to Ozone attack. AFB₁ and AFG₁ lack this double bond. On Ozone treatment AFB₁ and AFG₁ 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 AFB₁ irreversibly from a variety of food products such as milk, oil, peanut butter, peanuts and maize without leaving toxic by-products. AFB₁ 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., 2000⁶, 2000⁷, 1998). Acid producing bacteria’s such as Lactobacillus plantarum and Lactobacillus acidophilus were found to detoxify aflatoxin in maize (Linderfelser and Ciegler, 1970). Lactic acid bacteria and probiotic bacteria were reported to adhere to AFB₁ and remove it (Peltonen et al., 2008). *Tetrahymena pyriformis* at a dose rate of 22 x 10⁶ cells detoxified AFB₁, 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 AFB₁ by several fungal species was also reported. The drawback of AFB₁, detoxification by fungi was their long time incubation which was more than 120 hrs. AFB₁ degradation by bacteria, fungi and yeast are reported in the (Table.1).

**CONCLUSION**

AFB₁ 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. AFB₁ is one of the threats for mankind considering its carcinogenic, mutagenic and teratogenic effects. Prevalence of AFB₁ 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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