

## STRUCTURAL DIVERSITY AND BIOCHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF AFLATOXINS

Otto KETNEY \*<sup>1</sup>, Tita OVIDIU\*, Anca TIFREA\*

\*“Lucian Blaga” University of Sibiu, Faculty of Agricultural Sciences, Food  
Industry and Environmental Protection, Sibiu, Romania

**Abstract:** Among all mycotoxins, Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is considered to be the most carcinogenic, and it has been classified by the International Agency for Research on Cancer in Group 1 of human carcinogen. It signifies a high hazard because it contaminates a diversity of agricultural products such as nuts and derivatives, peanuts/hazelnuts, grains, seeds, cottonseed, milk, dairy food. In milk AFB<sub>1</sub> is metabolized to aflatoxin M (AFM<sub>1</sub>) which is 4-hydroxy derivative of AFB<sub>1</sub>, it is formed in the liver and excreted in the milk into the mammary glands of both human and lactating animals which have been fed with AFB<sub>1</sub> contaminated diet. After the food contamination, one part of the aflatoxin B<sub>1</sub> which was present in the food is eliminated through the milk. At the molecular level aflatoxin biosynthesis involves several levels of transcriptional and post-transcriptional control, so the main stages subsequent biochemical and genetic constituents of aflatoxin biosynthesis have been demonstrated recently. Recent studies over the last few decades have shown that the metabolism of AFB is an essential component of hepatocarcinogenic, however it was shown that AFB<sub>1</sub> is metabolized by cytochrome P450 oxidised to intermediates and other metabolites. Therefore, the biotransformation process may also lead to the formation of carcinogenic metabolites.

**Keywords:** mycotoxins, aflatoxin, milk, *Aspergillus*

### INTRODUCTION

According to the European Union and to the World Health Organization, food safety is everyone's responsibility, starting from the food origin until it gets on the customer's table. The food can contain a number of chemical compounds with toxic effect that can influence consumers' health. Substances having toxigenic effect on milk and milk products include

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<sup>1</sup> Corresponding author. Mailing address: University Lucian Blaga of Sibiu, Faculty of Agricultural Sciences, Food Industry and Environmental Protection, Str. I. Rațiu 7-9, 550012 Sibiu, Romania. E-mails: [otto.ketney@ulbsibiu.ro](mailto:otto.ketney@ulbsibiu.ro), [otto\\_ketney@yahoo.com](mailto:otto_ketney@yahoo.com)

afatoxins which are produced by means of biosynthesis of *Aspergillus* species, being developed on a substrate which can produce illness to those/people consuming the product.

Two recent research (Kensler et al., 2011; Wu et al., 2014), were limited at adverse effects on human health of exposure to aflatoxin. These tackle the mechanisms of toxicity to aflatoxin contamination body and related diseases. So to protect the health of humans and animals, over 100 nations have established maximum tolerable levels for aflatoxin in food (Wu and Guclu, 2012). These include and the European Union which established the level of AFM1 in milk consumption levels ( $0.05 \mu\text{g mL}^{-1}$ ) (European Union 2006).

Data according to which a large number of mycotoxins (over 200 species have been identified) exist in nature, is cited in literature (Richard, 2007; Creppy, 2002; Logrieco et al., 2002; Desjardins, 2006). Basically, there are no known areas around the globe but it is estimated that 25-60% of cereals and animal food throughout the globe are contaminated with known mycotoxins.

In order to reductions mycotoxins exist a number of ways such some physico-chemical methods shows advantages ,however in literature are still natural approach which involve heat treatment (Park, 2002), UV light (Ketney et al., 2010) or ionizing radiation (Herzallah et al., 2008) unfortunately they are still not applied on an industrial scale.

## STRUCTURAL DIVERSITY

The term "*Mycotoxin*" has Greek origin and it is a natural toxin produced by fungi including molds ("*μύκης*" *mikes*, *mukos*, "*fungus*") this is a natural toxin produced by fungi include molds, mostly fungi are aerobic (use oxygen). The term was introduced in 1962 by British researchers (Blount, 1961).

Over 100 species of fungi have been shown to produce mycotoxins such naturally associated with the development of various diseases in animals and humans , although from the point of view of toxigenic fungi and their spores are present everywhere, mycotoxicosis are primarily a problem in areas where the precipitation, relative humidity and temperature favor fungal growth and mycotoxin production.

From group of mycotoxins, aflatoxins are toxic secondary metabolites which are mainly produced by *Aspergillus* species, *Aspergillus flavus* and *Aspergillus parasiticus* (Machida et al., 2005; Peiwu Li et al., 2009) being found throughout the world, and they are present both into the ground and in the air (H. K. Abbas et al., 2005; Klich, 2002) they usually contaminate a large variety of food / feed in tropical and sub-tropical regions (Yunus et al., 2011).

On the basis of genetic and physiological morphological criteria, *Aspergillus Flavus* can be divided into two types taxonomy. S-type strains produces numerous small sclerotia (diameter less than 400 µm) and high levels of aflatoxin B series. L-type strains produce fewer large sclerotia and on average have a lower level of aflatoxins (Ehrlich et al., 2003).

## **CHARACTERISTICS OF MYCOTOXINS PRODUCED BY ASPERGILLUS SPECIES**

### **Structural characteristics of aflatoxin B group (B<sub>1</sub> and B<sub>2</sub>)**

From the structural point of view the most common aflatoxins are AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> AFG<sub>2</sub>, AFM<sub>1</sub> and AFM<sub>2</sub>, the term "B" and "G" refers to the colors blue and green products fluorescence UV light on TLC plates while the number 1 and 2 show the respective minor and major compounds (Bennett, 2003). However aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is a highly toxic compound with a lethal dose (LD<sub>50</sub> = 1-50 mg Kg<sup>-1</sup>) for most species, however is toxicity critical (LD<sub>50</sub> <1 mg Kg<sup>-1</sup>) for some highly sensitive species, such as rats (Kumar et al., 2012; Fiala et al., 2011; Fetaih et al., 2014; Shyamal et al., 2010) , rainbow trout (Arana et al., 2014; Alinezhad et al., 2011) and poultry (Raphaël et al., 2014; Magnoli et al., 2011; Chen et al., 2013; Hussain et al., 2010; He et al., 2013; Wan et al., 2013). The toxic effects of AFB<sub>1</sub> can be differentiated according to the level and duration of exposure to aflatoxin (Diaz et al., 2011)

Aflatoxins are fat-soluble compounds and are readily absorbed from the site of exposure, usually through the gastrointestinal tract and respiratory tract (Larsson and Tjälve, 2000; Agag, 2004). Contamination can be done in two major ways (a) ingestion of food contaminated with aflatoxin or aflatoxin ingestion or ingestion of aflatoxins in animal feed carried in milk and milk products (Agag, 2004) , (b) by inhaling dust of aflatoxins in especially AFB<sub>1</sub> in contaminated food industries and factories (Ali et al., 2013).

### **Structural characteristics of aflatoxins in group M (M<sub>1</sub> and M<sub>2</sub>)**

Biochemically speaking, Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) is the compound 4-hydroxy AFB<sub>1</sub>, which is formed in the liver and excreted in the milk in the mammary glands of lactating both human and animals that were fed with contaminated AFB<sub>1</sub> food (Fallah et al., 2009; Gürbay et al., 2010). Action level for aflatoxin B<sub>1</sub> is 20 parts per billion for dairy cows feed. Recently Battacone et al. (2003) observed that there was a linear relationship between dose AFB<sub>1</sub> and AFM<sub>1</sub> excretion in sheep milk. In general, it is believed that about 1-3% aflatoxin B<sub>1</sub> present in feed is eliminated as AFM<sub>1</sub> in milk these values depend on the animal, the milking type and on many other factors. After stopping the consumption of food containing AFB<sub>1</sub> must pass 72 hours

should pass in order for the aflatoxin concentration to be reduced to undetectable levels (Bilandžić et al., 2010).

Given these facts/given the above mentioned facts AFM1 was classified in group 1, regarding its risk (carcinogenic to humans) by the International Agency for Research on Cancer (IARC et al., 2002).

The Identification of AFM1 was made for the first time with layer chromatography (TLC) on extract of milk in 1964. Therefore, scientists such as De Jongh et al. (1964) showed by TLC a blue-violet fluorescent compound which had lower R<sub>f</sub> value than AFB1. These chromatographic results have revealed that during the lactating period, AFB1 bio-converted to another toxic compound called by Allcroft et al. (1966) "*toxic milk*" which was later assigned a name and namely "aflatoxin M". Several chromatographic studies in various solvent systems have indicated that this "*toxic milk*" contains two fluorescent components, a blue-violet to purple R<sub>f</sub> 0.34, and a lower R<sub>f</sub> value, that is 0.25. Afterwards, aflatoxins were named, depending on the physical characteristics of fluorescence (in the length of wave 365 nm) (Klich, 2007).

AFM1 determination is made by different techniques, the most common are thin layer chromatography (TLC) (Kamkar, 2006; Fallah, 2010; Carvalho et al., 2012), high performance liquid chromatography with mass detection (Huang et al., 2014; Han et al., 2010; Campone et al., 2013), electrochemical detection (Micheli et al., 2005; Rameil et al., 2010; Nguyen et al., 2013; Paniel et al., 2010) and chemiluminescence (Kanungo et al., 2011; Gan et al., 2013; Sarter and Zakhia, 2004; Vdovenko et al., 2014; Ping Li et al., 2014). Nevertheless the determination of AFM1 most often used international standard ISO 14501:2007 which is based on fluorescence or detected by mass spectrometry (Cavaliere et al., 2006; Afshar et al., 2013; Hashemi et al., 2014; Wang et al., 2012; Magnoli et al., 2011; Huang et al., 2014).

AFM1 is relatively stable milk products and is not affected by pasteurization (UHT or techniques) or cheese processing (Kav et al., 2011). Considering the fundamental role of milk and milk products in human nutrition, especially for children, this AFM1 in dairy products is considered a significant risk to food safety and public health (Cucci et al., 2007).

## **BIOSYNTHESIS**

Aflatoxin biosynthesis is a complicated involving several process, this process involves some levels of transcriptional and post-transcriptional control (HK Abbas et al., 2009; Chanda et al., 2009; Georgianna and Payne, 2009; Schmidt-Heydt et al., 2009). At a molecular level, the main

biochemical steps and genetic constituents of aflatoxin biosynthesis have been demonstrated recently (Yu, 2003; Bhatnagar et al., 2002).

The process is done in a similar way for several toxic secondary metabolites produced by fungi, aflatoxins are synthesized from polyketide molecules where condensation of ethyl esters produced from intermediates of  $\beta$ -keto-poly-thiols. The chain biosynthesis pathway is initiated by acetyl CoA and malonyl-CoA being an additional source of carbon.

Cleveland et al. (2003); Yabe and Nakajima (2004); Yabe et al. (1999) assigns a currently accepted scheme for the biosynthesis of aflatoxin which consists of the following steps: hexanoyl-initiating CoA biosynthetic pathway reaching the norsolorinic acid, norsolorinic acid that is converted in two steps through the intermediate process of averantin, after which it turns into averufin.

Averufin next step involves opening biosynthetic of the averufin ring, followed by the dehydration sequence, epoxidation and epoxide rearrangement to form the ethyl hemiacetal-versicolorin A through the versiconal A and versicolorin B, Versicolorin A is converted to dimethyl sterigmatocistin, then sterigmatocistin. Sterigmatocistin last major intermediate in oxidoreductase. A methoxy group, which is common to almost all aflatoxins appears from donor S-adenosyl-methionine (SAM) catalyzed by S-adenosyl-methyl-transferase. Bifuran ring formation in versicolorin A, sterigmatocistin and AFB1 appears involving hemiacetal acetate ester hydrolysis of the acetyl group versiconal eliminating followed by ring closure. The oxidation process of the terminal hydroxyl group to an aldehyde, results from the hemiacetal structure. The biosynthetic scheme for aflatoxin B1 is presented in Figure 1.

## BIOTRANSFORMATION

Studies over the last few decades have shown that the metabolism of AFB is an essential component of its hepatocarcinogenic and the biotransformation differences significantly contribute, depending on the species and interindividual differences in susceptibility to AFB1 (Eaton et al., 2001).

AFB1 is metabolized by cytochrome P450 (CYP450) in intermediates and other oxidized metabolites, including hydroxylated forms of aflatoxin M1 (AFM), aflatoxin Q1 (AFQ), and demethylated form of aflatoxin P1 (AFP) (Gross-Steinmeyer and Eaton, 2012). However, biotransformation can also lead to the formation of AFB1-exo-8, 9-epoxide (AFBO), which is a carcinogenic metabolite of AFB1 (David, 1994).

In general, metabolism or biotransformation of xenobiotics (foreign chemicals to the body) represent a process which aims to transforming the original molecules in more hydrophilic compounds readily excreted in the

urine (kidney) or bile (liver). It has traditionally been conceptualized as a process which takes place in two steps known as Phase I and II, although some authors claim that this classification may not be supported and should be removed (David et al., 2005).

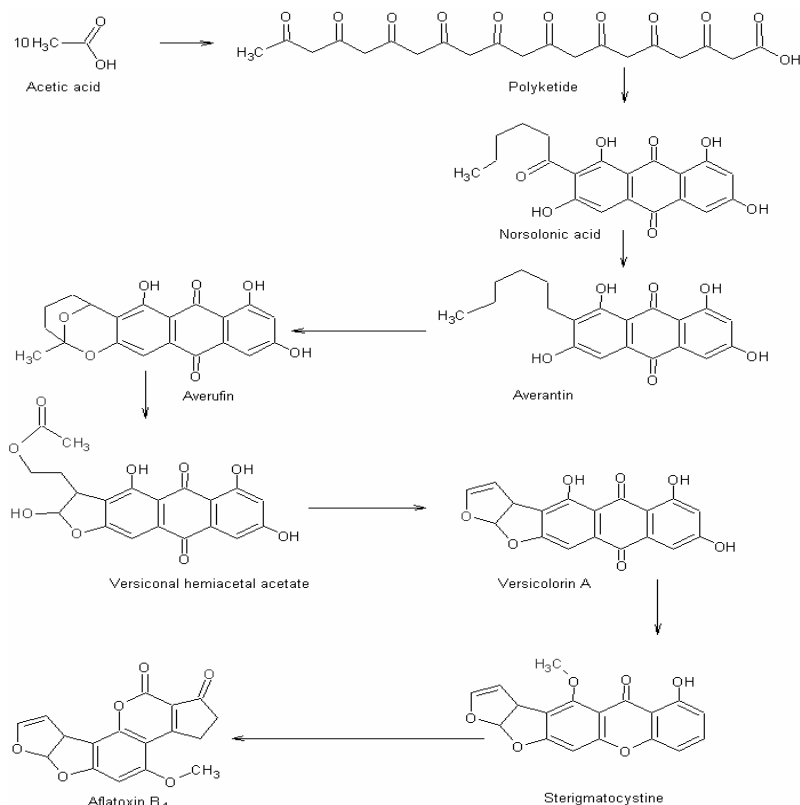


Figure 1 The biosynthetic scheme for aflatoxin B<sub>1</sub> (Deshpande, 2002)

Animals also metabolize O-demethylation AFB<sub>1</sub> by occurring AFP<sub>1</sub> (Figure 2), which was subsequently detected in the urine in several animal species. AFP<sub>1</sub> was shown to be less toxic than AFB<sub>1</sub>. Through in vitro metabolism by liver microsomes of monkeys and humans these have produced major metabolites of AFB<sub>1</sub>, which contain hydroxyl groups, which are arranged in β position of carbonyl groups of cyclopentanone ring in AFB<sub>1</sub>.

A general scheme for the primary metabolism of AFB<sub>1</sub> is based on results obtained in vitro studies using liver subcellular fractions isolated from different animal species were also observed (Figure 2). Most of the metabolites are less toxic than AFB<sub>1</sub>. From toxicologically most important intermediate toxic AFB<sub>1</sub> form is AFB<sub>1</sub>-2, 3-epoxide and AFB<sub>1</sub>-8, 9-epoxide (AFBO). Yet there are two stereoisomers of AFBO (endo- and exo-)

and exo -AFBO is capable of reacting with ADN to produce preferentially adducts of N7-guanine.

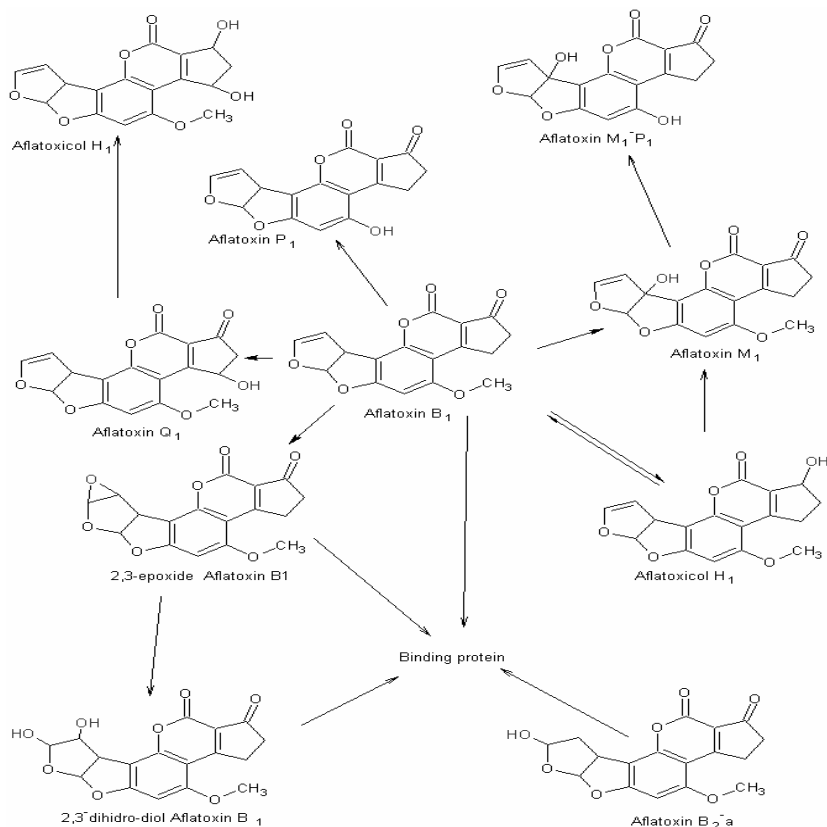


Figure 2 General scheme for the metabolism of aflatoxin B<sub>1</sub> primary (Deshpande, 2002)

The epoxide quickly hydrolysed to the initial state at AFB<sub>1</sub>-2, 3-dihydrodiol form, such AFB<sub>1</sub>-2, 3-dihydrodiol can be in accordance with the phenolate ion which is capable of forming Schiff bases with addition of an amino group of lysine (Figure 3)

An important characteristic in toxicity of AFB<sub>1</sub> is metabolic activation by CYP450 of AFB<sub>1</sub> to AFBO, which is an intermediate reaction which exerts their toxicological effects through the binding of toxic cellular components, such as proteins, ADN and ARN (Guengerich et al., 1998). Multiple isoforms of cytochrome P450 are capable of AFB<sub>1</sub> bioactivation, but evidence suggests that CYP3A4 and CYP1A2 are the main isoforms involved in human liver in this process (Kirby et al., 1993; Gallagher et al., 1994).

The most hydroxylated metabolites of AFB1 are formed from cytochromes P-450 which are AFM1, AFP1, AFQ1 and AFB2a (Figure 2). Additional metabolites that are generally formed in lesser amounts depending on different conditions, which include AFL and AFM1. These metabolites are considered as such detoxify AFB1 is more polar and thus more easily excreted. However recent studies show that the reduction of AFB1 by cytosolic reductase produce aflatoxicol (AFL) , which is considered to be a detoxification product by some authors that, at least in the case of fish, it may be oxidized back to AFB1 by a microsomal dehydrogenase , thus increasing the half-life physiological of AFB1 (Troxel et al., 1997).

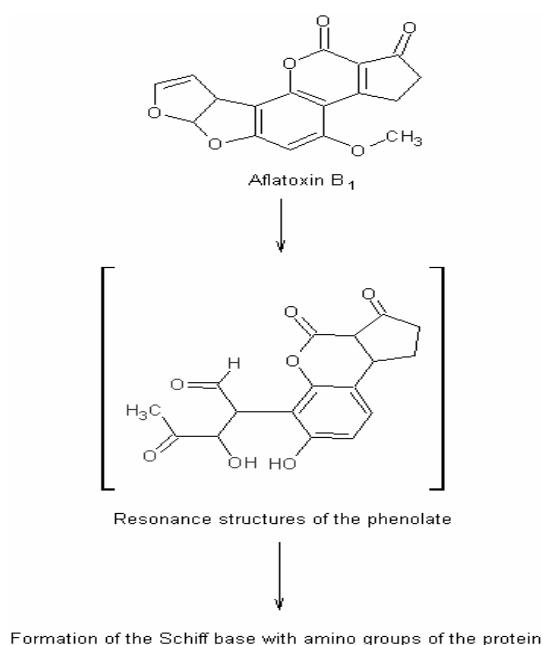


Figure 3 Schiff base formation between protein amino groups and aflatoxin B<sub>1</sub>-2, 3-epoxide (Deshpande, 2002).

## CONCLUSIONS

The biotransformation plays an important role in the biological activity and nature of aflatoxins. The detoxification mechanism involves mechanisms of biotransformation which are known as well. AFB1 represent by far the most toxic of the aflatoxins, and almost all the available information about bioactivity aflatoxins at animal is focused on AFB1 and its metabolites. Also aflatoxin is usually found in high concentration in contaminated food and animal feed.



It is important to note that not all the biotransformation occur of each species and that AFB1 metabolites vary with the species, the type of tissue and the age of the animal. The many of the same dietary factors or other agents known to alter the metabolism of another carcinogens agents similar with AFB1. The activity of the enzymes glutathione-S-transferase (GST) to AFBO suitable an important role in determining the resistance to some rodents AFB1. When a large part of the liver hepatocytes contain increased amounts of  $\gamma$ -glutamyl transpeptidase as a result of feeding hepatocarcinogenic substances such as AFB1, AFB1 conjugation in the gallbladder contains complex AFB1-Cys-Gly. In later stages of setting up mercapturate AFB1-peptidase activity and N-acetyltransferase. The Mercapturate of AFB1 was detected in the urine of rats and monkeys after administration of AFB1. Metabolism of aflatoxins other than AFB1 was studied and researched with less interest because of their potential toxic and low rate of occurrence.

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