Biological degradation of aflatoxins

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Abstract

Aflatoxins are cancerogenic compounds produced predominantly by certain strains of the Aspergillus genus. The ideal solution for minimization of health risk that aflatoxins pose is the prevention of foods and feeds contamination. Unfortunately, these contaminants can never be completely removed, and on that account, many studies have been carried out to explore an effective process of their detoxification to a threshold level. Biological decontamination seems to be attractive because it works under mild, environmentally friendly conditions. This review is focused on the biological detoxification of aflatoxins, especially aflatoxin B1, by microorganisms. There are briefly mentioned aflatoxin metabolic pathways in the human and animal body. Microorganisms such as soil or water bacteria, fungi, and protozoa and specific enzymes isolated from microbial systems can degrade aflatoxin group members with varied efficiency to less- or nontoxic products. Some aflatoxin-producing fungi from Aspergillus species have the capability to degrade their own synthesized mycotoxins. Yeasts and lactic acid bacteria work as biological adsorbents that prevent aflatoxin's transfer to the intestinal tract of humans and animals. Aflatoxin B1 absorbed into the organism could be metabolized by significantly different pathways. They lead to the production of the relatively nontoxic compounds, on the one hand, or to highly toxic active forms on the other hand.

Keywords: Aflatoxin; metabolism; biodegradation; decontamination; bacteria

Introduction

Aflatoxins are a group of highly toxic secondary metabolites produced mainly by Aspergillus species fungi. A. flavus produces only aflatoxins B, while A. parasiticus produces both aflatoxins B and G. Aflatoxins can be found mainly in cereals, oilseeds, tree nuts, spices, and milk. Among 18 different types of aflatoxins, such as B1, B2, G1, G2, P, Q, M1, M2, B2a, etc., were identified. The most commonly occurring ones in fungi cultures are aflatoxins B1, B2, G1, and G2, then aflatoxins M1 and M2 in milk.

Naturally occurring aflatoxins and aflatoxin B1 (AFB1) are classified by the International Agency for Research on Cancer (IARC) as group 1 carcinogens (“carcinogenic to humans”) (IARC, 2002). The AFB1 metabolite, 8,9-epoxide, forms DNA adducts primary with N7 of guanine (Cullen and Newberne, 1994). The toxic effect on living organisms is reviewed in a World Health Organization report (WHO, 1998). The toxicity of aflatoxin decreases in order B1, G1, B2, and G2. When AFB1 and aflatoxin B2 (AFB2) are consumed, they are metabolized to aflatoxin M1 (AFM1), respective M2 (AFM2), and distributed in tissues and biological fluids and milk (Zarba et al., 1992).

Biodegradation of aflatoxins, using microorganisms or enzymes, is one of the well-known strategies for the management of aflatoxins in foods and feeds. The methods of biodegradation are being actively studied and can be a highly promising choice, since it is efficient, specific, and...
environmentally friendly to reduce or eliminate the possible contaminations of aflatoxins in foods and feeds.

**Soil bacteria**

Many bacteria in soil are able to degrade aflatoxins. *Flavobacterium aurantiacum* NRRL B-184, a kind of bacteria from soils and water, showed a very high capability of detoxifying aflatoxins in feeds and foods (Ciegler et al., 1966a). The aflatoxin-contaminated substance and *F. aurantiacum* NRRL B-184 were mixed together and incubated at 28°C for 12 hours, all of the aflatoxin G was removed, as well as a part of aflatoxin B, which was diminished. Ducking assays implied detoxification of aflatoxin solutions by *F. aurantiacum* NRRL B-184 was complete, with no new toxic products being formed. Lillehoj showed that this bacterium can remove AFM, from milk in 1971 (Shapira, 2004). Later, it was observed that the radioactively labeled 14C-AFB1 is partially metabolized and partially adsorbed to *F. aurantiacum* cells (Line et al., 1994). D’Souza and Brackett (1998, 2000, 2001) have been monitoring the effects of cations and several chemical compounds on AFB1 degradation by these bacteria. For example, Cu2+, Mn2+, and Zn2+ lower the reduction capacity of *F. aurantiacum*. This confirms the influence of the enzymes in the degradation process. More applications of *F. aurantiacum* for decontamination of food/feed were discussed in a review (Bata and Låsztity, 1999).

Other microorganisms were also tested for their possible ability to degrade aflatoxins. The strain *Nocardia asteroides* reduces AFB1 by biotransformation to another fluorescent product (Arai et al., 1967), and *Corynebacterium rubrum* is able to detoxify aflatoxin as well (Shapira, 2004).

In another work, *Mycobacterium fluoranthovorans* sp. nov. DSM44556 is isolated from soils of a former coal gas plant, which was polluted with polycyclic aromatic hydrocarbons, was found to be capable of degrading AFB1 as a single carbon source (Hormisch et al., 2004). The AFB1 concentration was reduced to amounts of 70–80% of the initial concentration within 36 hours, and no AFB1 was detectable after 72 hours. In addition, the cell-free extracts of *M. fluoranthovorans* sp. nov. DSM44556 degraded AFB1, more efficiently (Teniola et al., 2005). More than 90% of the initial amount of AFB1 was degraded at 30°C within 4 hours, and no AFB1 was detected after 8 hours.

Teniola et al. (2005) investigated *Rhodococcus erythropolis* isolated from polycyclic aromatic hydrocarbon (PAH) soils for AFB1 degradation activity. Dramatic reduction of AFB1 was observed during incubation in the presence of *R. erythropolis*. Then, 17% residual AFB1 was left after 48 hours and only 3–6% was detectable after 72 hours. In addition, this work team also found AFB1 was effectively degraded by extracellular extracts from *R. erythropolis* (only 32% residual AFB1 was detectable after 72 hours) (Alberts et al., 2006). Results indicated AFB1 was most likely metabolized to degradation products with chemical properties different from that of AFB1, because the equipments they utilized could not reveal the formation of any breakdown products.

The high degradation rate and wide temperature range for degradation by both *Rhodococcus erythropolis* and *M. fluoranthovorans* sp. nov. DSM44556 indicate a potential and promising application for the degradation of AFB1 in the foods and feeds process.

**Fungi**

Fungi cannot only produce aflatoxins, but they are able to degrade them as well. Four fungal strains, *Aspergillus niger*, *Eurotium herbariorum*, a *Rhizopus* sp., and nonaflatoxin (AF)-producing *A. flavus*, are able to convert AFB1 to aflatoxicol (AFL) by reducing the cyclopentene carbonyl of AFB1. These fungi could convert AFB1 to aflatoxicol-A (AFL-A), then AFL-A was converted to aflatoxicol-B (AFL-B) by the actions of medium components or organic acids produced from the fungi. In addition, the interconversion between AFL-A and AFL-B was observed to have occurred independently of fungal metabolic activity (Nakazato et al., 1990). Fungi *A. niger* was capable of converting AFL to AFB1; afterward, AFB1 could be converted further to AFB1. However, the sum of AFL and AFB1 was found to be decreased with time, which suggested both AFB1 and AFL were further metabolized to unknown substances by the fungi (see Figure 1).

AFB1 and AFL production and degradation during fungi cultivation in liquid media by *A. flavus* strains (NRRL 2999, 3000, A-13570, and A-13367; also called M 001) was investigated (Ciegler et al., 1966b). The production was observed for the first 72 hours of growing and depended on aeration and mycelia dispersion. After this time, the lowering of aflatoxin concentrations was observed. The degradation was not dependent on carbohydrate concentration. On the other hand, the fragmentation of mycelia of previously aflatoxin nondegrading fungi (*A. flavus* NRRL 2999 and M 001) induced their ability to degrade aflatoxins. The aflatoxin reduction can be prevented by fermentation at low temperatures and a low agitation rate. Research made by Doyle and Marth (1978) proved that *A. parasiticus* NRRL 2999 and NRRL 3000 actively degraded aflatoxins. *A. flavus* NRRL 3353 was less active, and *A. flavus* NRRL 482 and *A. parasiticus* NRRL 3315 degraded minimal amounts of aflatoxins. Those aspergilli producing the greatest amounts of aflatoxins also degraded aflatoxins most rapidly. In the presence of asparagines, *Aspergillus candidus* converts AFB1 to aflatoxin D (Lafont and Lafont, 1974).

Fungi *Penicillium raistrickii* NRRL 2038 is able to transform AFB1 to a new compound that is similar to AFB1 (Ciegler et al., 1966a). Kusumaningtyas et al. (2006) found another fungi, *Rhizopus oligosporus*, was able to inhibit synthesis or to degrade AFB1, when...
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Cultured together with AFB$_1$-producing fungi *A. flavus*, *R. oligosporus* had the best degrading ability on Day 5. Fungi *R. arrhizus*, *R. stolonifer* NRRL 1477, and *A. arrhizus* NRRL 2585 transform AFB$_1$ to aflatoxicol (AFL), trivially named as aflatoxin R$_o$ (AFRo) (Cole et al., 1972). Incubation time longer than 3 days resulted in isomerization to AFR$_o$. A short review about other aflatoxin (AFG$_1$) degradation by *Rhizopus* species was published by the same work team (Cole and Kirksez, 1971). Metabolization of AFB$_1$ to AFR$_o$ was also reported for *D. dendroides* NRRL 2575, *Mucor alterans* NRRL 3358, *M. griseocyanus* NRRL 3359, *Absidia repens* NRRL 1336, and *Helminthosporium sativum* NRRL 3356 (Detroy and Hesseltine, 1969). Longer times of incubation led to the formation of new, unidentified blue-fluorescent product.

**Protozoa**

Cells of the protozoon *Tetrahymena pyriformis* W have the ability to degrade pure AFB$_1$ to another bright-blue fluorescent product and decrease the AFB$_1$ concentration to 25% in 30 hours. The course of detoxification of AFG$_1$ by this strain was different. During 10 hours, the concentration decreased to 80% and was constant for the next 20 hours (Teunissson and Robertson, 1967). One year later, it was concluded by Robertson et al. (1970) that *T. pyriformis* W reduced the carbonyl in the cyclopentane ring of AFB$_1$ to a hydroxyl group (Figure 2). The reduced aflatoxin appears to be identical to AFR$_o$.

**Digestive tract microorganisms**

Rumen bacteria have been reported to degrade aflatoxins. Kiessling et al. (1984) investigated the metabolism of AFB$_1$ by intact rumen fluid, rumen protozoa, and rumen bacteria *in vitro*. They found that AFB$_1$ was not degraded by rumen microorganisms. A slight decrease in the amount of AFB$_1$ occurred within 30 minutes; however, after this time, no further reduction was evident.

In another work, Westlake et al. (1987) studied four strains of *Butyryrivibrio fibrisolvens* (CE46, CE51, CE52, and CE56), one of the predominant rumen bacteria. Results showed that there was no significant degradation of AFB$_1$. The researchers proposed that microbial activity was solely responsible for aflatoxin’s degradation within the rumen. The efficiency of the rumen metabolism was demonstrated by the fact that only 2–5% of AFB$_1$ fed to cows reached the intestine (Engel and Hagemeister, 1978; Karlovsky, 1999).

AFB$_1$-contaminated corn mixed with poultry litter was incubated, and mycotoxin was destroyed by feces microorganisms in several weeks (Jones et al., 1996).
Yeast and lactic acid bacteria

The mechanism of reducing aflatoxins by yeasts and lactic acid bacteria is due to their adhesion to cell-wall components. Blanco et al. (1993), Jesperson et al. (1994), and Wiseman and Marth (1983) in (Westby et al., 1997) show no effect of lactic acid bacteria. However, many other researchers report very efficient aflatoxin reductions associated with fermentation.

Yeast Saccharomyces cerevisiae have capability to bind AFB₁. Further investigation showed that components of yeast cell wall, called oligomannanns after their chemical modification, esterification, were able to bind up to 95% of AFB₁ (Devegowda et al., 1996). In addition, they are more efficient than commonly used adsorbent HSCAS (hydrated sodium calcium aluminium silicate) (Mahesh and Devegowda, 1996). The addition of 0.05% of esterified glucomannans to the basal diet resulted to improved performance in broilers (Kumprecht et al., 1977; Aravind et al., 2003).

Significant decreasing of AFB₁ concentration during brewing process in the work of Chu et al. (1975) is probably caused by sorption of mycotoxin on S. cerevisiae yeasts. This hypothesis is supported by other researchers (Shetty et al., 2007; Celyk et al., 2003). A 19% reduction of spiked AFB₁ during dough fermentation in breadmaking was reported (El-Banna and Scott, 1983).

The addition of yeast cell walls into feed can lead to a decrease of aflatoxin toxic effect in broilers (Stanley et al., 1993; Santin et al., 2003).

AFB₁ was detoxified into AFB₂₉ during yogurt (Megalla and Hafez, 1982) and dairy product (Megalla and Mohran, 1984) fermentation.

Peltonen et al. (2001) investigated the ability of 12 Lactobacillus, 5 Bifidobacterium, and 3 Lactococcus bacteria strains to bind AFB₁. These bacteria are commonly used in the food industry as a starter culture in the production of fermented milk and meat products, such as yogurt, cheese, salami, etc. During the last few years, the selected strains of bifidobacteria and lactic bacilli are rising in use in combination with prebiotics as probiotic cultures applied in renewing of intestinal microflora.

El-Nezami et al. (1998) studied the ability of selected Lactobacillus strains to remove AFB₁ from the cultivation media. The probiotic strains L. rhamnosus GG and L. rhamnosus strain LC-705 showed significant ability to remove toxin from media (around 80% of added amount). The binding of AFB₁ to L. rhamnosus GG decreases its subsequent adhesion capability to Caco-2 cells, which means that these bacteria may reduce the accumulation of aflatoxins in the intestine via increased excretion of an aflatoxin-bacteria complex (Kankaankpää et al., 2000).

Enzymes

Specific enzymes that are capable of degrading aflatoxins have been purified from microbial systems. The detoxification by specific enzymes avoids the drawback of using the microorganism, which may, in addition to its degradation activity, change flavor or impair the nutritional value and acceptability of the product (Shapira, 2004).

A novel aflatoxin degradation enzyme had been isolated and purified from Pleurotus ostreatus by Motomura et al. (2003). AFB₁ was treated with culture supernatant from 19 mushroom strains. The supernatant from P. ostreatus showed aflatoxin-degradation activity, whereas other strains had no or only weak activity. In addition, the novel enzyme showed the best aflatoxin-degradation activity at 25°C with a pH of 4.0–5.0. Fluorescence measurements suggested that the specific enzyme cleaved the lactone ring of aflatoxin, although the degradation products of aflatoxin were not investigated clearly.

In another work (Liu et al., 2001), an enzyme named aflatoxin-detoxifizyme (ADTZ), which exhibited detoxification activity on AFB₁, was isolated and purified from Armillariella tabescens (E-20). AFB₁ seemed to be degraded into difuran ring-opening AFB₁, which was less toxic than AFB₁, and the optimum activity for the enzyme was at 35°C with a pH of 6.8. This work team had already isolated a multi-enzyme that was able to degrade AFB₁ from A. tabescens in the earlier time (Liu et al., 1998). In addition, they gave a proposed pathway of the degradation of AFB₁ by the multi-enzyme: AFB₁ was first transformed to AFB₁-epoxide, followed by hydrolysis of the epoxide to give the dihydrodiol. Then, the difuran ring would open in the subsequent hydrolysis step (Figure 3).

Metabolism of aflatoxins in animals and humans

Generally, there is a great diversity in the metabolism of aflatoxins among different animal species or even, in some cases, individual animals. There are four metabolic pathways of AFB₁: O-dealkylation to AFP₁, ketoreduction to AFL, epoxidation to AFB₁-8,9-epoxide (highly toxic, mutagenic, and carcinogenic), and hydroxylation to AFM₁, (highly toxic), AFP₁, AFQ₁, or AFB₂₉ (all relatively nontoxic). Thus, the main reactions in aflatoxins metabolism are hydroxylation, oxidation, and demethylation. Most of the metabolites of AFB₁ are able to be transformed to further metabolites. AFQ₁ is able to be transformed to AFH₁ in liver. For AFB₁-8,9-epoxide, there exists three further metabolic pathways in animals and humans: hydrolysis to form AFB₁-8,9-dihydrodiol, conjugation with to AFB₁-8,9-dihydro-8-(N'-guanyl)-3-hydroxy (AFB₁-N'-Gua), and conjugation with soluble nucleophilic molecules, such as glutathione (Yiannikouris and Jouany, 2002).
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**Figure 3.** Proposed pathway of degradation of AFB₁ by Armillariella tabescens.

**Figure 4.** Metabolic pathways of aflatoxin B₁ in animals (adapted from Yiannikouris and Jouany (2002)).
Various forms of P-450 serve different biotransformation capacities, depending on the animal species. AFB₁ is transformed to AFM₁, AFQ₁, and AFB₁-8,9-epoxide by mixed-function mono-oxygenase in rat liver. In addition, Yoshizawa et al. (1982) found AFM₁ was strictly mediated by cytochrome P-448, while AFQ₁ was catalyzed by both P-450 and P-448 in rat liver. Cytochrome P-450-dependent polysubstrate mono-oxygenase system, especially CYP3A4 and CYP1A2, are the major isoforms involved in human liver. However, in distinct contrast, lipoxygenase (LOX) and prostaglandin H synthase (PHS) were found to be the main enzymes of the biotransformation of AFB₁ in human lung, whereas P-450 played a relatively minor role (Donnelly et al., 1996).

Numerous researchers have investigated the metabolism of aflatoxins in vitro and in vivo. In an investigation by Roebuck and Wogan (1977), AFL was found to be the main metabolite of AFB₁ in duck liver, whereas AFB₁ was able to be converted to AFQ₁ and AFP₁ by monkey and human liver. In addition, AFB₁ was able to be converted to AFM₁ and AFM₂ in all species (duck, rat, mouse, and monkey) except humans. In vitro metabolism of AFB₁ by animal and human liver was also studied by Roebuck et al. (1978). Duck liver had a much higher level of activity than the tissues from other species. The significant pathway in duck liver was 2,3-desaturation to form AFB₁ spin., AFL was also found in duck liver, as well as AFB₁, AFM₁, and AFM₂. However, AFB₁ was only transformed to AFQ₁ and AFP₁ by rat, mouse, and human liver. Different with ducks, a peptide (or amino acid) conjugate of AFB₂ and a glucuronide conjugate of AFM₁ were the main metabolites of AFB₁ in chicken liver in vivo (Chipley et al., 1974).

In another work (Salhab and Edwards, 1977), in vitro metabolism of AFL by liver preparations from animals and humans was investigated. AFL was metabolized to AFB₁, AFQ₁, AFH₁, AFP₁, AFM₁, and AFM₂ by liver S12 fractions from monkey, dog, rat, mouse, and human. Human and hamster preparations were most active in the metabolic pathway from AFL to AFB₁, whereas rabbit and trout had the best ability to convert AFB₁ to AFL. However, whether AFH₁ was produced directly form AFL or via the AFB₁ and AFQ₁ intermediates was not identified (metabolic pathways in animals can be found in Figure 4).

Conclusion

There have been many studies of aflatoxin degradation carried out in laboratory conditions, but no biological system exists to be used in the full commercial sphere currently. Interesting results have been obtained by Flavobacterium aurantiacum application. This soil bacterium is supposed to be removing aflatoxins B, G, and M from substrate. The use of Rhodococcus erythropolis and Mycobacterium fluoranthovorans sp. nov. DSM44556 seems to be a promising opportunity for the degradation of AFB₁ in the foods and feeds process. Studies suggest that certain fungi, including Aspergillus or Rhizopus sp., convert aflatoxin B₁ to less toxic aflatoxicol, aflatoxin B₂, or B₂₆. Enzymes isolated from Pleurotus ostreatus and Armillariella tabescens showed aflatoxin-degradation activity as well. Saccharomyces cerevisiae and Lactobacillus spp. can reduce aflatoxin amount due to binding of toxin on its cell walls. Isolated rumen bacteria do not significantly reduce aflatoxin amount, as they seem to be able to metabolize toxins in bovine ruminal fluid.

The fate of aflatoxin B₁ differs in human and animal organisms and among other species as well. There are four metabolic pathways of AFB₁: O-dealkylation, ketoreduction, epoxidation, and hydroxylation. These reactions lead to the creation of highly toxic aflatoxin B₁-8,9-epoxide and aflatoxin M₁ or relatively nontoxic forms, such as AFP₁, AFQ₁, or AFB₂₆.

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