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Biological Control of Aflatoxins

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ABSTRACT

Mycotoxins are fungal secondary metabolites that if ingested can cause a variety of adverse effects on both humans and animals.Aflatoxins are cancerogenic compounds produced predominantly by certain strains of the Aspergillus genus. They have immunosuppressive, mutagenic, teratogenic and carcinogenic effects, especially on the liver. A variety of physical, chemical and biological methods have been developed for decontamination and control of aflatoxins from contaminated foods and feeds. In this paper, we review recent development in biological control of aflatoxin contamination.

Keywords: aflatoxin, biocontrol, biodegradation

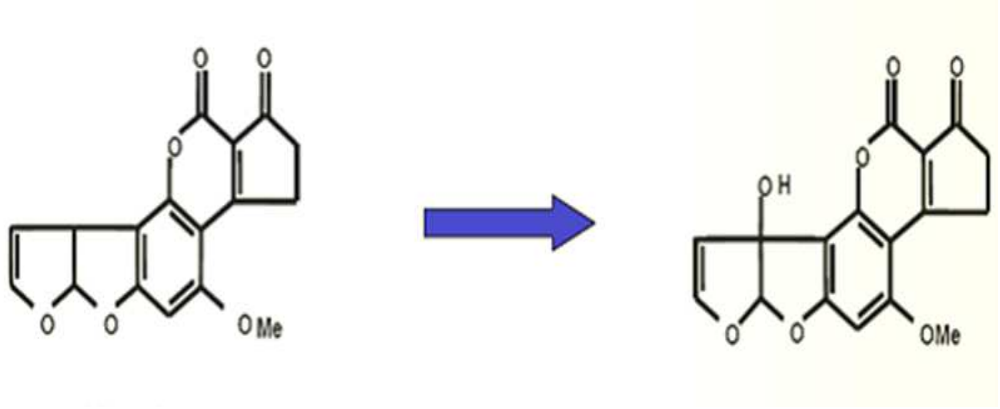
INTRODUCTION

Mycotoxins are toxic secondary metabolites naturally produced by molds *(Aspergillus*, *Fusarium*and *Penicillium*spp.) that may contaminate agricultural commodities when environmental conditions are favourable[1,2].Mycotoxins are well known to cause toxicities to humans and animals [3].After infesting crops, fungi synthesize the toxins, which will be transmitted to the final food products. Mycotoxins can be found in a wide variety of matrices, ranging from cereals, peanuts, spices, animal feeds, fruits and vegetables to meat, milk, eggs and many other derived products [4].Principally, there are three possibilities to avoid harmful effect of contamination of food and feed caused by mycotoxins: (1) prevention of contamination, (2) decontamination of mycotoxincontaining food and feedand (3) inhibition of absorption of mycotoxin content of consumed food into the digestive tract (2).The classes of mycotoxins with relevance to health are: aflatoxins,ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins and ergot alkaloids [4].The European Union has a maximum level of 2 µg/kg for AFB1 and 4 µg/kg for total aflatoxins in crops [5].Aflatoxins are a group of structurally-related toxic compounds produced by certain strains of the fungi *Aspergillusflavus* and*A. parasiticus*[6,7].Aflatoxins have sub-acute and chronic effects such as liver cancer, chronic hepatitis, jaundice, hepatomegaly and cirrhosis in humans, AFM1 is classified in Group 2 as a probable human carcinogen [8].*A. parasiticus* produces four major aflatoxins: B1, B2, G1 and G2, while AFB1 is the most toxic in the group and the toxicity is in the order of B1 > G1 > B2 > G2 [9, 10].Many strategies, including biological control, control of insect pest, development of resistant cultivar, have been investigated to manage aflatoxins in crops. Among them, biological control appears to be the most promising approach for control of aflatoxin in both pre-harvestedand post-harvested crops [11].Using microorganisms including bacteria, yeasts and nontoxigenic*Aspergillus* fungi are of the well-known strategies for the management of aflatoxins in foods andfeeds [11, 12]. In this paper, we review recent development in biological control of aflatoxin contamination.

DISTRIBUTION OF AFLATOXIN CONTAMINATION

Distribution in dairy products:

The investigation of aflatoxin contamination in dairy products indicated that aflatoxin M1(AFM1),Aflatoxin M1 is a major metabolite of aflatoxin B1 (AFB1), which is formed when animals ingest feed contaminated with aflatoxin B1[13]. Aflatoxin M1 in milk and milk products is considered to pose certain hygienic risks for human health. These metabolites are not destroyed during the pasteurization and heating process[14]. The amount of AFM1 which is found in milk depends on several factors, such as animal breed, lactation period, mammary infections etc. AFM1 could be detected in milk 12-24 h after the AFB1 ingestion, reaching a high level after a few days. When AFB1 intake is stopped, the AFM1 concentration in milk decreases to an undetectable level after 72 h (Fig. 1)[8,15].



Aflatoxin B1 Aflatoxin M1

Figure 1.Aflatoxin M1 is a major metabolite of aflatoxin B1

Maximum limits for aflatoxin M1 in milk and milk products in various countries shown in Table 1[16,17].There are some studies about the contamination of aflatoxin M1 in milk and dairy products in different cities of Iran and other countries [14,18,19,20,21,22].The contamination of milk and milk products with AFM1 display variations according to geography, country and season. The pollution level of AFM1 is differentiated further by hot and cold seasons, due to the fact that grass, pasture, weed and rough feeds are found more commonly in spring and summer than in winter[14].

Table 1. Maximum limits for aflatoxin M_1 in milk and milk products in various countries

Country	Maximum limit (µg/kg or µg/l)
France	0.05 Adult's milk 0.03 Children's milk
Turkey	0.05 Milk and products 0.25 Cheese
Czech Republic	0.1 Children's milk 0.5 Adult's milk
Belgium	0.050 Milk
USA	0.50 Milk
Switzerland	0.050 Milk and milk products 0.250 Cheese
Netherlands	0.020 Butter 0.200 Cheese
Germany	0.050 Milk
Australia	0.050 Milk

Galvano et al, reported that 80% of all yogurt samples in Italy were contaminated with AFM1, ranged between 1-3.1 ng/kg[23].Atasever et al,analyzed a total of 80 butter samples by Elisa, AFM1 was found in 66 (82.5%) samples at levels ranging from 10 to 121 ng/kg with mean concentration of 30.4ng/kg[24].In Turkey, a study done by Oruc and Sonal, found AFM1 in 89.5% of 57 cheese samples with ranges of 0-180 ng/l[25]. In Kuwait, 54 samples of dairy products were analyzed for aflatoxin M1, 28% were contaminated with AFM1[26].Sarimehmetoglu et al, detected AFM1 contamination in 327 (81.75%) of 400 cheese samples. The numbers of cheese samples that contained AFM1 over the legal limits of 0.25µg/kg were 110 (27.5%)[27].Akkaya et al, in Turkey,177 yogurt samples consisting of 104 samples of ordinary yogurt, 21 of fruit yogurt, and 52 of strained (Torba) yogurt were tested for AFM1 by ELISA method. The highest AFM1 concentration was 150 ng/kg in strained yogurt, 100 ng/kg in ordinary yogurt as well as in fruit yogurt. 11.53% of ordinary yogurt (12 samples), 9.52% of fruit yogurt (2

samples), and 21.15% of strained yogurt (11 samples) had higher AFM1 levels, than the acceptable levels (50 ng/kg for yogurt)[28]. Martins and Martins analyzed a total of 182 samples of national cheese (Portugal) by TLC, observing that all of them were not contaminated by AFM1 [29].According to observations, the levels of contamination of milk and dairy products by AFM1 seem to vary in many studies. These variations may be related to different reasons such as dairy products manufacturing procedures, different milk contaminations, type of dairy products, geographical region, the country, the season and the analytical methods employed.

Distribution in pet food and cereals:

Mycotoxin contamination in pet food poses a serious health threat to pets. Cereal grains and nuts are used as ingredients in commercial pet food for companion animals such as cats, dogs, birds, fish and rodents[30]. Grains (cereals and oilseeds) and nuts in general are subject to mold attack, in preharvest and postharvest. Aflatoxin contamination has been reported for grains as corn, soya, wheat, rice, cottonseed, nuts such as peanuts, almonds, hazelnuts, walnuts, cashew nuts, pecans, and pistachio nuts.Aflatoxin contamination having been observed in several foodstuffs, the contamination of maize, peanuts, and oilseeds can be considered, in terms of diet exposure, the most important worldwide[31,32]. Table 2, shows contamination, frequencies, and concentrations Aflatoxin B1 in grains or nutsfrom various countries [33].

Table 2.Concentrations Aflatoxin B1 in grains or nut

Country/commodity	Positive AFB1 samples (%)	Contamination rate (ppb)
Bangladesh/Maize	67	33.0(mean)
Brazil/Corn	38.3	0.2-129.0
China/Corn	76	>20.0
India/Maize	26	>30.0
Egypt/Soybean	35	5.0-35.0
Malaysia/Wheat	1.2	>25.62
Mexico/Corn	87.8	5.0-465.0
Nigeria/Corn	45	25.0-770.0
Senegal/Peanut oil	85	40.0 (mean)
Qatar/Pistachio	8.7 to 33	>20.0
Korea/Corn	19	74.0

Methods in Mycotoxin analysis:

Common analytical methods for mycotoxins can be divided in two categories: screening methods and confirmatory methods. The first category includes rapid methods such as ELISA, which has a wide range of applicability: aflatoxins, fumonisins, ochratoxin A, zearalenone and trichothecenes. New screening techniques will be developed, for the purpose of being used in prevention strategies. Biosensor-based techniques with surface plasmon resonance detection are beginning to be used.Biosensors based on the use of monoclonal or polyclonal antibodies have seen a great development in the field of small molecules analytical determination and specifically in the mycotoxins analyses. Main advantages of biosensors technology in comparison with traditional analytical methods are fast detection, low cost assay, high sensitivity, their high selectivity, easy preparation and operation assay method. From the confirmatory methods, gas chromatography and high performance liquid chromatography, often with mass-spectrometric detection, are most commonly used in the present days. Thin layer chromatography was among the first methods used for mycotoxin analysis. Automation, high performance separation and generally lower detection limits are the advantages of GC and HPLC compared to TLC[33,34].

Biological control of mycotoxins:

Several approaches have been developed for decontamination of mycotoxins in foods. Though many approaches are available for mycotoxin decontamination, most of them are not widely available due to high cost or practical difficulties involved in detoxification process[3]. Several strategies, including chemical, physical and biological control methods have been investigated to manage aflatoxins in foods. Biological detoxification of mycotoxins works mainly via two major processes, sorption and enzymatic degradation, both of which can be achieved by biological systems[2, 11]. Live microorganisms can absorb either by attaching the mycotoxin to their cell wall components or by active internalization and accumulation. Dead microorganisms too can absorb mycotoxins and this phenomenon can be exploited in the creation of biofilters for fluid decontamination or probiotics to bind and remove the mycotoxin from the intestine. Enzymatic degradation can be performed by either extra or intracellular enzymes. The degradation can be complete, the final product being CO_2 and water. Alternatively, enzymatic modification can alter, reduce or completely eradicate toxicity[35]. 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.In the 1960, Ciegler et al and Lillehoj et al, screened over 1000 microorganisms for the ability to degrade aflatoxins. Only one bacterium, *Flavobacteriumaurantiacum* B-184, was able to irreversibly remove aflatoxin from solutions [36, 37].A few strains

of lactic acid bacteria (LAB) have been reported to bind aflatoxins B1 or M1 in contaminated media or in a food model and several studies have suggested that the antimutagenic and anticarcinogenic properties of probiotic bacteria can be attributed to their ability to non-covalently bind hazardous chemical compounds such as aflatoxins in the colon.Nezami et al, using an in vivo system were able to demonstrate reduced aflatoxin B1 uptake by the chicken duodenum in the presence of *Lactobacillus* and *Propionibacterium* strains [38].Haskard et al, studied the effect of different variables on binding of AFB1 to *L. rhamnosus*. They reported temperature, sonication and pH had no significant effect on the release of bound AFB1 by *L. rhamnosus*, while salt (NaCl and $CaCl_2$) concentrations showed minor effects [39]. Teniola et al, investigated *Rhodococcuserythropolis* isolated from polycyclic aromatic hydrocarbon soils for AFB1 degradation activity. Dramatic reduction of AFB1 was observed during incubation in the presence of *R. erythropolis* cells [40].Dsouza et al, who found that copper and zinc ions may inhibit the degradation of aflatoxin B1 by *Flavobacteriumaurantiacum* [41].In study Guan et al, twenty five single colony bacterial isolates were obtained from 65 samples collected from various Sources. All these isolates were able to reduce concentrations of AFB1 in the liquid medium tested after 72 h incubation at 37 °C with various degrees of effectiveness. Ten isolates reduced AFB1 in the medium by over 70% (Table 3) [42].

Table 3.AFB1 degradation by individual microbial isolates from various Sources

Isolate	Source	Degradation (%)
Stenotrophomonasmaltophilia(35-3)	South American tapir feces	82.50
Bacillus sp.	Hog deer feces	80.93
*Brevundimonas*sp.	Yellow cheek feces	78.10
Bacillus sp.	Farm soil	77.80
*Klebsiella*sp.	Rabbit feces	77.57
*Brevundimonas*sp.	Goral feces	76.83
*Enterobacter*sp.	Hog deer feces	75.92
*Brachybacterium*sp.	Rabbit feces	74.83
*Rhodococcus*sp.	Ostrich feces	73.92
*Cellulosimicrobium*sp.	Farm soil	73.75

Palumbo et al, reported that a number of *Bacillus*, *Pseudomonas*, *Ralstonia* and *Burkholderia* strains could completely inhibit *A. flavus* growth. *B. subtilis* and *P. solanacearum* strains isolated from maize soil were also able to inhibit aflatoxin accumulation [43].Munimbazi and Bullerman, reported that more than 98% inhibition in aflatoxin production by *A. parasiticus* was caused by *B. pumilus*[44].*Flavobacteriumaurantiacum* NRRL B-184, a kind of bacteria from soils and water, showed a very high capability of detoxifingaflatoxins in feeds and foods [45].Liu et al, an enzyme named aflatoxin-detoxifizyme (ADTZ), which exhibited detoxification activity on AFB1, was isolated and purified from *Armillariellatabescens*[46].Bown et al, observed reduction of aflatoxin content in maize after application of atoxigenic*A. flavus*[47]. In study Motomura et al, AFB1 was treated with culture supernatants from 19 mushroom strains. The supernatant from *P. ostreatus*showed aflatoxin degradation activity [48]. Boller and Schroeder, reported that *A. cheralieri* and *A. candidus* that dominated the mycoflora in rice also showed marked inhibition in aflatoxin production by *A. parasiticus*[49].Based on the available reports, we can conclude that microorganisms are themain living organisms applicable for mycotoxin decontamination in foods.

CONCLUSION

Mycotoxins pose a serious threat to animal and human healthand efforts continue to be devoted, worldwide, to preventing or eliminating them. First step towards preventing mycotoxin formation is to control or prevent the growth of storage fungi. According to results of experiments to date, microorganisms are the main living organisms applicable for mycotoxin biodegradation. 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 aflatoxins transfer to the intestinal tract of humans and animals.Finally, it can be concluded that the review revealed a high incidence ofaflatxoin contaminated in agricultural commodities and that biological removalof aflatoxinfrom food and feed may be used on large scale to minimizeeconomic loss due aflatoxin contamination and to improve animal and human healthcondition.

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