Microbiological Control of Mycotoxins: Present Status and Future Concerns

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Mycotoxins are naturally occurring toxic substances in foods, posing considerable health risk to humans. Microbiological decontamination of mycotoxins using microorganisms is one of the well-known strategies for the management of mycotoxins. Numerous organisms, including bacteria, fungi and yeasts have been tested for their ability in reducing mycotoxigenic fungi and resultant mycotoxin contamination in foods. Great successes in reducing aflatoxin contamination in fields of different crops have been achieved by application of atoxigenic strains of *Aspergillus*. However, in this chapter, we review the developments in biological control of mycotoxin contamination with particular emphasis on aflatoxins, fumonisins, ochratoxins and patulin to develop further proper management strategies to control mycotoxins in foods.

Key words Mycotoxins; biological control; bacteria; fungi; yeasts; foods

1. Introduction

Mycotoxins produced by *Aspergillus, Fusarium* and *Penicillium* spp. are natural contaminants in foods [1]. Mycotoxins are well known to cause toxicities to humans and animals [2]. Occurrence of mycotoxin contamination in foods is more prevalent in the tropical and subtropical countries resulting in acute and chronic mycotoxicoses in humans and animals [2]. Wild [3] reported that many West African countries, over 98% of the tested people were positive to aflatoxin-DNA adducts indicating aflatoxin exposure in the population. The importance of this situation is highlighted with an outbreak of aflatoxicosis as recent as 2004 in Kenya [2, 4]. Many of the developed countries have regulations for mycotoxins in food grains and its products [2]. However, the risk of mycotoxin exposures continues in the developing countries due to lack of food security, poverty and malnutrition [2, 5].

Several approaches have been developed for decontamination of mycotoxins in foods [2, 6]. 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 [2]. Many species of bacteria, fungi and yeasts have been shown to enzymatically degrade mycotoxins [7-13]. However, question remains on the toxicity of products of enzymatic degradation and undesired effects of fermentation with non-native microorganisms on quality of food [2]. In this chapter the biocontrol of aflatoxins, fumonisins, ochratoxins and patulin by bacteria, fungi and yeasts will be discussed more in depth to fill the existing gaps and to develop further proper management practices using biocontrol agents to ensure food safety and to protect consumer's health.

2. Microbiological control of aflatoxins

Aflatoxins are potent toxic secondary metabolites produced mainly by *Aspergillus flavus, A. parasiticus* and rarely *A. nomius* [1]. Aflatoxin contamination of crops is a worldwide food safety concern [1]. Several strategies, including chemical, physical and biological control methods have been investigated to manage aflatoxins in foods. Among them, biological control appears to be the most promising approach for control of aflatoxins. 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. (Table 1) under laboratory conditions. For example, Palumbo et al. [14] 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 [15]. In most cases, although these strains were highly effective against fungal growth and resultant toxins under laboratory conditions, they do not give good efficacies in fields because it is difficult to bring the bacterial cells to the *Aspergillus* infection sites on commodities under field conditions [16]. In our laboratory, *B. subtilis, P. fluorescens* and *Rhodococcus erythropolis* effectively inhibited the mycelial growth and subsequent aflatoxin B_1 (AFB1) production by *A. flavus* [8]. Biological degradation of AFB1 by *R. erythopolis* and *Mycobacterium fluoranthenivorans* was greater than 90% within 4 hours at 30^0C, while after 8 hours AFB1 was practically undetectable. The degradation of AFB1 by these bacteria most probably occurred through a cascade of enzyme reactions with loss of fluorescence over time [17]. The biodegradation of AFB1 by *R. erythropolis* was examined in liquid cultures. The degradation was enzymatic and the enzymes responsible for the degradation of AFB1 were extracellular and constitutively produced [18].

B. pumilus has been associated with inhibition of aflatoxin, cyclopiazonic acid, ochratoxin A and patulin production. Munimbazi and Bullerman [19] reported that more than 98% inhibition in aflatoxin production by *A. parasiticus* was

caused by *B. pumilus*. El-Nezami et al. [20] reported the ability of lactic acid bacteria to remove AFB1 from artificially contaminated liquid media. The removal was strain dependent and very fast, with two strains of *L. rhamnosus* removing about 80% of the toxin at the beginning of the incubation time period. The two best strains were further tested and their ability to remove aflatoxin was found to be dependent on temperature, with maximum removal at 37^0C, and a bacterial concentration of at least 2×10^9 CFU/mL necessary for significant removal.

Table 1. Bacterial and fungal strains on aflatoxin producing *Aspergillus* spp.

Bacteria/fungi	*Aspergillus* spp.	Reference
Achromobacter xylosoxidans	*A. parasiticus*	[21]
Acremonium zeae	*A. flavus*	[22]
A. flavus	*A. flavus, A. parasiticus*	[16, 23, 24]
B. subtilis	*A. flavus*	[8, 25]
B. pumilus	*A. flavus, A. parasiticus*	[26]
Lactobacillus spp.	*A. flavus, A. parasiticus*	[27]
Burkholderia cepacia	*A. flavus*	[28]
L. casei	*A. flavus*	[29]
L. lactis	*A. flavus, A. parasiticus*	[30]
L. sanfrancisco	*Aspergillus* spp.	[31]
Nannocystis exedens	*A. flavus, A. parasiticus*	[32]
P. fluorescens	*A. flavus*	[8]
R. erythropolis	*A. flavus*	[8, 17]
Streptococcus lactis C10	*A. parasiticus*	[33]
S. thermophilus 489	*A. fumigatus, A. parasiticus*	[34]
T. viride and *T. harzianum*	*A. flavus*	[8, 35]

Several researchers have tested the lactic acid bacteria and bifidobacteria for their ability to bind AFB1 in liquid media. For example, Peltonen et al. [36] found that between 5 and 60% of the aflatoxin in solution was bound by the bacteria, with *L. amylovorus* and *L. rhamnosus*. Haskard et al. [37] 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. Of the variables studied, urea had the greatest effect suggesting that hydrophobic interactions play a major role in binding [38]. According to available literature, the stability of the interactions involved in the binding of mycotoxins by bacterial cells depends on strain, treatment and environmental conditions [37, 39]. Therefore, it is important to understand how different bacterial species behave under different environmental conditions to evaluate their potential to be used as decontaminating agents in food processes and as binding agents in the gastrointestinal tract of humans and animals.

Great successes in biological control of aflatoxin contamination in different crops have been achieved through the application of competitive atoxigenic strains of *A. flavus* and *A. parasiticus* [40]. In many field experiments, particularly with peanut and cotton, significant reductions in aflatoxin contamination have been observed by the use of atoxigenic *Aspergillus* strains [16, 23, 24]. Cotty [41] tested atoxigenic *A. flavus* strains for their ability in reducing aflatoxin contamination of cottonseed. Results from greenhouse experiments showed that six of seven atoxigenic strains significantly reduced the amount of aflatoxin produced by the toxigenic strains in cottonseed when they were co-inoculated with toxigenic strains, and that the strain AF36 was the most effective in reducing aflatoxin contamination [42]. Bown et al. [43] observed reduction of aflatoxin content in maize after application of atoxigenic *A. flavus*. In this study, when corn ears were either co-inoculated with AF36 and a toxigenic strain of *A. flavus* or inoculated with AF36 at 24 h prior to inoculation with the toxigenic strain, subsequent aflatoxin concentrations were significantly reduced, compared to inoculation with the toxigenic strain alone. Recently, two products of atoxigenic strains have received U.S. Environmental Protection Agency (EPA) registration as biopesticides to control aflatoxin contamination in cotton and peanuts in USA [16].

Several field experiments have shown that a natural strain (*A. flavus* NRRL21882) isolated from peanut in Georgia, was very effective in controlling aflatoxin contamination in peanuts [40]. In 1999, field plots of peanuts were treated with atoxigenic strains of *A. flavus* (NRRL 21882) and *A. parasiticus* (NRRL 21369) at 67 days after planting. At harvest, peanuts were contaminated with high levels of aflatoxins (516.8 µg/kg) in the untreated plots, but only 54.1 µg/kg in the atoxigenic treatments. After storage, aflatoxins in non-field treated peanuts contained 9145.1 µg/kg compared with 374.2 µg/kg for that in field-treated peanuts. These results indicated that field application of the atoxigenic strains had a carry-over effect and reduced aflatoxin contamination that occurred in storage [44]. Recently, a commercial biopesticide product (called afla-guard) has been developed based on the *A. flavus* strain NRRL 21882. This strain is the active ingredient in an EPA-registered biopesticide afla-guard. Additionally, the atoxigenic *A. flavus* strains CT3 and K49 have been tested in the USA and showed good efficacies in reduction of aflatoxin contamination in corn [45]. Since applications of atoxigenic *Aspergillus* strains have shown a great success in controlling aflatoxin

contamination in the USA, similar studies were also conducted in several other countries [40]. In Australia, application of atoxigenic strains could reduce aflatoxin formation in peanuts by 95% [24]. Recently, in China, Yin et al. [40] found a highly competitive strain AF051 after screening of 30 atoxigenic strains of *A. flavus*. Field tests showed that this strain reduced naturally *Aspergillus* populations by up to 99% in the soil of peanut fields. Cardwell and Henry [46] found that atoxigenic strain BN30 was very effective in reducing the amount of toxin produced in maize when co-inoculated with the highly toxigenic S-strain from Africa. These results indicate that applications of atoxigenic strains could be used in different agro-ecozones for the control of aflatoxin contamination in different food crops.

Saprophytic yeast species such as *Candida krusei* and *Pichia anomala* have shown promise as biocontrol agents for decontamination of aflatoxins [40]. Similar to bacterial agents, these yeast strains were able to significantly inhibit *Aspergillus* growth and resultant toxins under laboratory conditions [47, 48]. Shetty et al. [49] found that the ability of *S. cerevisiae* to bind AFB1 was strain specific with 7 strains binding 10-20%, 8 strains binding 20-40% and 3 strains binding more than 40% of the added AFB1. Though the yeasts are considered to be potential biocontrol agents for management of aflatoxins, further field experiments are necessary to test their efficacies in reducing aflatoxin contamination under field conditions. There are many reports on the use of physically separated yeast cell walls obtained from brewery as feed additive in poultry diet resulting in amelioration of toxic effects of aflatoxins [2, 50]. When dried yeast and yeast cell walls were added to rat-ration along with AFB1, a significant reduction in the toxicity was observed [51]. In an *in vitro* study with the cell wall material, there was a dose dependent binding of as much as 77% (w/w) and modified mannan-oligosaccharides derived from the *S. cerevisiae* cell resulted in as much as 95% (w/w) binding [52].

3. Microbiological control of fumonisins

Fungi belonging to the genus *Fusarium* are associated with the production of fumonisins. Among the fumonisins, fumonisin B$_1$ (FB1) in particular is of international, agroeconomic, and food safety concern. For example, high doses of FB1-infested corn feed have been shown to cause pulmonary edema in swine, while lower doses lead to hepatic disease [1, 53]. A number of reports in recent years have focused on interactions between *Fusarium* and potential bacterial antagonists (Table 2). Stiles and Bullerman [54] studied the effect of *L. rhamnosus* on growth and mycotoxin production by *Fusarium* species, including *F. proliferatum, F. verticillioides* and *F. graminearum*. The results showed that production of FB1 was reduced up to 63.2%, FB2 up to 43.4% and deoxynivalenol and zearalenone up to 92% and 87.5%, respectively. *L. rhamnosus* was evaluated for its potential to remove or degrade zearalenone and α-zearalenol and both viable and non-viable cells were able to remove about 50% of the toxin from solution, indicating that binding rather than metabolism was the mechanism in action [20]. When *L. rhamnosus* was exposed to both toxins at the same time, its ability to remove zearalenone and α-zearalenol from solution was significantly reduced, indicating that these toxins may share the same binding site on the bacterial cell [20].

El-Nezami et al. [20] studied the ability of *Lactobacillus* and *Propionibacterium* strains to remove trichothecenes from liquid media. Some strains showed to remove deoxynivalenol, diacetoxyscirpenol and fusarenon, with the amount of removal varying from 18 to 93% and one strain removed only deoxynivalenol and diacetoxyscirpenol, with removal varying from 10 to 64%. Niderkorn et al. [55] screened lactic and propionic acid bacteria for their ability to remove deoxynivalenol and fumonisin from solution and they found that it was strain specific, with propionic acid bacteria being less efficient than LAB. *L. rhamnosus* removed up to 55% of deoxynivalenol, while *Leuconostoc mesenteroides* removed about 82% of FB1 and *L. lactis* removed 100% of FB2. *B. amyloliquefaciens* and *Microbacterium oleovorans* isolates were shown to effectively reduce *F. verticillioides* propagules and fumonisin content in maize kernels at harvest when applied as seed coatings [56]. The effects of four bacterial biocontrol agents on *F. verticillioides* infection and fumonisin accumulation in the maize agro-ecosystem were evaluated in a 2-year field study. Seed treatment with *B. amyloliquefaciens* and *Enterobacter hormaechei* reduced the infection by the fungus and FB1 contents in both years of the study [13]. Very few scattered reports are available on the use of yeast and fungal strains for degradation of fumonisins. *S. cerevisiae* strains were tested for their ability to degrade zearalenone and fumonisins in Sabouraud broth. Two strains were capable to degrade zearalenone totally, one strain decreased the mycotoxin concentration up to 25% and one strain up to 75% of original amount. Two strains were capable to degrade fumonisins partially [57]. The potential effects of ethanol fermentation by yeasts on *Fusarium* mycotoxins were studied by Flesch and Voight-Scheuerman [58] who investigated the decomposition of trichothecenes during alcoholic fermentation of grape juice.

Table 2. Bacterial and fungal strains on fumonisin producing *Fusarium* spp.

Bacteria/fungi	*Fusarium spp.*	Reference
Azotobacter armeniacus	*F. verticillioides*	[59]
B. subtilis	*F. verticillioides, F. moniliforme, F. graminearum*	[60-61]
Bacillus spp.	*F. verticillioides, F. graminearum*	[62-64]
B. mojavensis	*Fusarium* spp.	[60]
Burkholderia cepacia	*F. verticillioides*	[28]
Clonostachys rosea	*F. verticillioides, F. proliferatum*	[65]
L. sanfrancisco	*Fusarium* spp.	[31]
L. plantarum	*Fusarium* spp.	[66]
L. lactis subsp. lactis	*Fusarium* spp.	[30]
P. fluorescens	*F. graminearum, F. culmorum, F. verticillioides*	[61, 67, 68]
Trichoderma spp.	*F. graminearum*	[69]

4. Microbiological control of ochratoxins

Ochratoxin A (OTA) produced by *Aspergillus* and *Penicillium* spp. is a natural contaminant in cereals and beverages [1]. According to the available literature grape and wine are considered as the second major source of OTA intake after cereals. Thus, it is understandable that considerable research has been done concerning detoxification of these two food groups [70]. Several bacterial and fungal strains belonging to *Streptococcus, Bifidobacterium, Lactobacillus, Butyribrio, Phenylobacterium, Pleurotus, Saccharomyces, Bacillus* and *Acinetobacter* genera and certain fungi belonging to *Aspergillus* (*A. fumigatus, A. niger, A. carbonarius, A. japonicus, A. versicolor, A. wentii* and *A. ochraceus*), *Alternaria, Botrytis, Cladosporium, Phaffia, Penicillum* and *Rhizopus* (*R. stolonifer* and *R. oryzae*) genera, are able to degrade OTA *in vitro* up to more than 95% [71]. Moreover, some of them have shown detoxifying properties in *in vivo* assays. Fuchs et al. [72] screened 30 different LAB strains for their ability to remove OTA from solution, and they reported 95% removal of OTA in liquid media by *L. acidophilus.* Based on early research, it was reported that OTA is degradable by rumen bacteria [70, 73]. Thirty seven bacterial species, 10 strains of yeasts and 12 moulds were screened in order to detect microorganisms able to degrade OTA [74]. *Acinobacter calcoaceticus* was able to degrade OTA in ethanol-minimal salts medium at both 25 and 30°C. It was suggested that the end product of degradation of OTA by *A. calcoaceticus* is a less toxic compound, ochratoxin α (OTα) [70]. Bejaouii et al. [75] studied the degradation of OTA by 40 isolates of *Aspergillus* section *Nigri* species isolated from French grapes. They reported significant reduction of OTA in liquid medium.

Abrunhosa et al. [71] observed poor OTA-hydrolyzing activity using protease preparations and enzyme extract of *Aspergillus* strains. The OTA content and its fate during wine-making and possibilities of its degradation have been intensively studied [70]. For example, presence and fate of this mycotoxin in grapes, wine and beer were published by Mateo et al. [76] and Rousseau [77]. Although the decrease of OTA content in liquid phase during vinification process is observed by the majority of researchers, reports are controversial regarding the mechanism of OTA removal [70]. That is, is it a result of malolactic fermentation due to the action of lactic acid bacteria, [78] or is it adsorption to yeast cell walls [79]. After success in clarifying the mechanism and degradation products of ochratoxin, three directions in recent research may be observed [70, 80] 1) study of different moulds able to degrade OTA, [12] 2) screening of bacterial strains for OTA degradation [81] and 3) isolation and identification of enzymes involved in OTA degradation process [82]. Although the results of these studies showed very promising for reducing OTA contamination, studies on model systems do not guarantee the degradation of OTA *in situ*, using foods [70]. Therefore, further studies are needed to identify the main degradation products and to investigate the activity of these microbes in foods used for human consumption.

Grunkemeier [83] observed a mixture of sterilized yeast and fermentation residue of beer was shown to be an efficient binder of OTA in an *in vitro* adsorption study. Our experimental results showed that yeast strains are able to degrade OTA more than 80% during their growth under *in vitro* conditions [9]. Bejaouii et al. [84] suggested that oenological strains of *Saccharomyces* yeasts can be used for the decontamination of OTA in synthetic and natural grape juice. Heat treated cells showed higher adsorption (90%, w/w) than to viable cells (35%, w/w) indicating physical nature of binding and cell density played an important role in adsorption efficiency. This work indicated that yeast could be a potential decontaminating agent for OTA in grape juice. In addition, dead yeast cells can potentially be used for removing OTA from grape juice, as dead yeasts do not pose any quality or safety problems [84].

5. Microbiological control of patulin

Patulin contamination of fruit-based foods and beverages is an important food safety issue due to the high consumption of these commodities throughout the world [1, 70]. Based on the experimental results available, it has been concluded that patulin produced by *Penicillium* spp. is genotoxic, although no adequate evidence of carcinogenity in experimental animals exists [85]. Apple and apple products are main sources of patulin contamination [1]. However, patulin has also been found in other fruits, such as pears, peaches, apricots, grapes and cheese [86, 87]. Extensive research has been carried out to study the effect of yeasts on biodegradation of patulin since 1990's. For example, Burroughs [88] found that about 90% of the patulin reduction from the medium after 3 days. Stinson et al. [89] observed decrease of patulin content in fermented apple juice contaminated with yeast during alcoholic fermentation. Recently, Moss and Long [90] reported that commercial yeast *S. cerevisiae* transformed patulin into ascladiol. As reported by Moss [91], acute toxicity of ascladiol amounted to only one-fourth of the strength of patulin. Richelli et al. [92] found the ability of *Gluconobacter oxydans* to degrade patulin more than 96% after twelve-hour treatment, due to change of chemical structure and the degradation product (ascladiol) of this mycotoxin. The genus *Gluconobacter*, whose taxonomy is at present under worldwide review, is made up of five different species, [93] which have no health risk, and that are commonly used in food manufacturing. Apple juice inoculated with this bacterium and incubated for 3 days still tasted like juice and was drinkable [70, 93].

Microbiological control of patulin result largely from the observation that patulin is almost always completely degraded during yeast fermentation [94]. According to available literature, this method is much better understood compared with other decontamination methods [94]. Recently, Coelho et al. [95] reported more than 90% of patulin reduction during yeast fermentation. Stinson et al. [96] examined and found that out of eight yeast strains tested, six reduced patulin levels to below detectable levels, while all eight strains resulted in a 99% or better decrease in total patulin content. However, a control, stored the same period of time (two weeks), had only a 10% reduction. Moss and Long [90] observed reduction of patulin levels during fermentative growth but not aerobic growth by three strains of *S. cerevisiae*. This reduction resulted in the production of two major products: *E*-ascladiol, patulin's immediate biosynthetic precursor, and its isomer *Z*-ascladiol. *E*-ascladiol is itself a mycotoxin, which has reduced toxicity compared with patulin and also reacts with sulfhydryl-containing compounds [94, 97]. Our laboratory experimental results showed that *Metschnikowia pulcherrima* strains isolated from apples had strong patulin degrading activity (100%) within 48 h [unpublished data]. Biological control with yeast is limited to products that can be fermented. More over, yeasts are itself sensitive to patulin and at concentrations greater than 200 μg/mL, yeast has been completely inhibited [98]. Until today no reports are available on the direct enzymatic degradation of patulin. Reducing enzymes such as those involved in yeast fermentation, as well as lactone degrading enzymes such as β-lactamase, may well be able to degrade patulin alone [94]. Gourama [99] reported the inhibitory activity of cell-free supernatants of *L. casei* strains on the growth of *Penicillium* spp. and the production of toxins (patulin and citrinin). Fuchs et al. [72] screened thirty different LAB strains for their ability to remove patulin from solution, and they reported at least 80% removal of patulin by *Bifidobacterium animalis*.

6. Conclusion and Future concerns

In this chapter, we have tried to provide information on microbiological control of various mycotoxins using bacteria, fungi and yeasts to fill the existing gaps. Based on the available reports, we can conclude that microorganisms are the main living organisms applicable for mycotoxin decontamination in foods. Results of various researchers showed that yeasts and bacterial strains had differences in decontamination of mycotoxins [70, 100]. For example, *Kluyveromyces marxianus* was sensitive to all trichothecene toxins, but this yeast was not inhibited by other mycotoxins, and *B. brevis* was sensitive to eight mycotoxins, including zearalenone and OTA, but was not affected by high concentrations of trichothecene toxins [100]. However, the results achieved in microbiological decontamination of mycotoxins until today may be treated as a first step in development of practical commercial technologies. Further intensive screening of microbes may lead to detection of efficient and applicable microorganisms. Based on the available reports of mycotoxin-degrading microorganisms in digestive tract of animals, the activity of these microorganisms may be increased and they may be used *in vivo* for degradation of mycotoxins in food [70]. With the application of molecular biology techniques, the potential mycotoxin degrading microbial strains can be engineered to significantly improve the quality and safety of foods from mycotoxins contamination to protect consumer's health. Finally a most useful practical technology should be developed from economical point of view.

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