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REVIEW ARTICLE

Biological detoxification of mycotoxins: a review

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Abstract Mycotoxins are secondary fungal metabolites and are reported to be carcinogenic, genotoxic, teratogenic, dermato-, nephro- and hepatotoxic. Several studies have shown that economic losses due to mycotoxins occur at all levels of food and feed production, including crop and animal production, processing and distribution. Therefore, there is a great demand for a novel approach to prevent both the formation of mycotoxins in food and feed and the impact of existing mycotoxin contamination. Recently, investigators have reported that many microorganisms including bacteria, yeast, moulds, actinomycetes and algae are able to remove or degrade mycotoxins in food and feed. We have reviewed various strategies for the detoxification of mycotoxins using microorganisms such as bacteria, yeast and fungi.

Keywords Mycotoxins · Detoxification · Bacteria · Fungi · Yeast

Introduction

Mycotoxins are highly toxic secondary metabolic products of various moulds, mainly those belonging to the genera *Fusarium, Aspergillus* and *Penicillium*. It has been estimated that at least 300 of these fungal metabolites are potentially toxic to animals and humans (Heidler and Schatzmayr 2003). The major agriculturally important mycotoxins include aflatoxins (AFs), fumonisins (FB_1, FB_2), the trichothecene mycotoxins (deoxynivalenol [DON], nivalenol [NIV], T-2 and HT-2) and ochratoxin A (OTA), each of which is produced by several fungal species. The impact of these classes of mycotoxins on human and animal health has been extensively studied (Council for Agricultural Science and Technology 2003).

The diversity of mycotoxin structures induces diverse toxic effects. For example, the aflatoxin structure permits the formation of DNA adducts with guanine, inducing cancerous cell formation (Bren et al. 2007), whereas fumonisins inhibit ceramide synthase (Soriano et al. 2005), inducing an adverse effect on the sphinganin/sphingosin ratio. The deamination of FB_1 induces a loss of toxicity, indicating that amines play a role in fumonisin toxicity. Ochratoxins affect protein synthesis and inhibit ATP production, and its toxicity is associated with its isocoumarin moiety (Xiao et al. 1996). DON and T-2 toxin induce apoptosis in haemopoietic progenitor cells and immune cells (Parent-Massin 2004), and they also inhibit protein, ADN and ARN synthesis (Richard 2007), whereas their toxicity is induced by the epoxy structure (Sundstøl-Eriksen et al. 2004).

Because of the detrimental effects of mycotoxins, some strategies have been developed to prevent the growth of mycotoxigenic fungi and also to decontaminate and/or detoxify foods and feeds (Kabak et al. 2006). These strategies include: 1) the prevention of mycotoxin contamination, 2) the detoxification of mycotoxins present in foods and feeds, and 3) inhibition of mycotoxin absorption in the gastrointestinal tract.

Although numerous physical and chemical detoxification methods have been tested, none really fulfills the necessary efficacy and safety (Mishra and Das 2003), and since cost-effective methods to detoxify mycotoxin-contaminated grains and foods are urgently needed to minimize potential losses to the farmer and toxicological hazards to the consumer (Young et al. 2007), it is a necessity to find new suitable methods for the decontamination of mycotoxins. Therefore, the development of (micro) biological detoxification measures is essential to improve the safety of these foods for human consumption

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(Sweeney and Dobson 1999). Microbes or their enzymes could be applied for mycotoxin detoxification; such biological approaches are now being widely studied (Sweeney and Dobson 1998; Bata and Lasztity 1999). In this review the biological control of several mycotoxins by bacteria, fungi and yeasts will be discussed to develop appropriate managing practices to ensure food safety and consumer's health.

Detoxification of mycotoxins by transformation

Scientists have come to favor the detoxification of mycotoxins by biological transformation, which can be defined as the degradation or enzymatic transformation of mycotoxins to less toxic compounds. A wide range of microorganisms belonging to bacteria, moulds and yeasts have shown a capacity to biotransform mycotoxins. Such microbes act in the intestinal tract of animals prior to resorption of the mycotoxins. Biological transformation reactions include acetylation, glucosylation, ring cleavage, hydrolysis, deamination, and decarboxylation.

Bacillus species The capacity of selected *Bacillus* strains to produce and secrete large quantities (20–25 g L^{-1}) of extracellular enzymes has placed them among the most important industrial enzyme producers (Schallmey et al. 2004). Petchkongkaew et al. (2008) isolated *B. licheniformis* from soybean and found that it removed 92.5 % of OTA after 48 h at 37 °C. Moreover, Tinyiro et al. (2011) found that *B. subtilis* 168 and *B. natto* were more efficient in the removal of zearalenone (ZEN) from liquid medium, and that more than 75 % of ZEN was eliminated after incubation, whereas Cho et al. (2010) reported that *B. subtilis* strain degraded 99 % of ZEN in liquid medium. They added that ZEN decontamination is never complete unless the presence of its oestrogenic analogues such as α-ZEN is ruled out.

On investigating the decontamination ability of various *Bacillus* species (*B. clausii*, *B. subtilis*, *B. lentus*, *B. pumilus*, *B. megaterium*, *B. firmus*) on four genotoxins (4-nitroquinoline-1-oxide, *N*-methyl-*N*-nitro-nitrosoguanidine, 2-amino-3, 4-dimethylimidazo [4, 5-f] quinoline and AFB_1), results suggested that *Bacillus*-based probiotics could be useful for reducing the gastrointestinal risk originating from genotoxic agents (Cenci et al. 2008). The authors added that the inhibition of AFB_1 activity was generally associated with a decrease in optical density, thus indicating that the genotoxicity inhibition may be related to a reduction of bioavailable compounds concentration in the water phase as a consequence of genotoxin absorption by the cell.

Brevibacterium species Brevibacterium species are found in diverse habitats, including soil, poultry, fish, human skin, and food. They differ from other bacteria for their ability to metabolize compounds of heterocyclic and polycyclic ring structures. *Brevibacterium* is widely used in food technology; *B. linens*, *B. casei*, and *B. iodinum* have been isolated from milk and cheese curds, and contribute to the aroma, surface coloration, and the ripening of several types of cheese (Kollöffel et al. 1999; Onraedt et al. 2005). Rodriguez et al. (2011) demonstrated that *Brevibacterium* species (*B. epidermidis* DSM 20660^T, *B. iodinum* DSM 20626^T, *B. linens* DSM 20425^T, *B. casei* DSM 20657^T, *B. casei* DSM 9657, and *B. casei* DSM 20658) are able to totally degrade OTA, even at a concentration as high as 40 mg L^{-1}. Results strongly suggest that *Brevibacterium* species possess an enzyme, possibly a carboxypeptidase, which hydrolyzes the amide bond in the OTA molecule. In agreement, Sørhaug (1981) and Fernández et al. (2000) reported that *B. linens* has highly active and multiple proteolytic enzymes, that are mainly extracellular, and intracellular proteases or peptidases.

Eubacterium species From ruminal fluid, Binder et al. (2000) isolated an anaerobic bacterium belonging to the genus *Eubacterium* BBSH 797, which was able to degrade trichothecenes to de-epoxy metabolites. During its metabolism, BBSH 797 produced specific enzymes that eliminated toxicity of trichothecenes by selective cleavage of their toxic 12,13-epoxy group (Fuchs et al. 2000; 2002). Binder et al. (1997, 1998) reported that DON is also degraded by *Eubacterium* species DSM 11798, which transformed DON into its metabolite DOM-1, the nontoxic, de-epoxide of DON (Fig. 1). The detoxifying strain *Eubacterium* BBSH 797 was the first microbe used in a mycotoxin-deactivating feed additive (Binder et al. 2000). This microbial feed additive has been tested for in vivo efficacy in broilers (Awad et al. 2004) and was shown to be effective in counteracting the toxic effects of DON on intestinal glucose transport. Awad et al. (2006) indicated that supplementation of probiotic bacteria (*Eubacterium*) was beneficial in counteracting the toxicity of DON in commercial broilers at the gut level. The *Eubacterium* sp. strain BBSH 797 has been developed into a commercial product—Mycofix plus (Biomin®)—for detoxifying trichothecenes in animal feed (Schatzmayr et al. 2006).

Flavobacterium aurantiacum The first evidence of the ability of live cells of *F. aurantiacum* to degrade AFB_1 was reported by Ciegler et al. (1966). Lillehoj et al. (1967) reported that an inoculum level of 1.0×10^{11} resting cells per millilitre with 7.0 μg mL^{-1} of AFB_1 during a 4-h period facilitated complete AFB_1 removal from a buffered aqueous medium. They added that the optimum temperature for AFB_1 degradation by *F. aurantiacum* has been reported to be 35 °C, and the optimum pH was reported to be 6.75. Hao and Brackett (1998) stated that *F. aurantiacum* removed AFB_1 from partially defatted peanut milk higher than from phosphate buffer, whereas about 74 % of AFB_1 was removed from partially

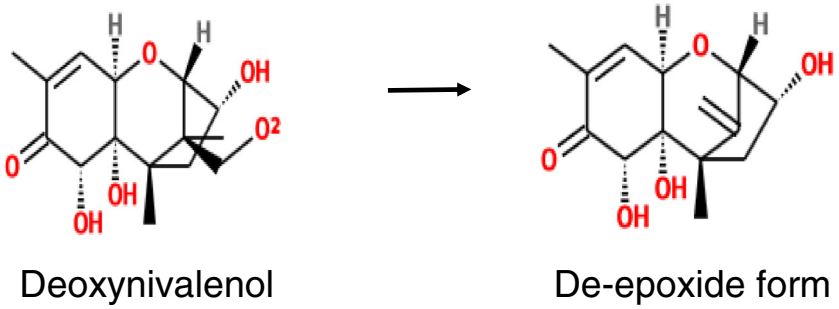

Fig. 1 Biotransformation of deoxynivalenol to de-epoxide form

defatted peanut milk within 24 h. Similarly, Özkaya (2001) observed that *F. aurantiacum* strain NRRL-B-184 removed 79.9–98.9 %, 92.6–99.8 %, and 88.7–100 % AFB_1 from PBS, peanuts and red pepper, respectively, within 48 h.

In a study to determine the exact fate of AFB_1 in the presence of *F. aurantiacum*, Line et al. (1994) determined the amount of radioactivity in both organic and aqueous phases after reaction of AFB_1 with *F. aurantiacum* using ^{14}C-labeled AFB_1, and found that only 24 % of the radioactivity remained after 6 h, whereas controls containing no bacterium retained the entire radioactivity, thus providing the first evidence that AFB_1 was being degraded by the bacterium. In a similar study, Line and Brackett (1995) observed that AFB_1 was partially metabolized and partially adsorbed to *F. aurantiacum* cells. Meanwhile, Smiley and Draughon (2000) demonstrated that the crude protein extracts from *F. aurantiacum* degrades AFB_1 suggesting that the degradation of AFB_1 is linked to a protein that has a characteristic typical of enzymes, and added that the component within the crude protein extract of *F. aurantiacum* is responsible for the degradation of AFB_1.

The role of trace metal ions in microbial AFB_1 degradation was studied by D'Souza and Brackett (1998) who found that incubating *F. aurantiacum* cells at 30 °C with 1 and 10 mM Cu^{2+}, Mn^{2+}, and Zn^{2+} significantly decreased AFB_1 degradation after 4 and 24 h. They added that the addition of 1 mM EDTA countered the inhibition by 1 mM Mn^{2+} after 4 and 24 h, but 1 mM OPT did not counter the inhibition by 10 mM Mn^{2+} after 4 and 24 h. This effect is probably connected to an influence on the enzyme system involved in the degradation process. In another study by D'Souza and Brackett (2000), the divalent cations Ca^{2+} and Mg^{2+} were shown to stimulate AFB_1 degradation by *F. aurantiacum*. In a study undertaken by D'Souza and Brackett (2001) to determine the effects of reducing conditions (L-cysteine) and seryl (phenylmethylsulfonyl fluoride) and sulfhydryl (divalent cadmium) group inhibitors on AFB_1 degradation by *F. aurantiacum*, results indicated that the addition of 0.1, 1, or 10 mM L-cysteine did not have any significant effect on AFB_1 degradation, whereas 1 mM phenylmethylsulfonyl fluoride significantly decreased AFB_1 degradation.

It was suggested that this microorganism might be of potential value in the biological detoxification of foods and feeds. The importance of biological methods of AFB_1 degradation will likely increase if consumer resistance to chemical treatments continues to grow; however, the bright orange pigmentation associated with this bacterium would likely limit its applicability for food and feed fermentations (Bata and Lasztity 1999). Recently, the name and classification of F. aurantiacum was changed, and it is now known as *Nocardia corynebacteroides* (NRRL 24037). Tejada-Castaneda et al. (2008) showed that *N. corynebacteroides* is safe for broilers, and has the ability to reduce the amount of AFB_1 by forming other compounds that have a lower toxicity, such as AFB_2, and by making others, such as AFG_2 and aflatoxicol (AFL), disappear. This may be done by transforming them into other compounds with a different solubility. Cole and Cox (1981) added that the toxicity of AFB_2 is approximately 15 times less than that of AFB_1, and that AFL is 18 times less than AFB_1 for ducklings. Galtier (2003) described that hydroxylated or dealkylated products such as AFM_1, AFP_1, or AFQ_1 are quickly conjugated to glucuronic acid or sulfate and are then excreted with bile or urine in several animal species.

Mycobacterium fluoranthenivorans Actinomyceta, such as *M. fluoranthenivorans* sp. nov. DSM 44556^T isolated from soils of a former coal gas plant that was polluted with polycyclic aromatic hydrocarbons, was found to be capable of degrading AFB_1 as a single carbon source (Hormisch et al. 2004). The AFB_1 concentration was reduced to amounts of 70–80 % of the initial concentration within 36 h, and no AFB_1 was detectable after 72 h. In addition, the cell-free extracts of *M. fluoranthenivorans* sp. nov. DSM 44556^T degraded AFB_1 more efficiently, whereas after 24 h AFB_1 was not detectable (Teniola et al. 2005).

Myxobacteria species Most of the myxobacteria are able to lyse a variety of bacteria and fungi and to obtain nutrients for growth from the products of the lysis (Iizuka et al. 1998). They are a kind of higher prokaryotic organism and are reported to be a rich source of secondary metabolites (Reichenbach and

Dworkin 1992; Reichenbach and Hoefle 1993). To date, more than 80 basic structures and 450 structural variants have been described from microbes, most of which are exclusively produced by myxobacteria (Reichenbach 2001). One of the unique characteristics that myxobacteria possess is the prolific production of extracellular lytic enzymes and antibiotics, many of which are being used as human drugs (Bode et al. 2003; Jansen et al. 2003; Leibold et al. 2004; Kunze et al. 2005). Guan et al. (2010) reported that the bacterial culture of *Myxococcus fulvus* ANSM068 reduced AFB_1 by 80.7 % after co-incubation at 30 °C for 72 h. Meanwhile, the culture supernatant of *M. fulvus* ANSM068 was able to reduce AFB_1 by 76.6 %. However, no significant reduction was observed in the treatments with the cells and cell extracts. This might be due to AFB_1 biotransformation instead of binding through the modification of the lactone ring on the AFB_1 molecule by enzymes (Zhao et al. 2010), thus *M. fulvus* or its metabolites could be potentially applied in the detoxification of AFB_1 in contaminated food and feed.

Zhao et al. (2010) added that culture supernatant of *M. fulvus* was able to degrade AFB_1, AFG_1 and AFM_1 effectively in solution by 71.89, 68.13 and 63.82 %, respectively, after 48 h of incubation. The authors purified the myxobacteria aflatoxin degradation enzyme (MADE), and noticed that an enzyme activity of 50 U mL^{-1} was, respectively, associated with 48.00 % AFG_1 and 46.30 % AFM_1 degradation, whereas enzyme activity of 75 U mL^{-1} coincided with 72.37 % degradation of AFG_1 and 70.71 % degradation of AFM_1.

Pseudomonas species El-Deeb (2005) reported that *Pseudomonas* species of soil bacteria were able to remove ZEN, and they added that the product was not identified, but was assumed to be less toxic than ZEN. Meanwhile, cell-free supernatants of *Pseudomonas* species (*P. putida* DSM 291^T, *P. putida* KT2442) caused a decrease in OTA concentration by 8.45 and 25.70 %, respectively (Rodriguez et al. 2011). An enzyme from *Pseudomonas* species responsible for the transformation into a less toxic product was encoded by a plasmid (Skrinjar et al. 1996).

Rhodococcus erythropolis *Rhodococcus erythropolis* is considered to play a role in the removal of toxic polyaromatic pollutants from the environment (Sakai et al. 2003). Teniola et al. (2005) investigated *R. erythropolis* isolated from polycyclic aromatic hydrocarbon (PAH) soils for AFB_1 degradation activity. Remarkable reduction of AFB_1 was observed during incubation, and it was noticed that up to 70 % of AFB_1 was eliminated within 1 h of applying cell-free extracts, and over 90 % degradation was observed within 4 h. They also succeeded in isolating the extracellular enzyme from *R. erythropolis* responsible for the transformation of AFB_1. Moreover, Alberts et al. (2006) reported that treatment with *R. erythropolis* extracellular extracts led to a 66.8 % reduction of AFB_1 from 0 to 72 h, that the degradation of AFB_1 was enzymatic, and the enzymes responsible are extracellular and constitutively produced.

In agreement, Yamada et al. (1998) stated that various enzymes are produced by *R. erythropolis* and are involved in the catabolic pathways of aromatic compounds such as polychlorinated biphenyls. These enzymes include ring cleavage biphenyl dioxygenases, dihydrodiol dehydrogenases, and hydrolases, and the genes coding for these enzymes are clustered and degradation of AFB_1 occurs via a cascade of reactions, since AFB_1 is a polyaromatic compound and could be degraded in a similar manner.

Rhodosporidium kratochvilovae The fate of PAT after 0, 24, 48, and 72 h of in vitro incubation with the yeast *R. kratochvilovae* LS11 was studied by Castoria et al. (2011), who found that the concentration of PAT progressively decreased, whereas desoxypatulinic acid progressively increased with time, paralleling the decrease of PAT. They added that the lower toxicity of desoxypatulinic acid is proposed to be a consequence of the hydrolysis of the lactone ring and the loss of functional groups that react with thiol groups. The formation of desoxypatulinic acid from PAT represents a novel biodegradation pathway that is also a detoxification process.

Trichosporon mycotoxinivorans Molnar et al. (2004) isolated, characterized and studied the capability of a new yeast strain, *T. mycotoxinivorans*, to degrade OTA in a mineral solution (minimal medium) containing 400 μg L^{-1} of OTA. It was noticed that after 2.5 h, the entire OTA had been deactivated by *T. mycotoxinivorans*, whereas after 48 h just 30 % of OTA was converted into the nontoxic OTα (Fig. 2). The authors developed this microorganism into a commercial product for detoxifying OTA in animal food. *T. mycotoxinivorans* was also able to decarboxylate ZEN (Molnar et al. 2004; Vekiru et al. 2010). The metabolizations of ZEN by *T. mycotoxinivorans* lead to a compound that was no longer estrogenic (Fig. 3) (Schatzmayr et al. 2006).

Aspergillus species A preliminary study was performed to screen 12 black *Aspergillus* strains for their ZEN transformation activity. HPLC analyses showed that ZEN was removed by two *A. niger* strains. ZEN was completely metabolized in 48 h by the mycelium even with high concentrations of ZEN in culture medium (Jard et al. 2009). The ability to degrade OTA was reported by Varga et al. (2000), who found that various *Aspergillus* strains such as *A. fumigatus*, *A. japonicus*, and *A. niger* degraded OTA in liquid YES media, with *A. niger* degrading OTA to OTα and phenylalanine in solid media and liquid cultures, whereas OTα was degraded into an unknown compound. Bejaoui et al. (2006) studied the degradation of OTA by 40 isolates of *Aspergillus* section *Nigri*

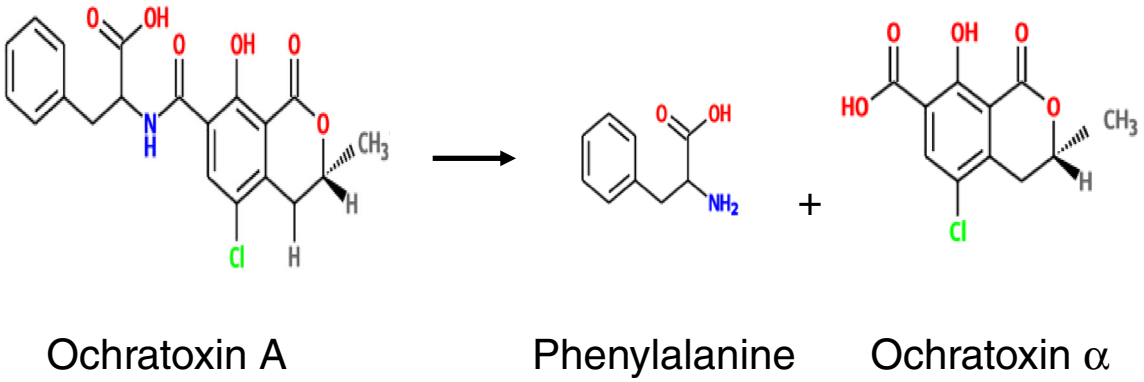

Fig. 2 Detoxification of ochratoxin A by cleavage of phenylalanine moiety

species isolated from French grapes, and reported significant reduction of OTA in liquid medium. Similarly, Abrunhosa et al. (2002) reported that *Aspergillus* spp. isolated from grapes and belonging to *Aspergillus* section *Nigri* degraded OTA.

Rhizopus species *Rhizopus* isolates, such as *R. arrhizus* (Cole et al. 1972), *R. oryzae* (Faraj et al. 1993; Varga et al. 2005), have been reported to degrade AFB_1, whereas several *Rhizopus* species (Cole and Kirksez 1971) have been shown to remove AFG_1. Bol and Smith (1989) studied detoxification of AFB_1 by food-grade *Rhizopus* strains. They observed that 87 % of *Rhizopus* strains tested were positive for AFB_1 defluorescence on incubation at 25 °C. Knol et al. (1990) demonstrated that *R. oryzae* NRRL 395 eliminated AFB_1 from peanut meal, which has been quite effective with major decrease (from 260 to 70 μg kg^{-1}) of AFB_1 content in this raw material. In addition, several *Rhizopus* species (Nout 1989; Faraj et al. 1993) were found to reduce aflatoxin levels. Kusumaningtyas et al. (2006) found that *R. oligosporus*, was able to inhibit synthesis or to degrade AFB_1 when cultured together with AFB_1-producing fungi *A. flavus*.

On studying a total of 55 *Rhizopus* isolates for mycotoxin degradation, a number of *Rhizopus* isolates were found to be able to degrade ZEN, PAT and OTA in a liquid medium below detection limit (Varga et al. 2005), whereas none of the examined isolates could degrade AFB_1. Varga and Toth (2005) revealed that several *Rhizopus* isolates, including *R. stolonifer*, *R. oryzae* and *R. microsporus* strains, completely degraded ZEN. The OTA degradation kinetics of *Rhizopus* isolates and the detection of the degradation product OTα suggested that a carboxypeptidase A activity may be responsible for OTA decomposition in these isolates (Abrunhosa et al. 2002). The metabolite OTα has been evaluated in several studies and was found to be less toxic than OTA (Bruinink et al. 1998).

Other biological materials *Agrobacterium–Rhizobium* strain E3-39, isolated from soil samples, transformed DON into 3-keto-DON under aerobic conditions. The transformation activity of the strain was considerably persistent and the enzyme(s) responsible for the microbial transformation was found in cell culture and cell-free filtrate, but not in cell extract (Shima et al. 1997). Very few studies have been done on the biological degradation of FB_1. Jard et al. (2011) reported that the main microorganism capable of degrading FB_1 is the black yeast, *Exophiala spinifera*. They added that the transformation of FB_1 into AP_1 is performed by an extracellular carboxylesterase. This enzyme has been cloned and was shown to be efficient in transgenic maize, as the plant became resistant to fumonisin (Duvick et al. 2003).

The strain *Nocardia asteroides* was found to reduce AFB_1 by biotransformation to another fluorescent product (Arai et al. 1967), as well as *Corynebacterium rubrum* which was able to detoxify aflatoxin (Shapira 2004). Meanwhile, culture supernatant of *Stenotrophomonas altophilia* showed strong AFB_1 -degrading activity and it was more effective than viable cells and cell extracts, and was able to degrade 78.7 % AFB_1 after 72 h incubation compared to 17.5 % and 9.6 % by viable cells and cell extracts, respectively (Guan et al. 2008).

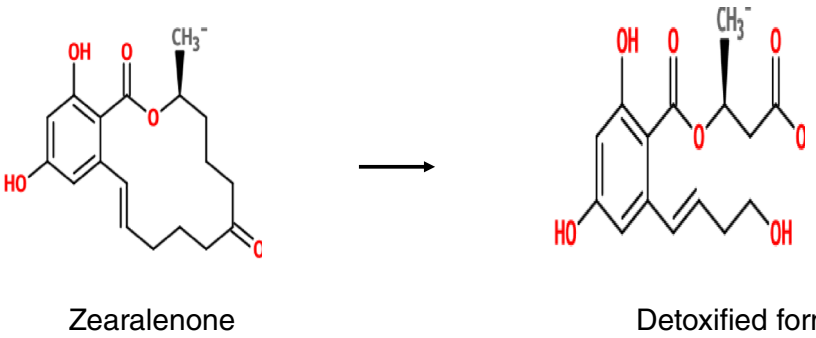

Fig. 3 Biotransformation of zearalenone to less toxic compound

On the other hand, bacteria such as *Acinetobacter calcoaceticus* (Hwang and Draughon 1994) and *Phenylobacterium immobile* (Wegst and Lingens 1983) were found to convert OTA to a much less toxic compound in liquid cultures. El-Sharkawy and Abul-Hajj (1988) reported that *Gliocladium roseum* detoxified ZEN by opening the ring structure with subsequent decarboxylation in yields ranging between 80 and 90 %, where it is known that the 12,13-epoxide ring is responsible for trichothecene toxic activity and that the removal of this epoxide group involves a significant loss of toxicity.

Fungal strains (*Eurotium herbariorum* and non-aflatoxin-producing *A. flavus*) were able to convert AFB_1 to aflatoxicol (AFL) by reducing the cyclopentenone carbonyl of AFB_1. AFB_1 was converted into aflatoxicol-A (AFL-A), which was then converted into aflatoxicol-B (AFL-B) by the actions of medium components or organic acids produced from the fungi (Nakazato et al. 1990).

Trichoderma sp. 639, *Phoma* sp., *Sporotrichum* sp. ADA, *Sporotrichum* sp. SF, and *Alternaria* sp. have been shown to degrade AFB_1 to levels between 65 and 99 % in 5 days at 28± 2 °C (Shantha 1999). On the other hand, fungal isolates, such as *Dactylium dendroides* (Detroy and Hesseltine 1968; 1970), *Trichoderma viride*, *Mucor ambiguus* (Mann and Rehm 1976), *Phoma* sp. (Shantha 1999), and *Trametes versicolor* (Zjalic et al. 2006), have also been reported to degrade AFB_1. Extracellular enzymes from the macroscopic fungus *Pleurotus ostreatus* were shown to be able to cleave the AFB_1 lactone ring leading to a loss of toxicity (Motomura et al. 2003), while *Armillariella tabescens* was able to open the AFB_1.

Decreasing the bioavailability of mycotoxins by adsorption

The most commonly used technique for reducing exposure to mycotoxins is to decrease their bioavailability by the inclusion of various mycotoxin-binding agents or adsorbents, which reduce mycotoxin uptake. An important criterion for the evaluation of mycotoxin adsorbants is their effectiveness at different pH levels (acidic and neutral). Thus, the adsorbant must be efficient throughout the entire gastrointestinal tract, and the mycotoxin–adsorbant complex remains stable to prevent desorption of the toxin during the digestion.

Bifidobacterium species *Bifidobacterium* is a genus of Gram-positive, non-motile, often branched anaerobic bacteria. They are ubiquitous, endosymbiotic inhabitants of the gastrointestinal tracts and mouths of mammals and other animals, and some of these bifidobacteria are used as probiotics. Peltonen et al. (2001) studied the AFB_1 binding ability of five *Bifidobacterium* strains in phosphate buffered saline (PBS), and they noticed that *Bifidobacterium* strains bound 18.0–48.7 % of AFB_1. Recently, Fuchs et al. (2008) investigated the detoxification of two abundant mycotoxins, namely OTA and patulin (PAT), by *Bifidobacterium* and they found that two *Bifidobacterium longum* (LA 02, VM 14) strains were highly effective and caused a decrease of OTA by approximately 50 %, whereas with PAT, the strongest effect (ca. 80 % decrease) was seen with a *B. animalis* (VM 12) strain. Recently, Hateb et al. (2012a) reported that the maximum PAT uptake was achieved by *B. bifidum* 671 by (52.9 %) for viable and (54.1 %) for nonviable cells after 24-h incubation.

On studying the ability of two dairy strains of *B. bifidum* to remove AFM_1 from PBS and reconstituted milk, results revealed that viable (10^8 CFU mL^{-1}) and heat-killed *Bifidobacterium* ranged from 14.04 to 28.07 %, and from 12.85 to 27.31 % in PBS and reconstituted milk, respectively (Kabak and Var 2008). Previous studies also revealed that the binding abilities of AFM_1 by *B. longum* and *B. bifidum* reached 26.7 and 32.5 %, respectively (Kabak and Var 2004).

On the other hand, Lankaputhra and Shah (1998) studied the antimutagenic activity of live and killed cells of nine strains of Bifidobacteria on eight chemical mutagens and promutagens (*N*-methyl, *N*′-nitro, *N*-nitrosoguanidine; 2-nitroflourene; 4-nitro-*O*-phenylenediamine; 4-nitroquinoline-*N*-oxide; AFB_1; 2-amino-3-methyl-3H-imidazoquinoline; 2-amino-1-methyl-6-phenyl-imidazo (4, 5-b) pyridine, and 2-amino-3-methyl-9H-pyrido (3, 3–6) indole). Authors reported that all six strains of Bifidobacteria inhibited AFB_1 poorly.

Lactic acid bacteria

Aflatoxins

Specific dairy strains of lactic acid bacteria (LAB) have been shown to remove AFB_1, the most common aflatoxin, effectively from liquid solutions (El-Nezami et al. 1996, 1998a). Several studies were done regarding removal of aflatoxin by adhesion to probiotic bacteria (Peltonen et al. 2000) as well as bio-competitive inhibition of aflatoxin production by geocarpospheric bacteria (Chaurasia 1995). El-Nezami et al. (1998b) evaluated the ability of five *Lactobacillus* species to bind aflatoxins in vitro, and have shown that probiotic strains such as *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC-705 were very effective for removing AFB_1, with more than 80 % of the toxin trapped in a 20 μg mL^{-1} solution. It was assumed that AFB_2, AFG_1, and AFG_2 were less sensitive to this binding process (El-Nezami et al. 2002b). Peltonen et al. (2000) added that the removal of AFB_1 from buffered solution by probiotic bacteria ranged from 5.8 to 31.3 %. Peltonen et al. (2001) investigated the AFB_1 binding ability of 12 *Lactobacillus* and three *Lactococcus* strains in PBS. In their study, *Lactobacillus* strains bound 17.3–59.7 % AFB_1, and *Lactococcus* strains bound 5.6–41.1 % AFB_1. Khanafari et al. (2007) studied the efficacy of *Lactobacillus*

plantarum (PTCC 1058) to bind AFB_1, and reported that 45 % of AFB_1 was removed from solution after 1 h, and that the autoclaved bacteria did not remove AFB_1 efficiently (31 % in 1 h). The authors added that probiotic bacteria retained 92 % of AFB_1 added after three washes. In vitro binding of AFB_1 by LAB was described as a fast (no more than 1 min) and reversible process (Bueno et al. 2006) that is strain- and dose-dependent (Kankaanpää et al. 2000).

Hernandez-Mendoza et al. (2009) screened eight strains of *Lactobacillus casei* for their ability to bind AFB_1, and the percentage bound ranged from 14 to 49 %. Previous investigations into levels of AFB_1 binding by *Lactobacillus casei* have reported values ranging 0.6 to 46 % (Bolognani et al. 1997; El-Nezami et al. 1998a; Peltonen et al. 2000, 2001; Haskard et al. 2001; Lahtinen et al. 2004; Hwang et al. 2005; Zinedine et al. 2005). Hernandez-Mendoza et al. (2010) suggested that the presence of *Lactobacillus casei* Shirota can decrease aflatoxin absorption at the intestinal level even after a long period of toxin exposure, which consequently circumvents its toxic effects. They added that *Lactobacillus casei* Shirota had the ability to bind AFB_1 into the bacterial cell envelope, and that the images also revealed that aflatoxin binding produced structural changes that modified the bacterial cell surface.

Halttunen et al. (2008) examined the ability of a combination of LAB strains to remove mycotoxins, and revealed that the toxin-removing capacity of a combination of strains of LAB is not the sum of their individual capacities. Thus suggesting that pure, single strains should be used when the goal is to remove single compounds, and that the use of combinations of strains may be beneficial when several compounds are removed together.

Lankaputhra and Shah (1998) studied the antimutagenic activity of live and killed cells of six strains of *Lactobacillus acidophilus* on eight chemical mutagens and promutagens (*N*-methyl, *N*′-nitro, *N*-nitrosoguanidine; 2-nitroflourene; 4-nitro-*O*-phenylenediamine; 4-nitroquinoline-*N*-oxide; AFB_1; 2-amino-3-methyl-3H-imidazoquinoline; 2-amino-1-methyl-6-phenyl-imidazo [4, 5-b] pyridine, and 2-amino-3-methyl-9H-pyrido [3, 3–6] indole). The authors reported that all *Lactobacillus acidophilus* strains, except *Lactobacillus acidophilus* 2415, inhibited AFB_1 at high concentration (>50 %). The mechanism of antimutagenic activities of fermented dairy products or probiotic bacteria has not been clearly understood (Nadathur et al. 1994). Binding of mutagens to microbial cells has been suggested to be a possible mechanism of antimutagenicity (Orrhage et al. 1994). Recently, Cenci et al. (2008) investigated the effect of *Lactobacillus rhamnosus* GG against genotoxins (4-nitroquinoline-1-oxide, *N*-methyl-*N*-nitro-nitrosoguanidine, 2-amino-3, 4-dimethylimidazo [4, 5-f] quinoline and AFB_1), and revealed high antigenotoxicity against AFB_1 (80.8 %).

The binding abilities of AFM_1 by viable *Lactobacillus* strains at 10^8 CFU mL^{-1} in PBS ranged from 10.22 to 26.55 % depending on the contamination level and incubation period (Kabak and Var 2008). They added that the percentage of AFM_1 removal by heat-killed bacteria from PBS ranged from 14.04 to 28.97 %. Similarly, Kabak and Var (2004) found that the binding abilities of AFM_1, by *Lactobacillus* strains in PBS and reconstituted milk, ranged from 25.7 to 30.5 %. These results were considered to be lower than those reported by Pierides et al. (2000), who found that the AFM_1 binding abilities of viable *Lactobacillus* strains within 15–16 h ranged from 18.1 to 50.7 %.

Ocharatoxin A

Degradation of OTA was observed in milk due to the action of *Lactobacillus* and *Streptococcus* species (Skrinjar et al. 1996). Turbic et al. (2002) showed that OTA (36–76 %) were removed by *Lactobacillus rhamnosus* strains in high and moderate amounts. Meanwhile, Heidler and Schatzmayr (2003) clearly demonstrated that bacteria *Lactobacillus vitulinus* isolated from rumen fluid was able to cleave OTA into the non-toxic metabolite ochratoxin α (OTα) and the amino acid phenylalanine. Similar studies demonstrated that *Lactobacillus* strains were able to eliminate 0.05 mg L^{-1} OTA added to culture medium-in particular, *Lactobacillus bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus acidophilus*, eliminated up to 94, 72, and 46 %, respectively, of OTA (Böhm et al. 2000).

Del Prete et al. (2007) studied the in vitro interaction between OTA and wine LAB (*Lactobacillus, Pediococcus*, and *Oenococcus oeni* species). Results showed a decrease in OTA concentration caused by several wine LAB strains grown in liquid synthetic culture media. The decrease in OTA concentration ranged from 8.23 to 28.09 %. It was also noticed that commercial oenococcal strains were more effective than other wine LAB analyzed, with an OTA reduction ranging from 17.35 to 28.09 %.

In a study on the elimination of OTA by LAB strains of intestinal and plant origin, Piotrowska and Zakowska (2005) screened 29 strains of LAB belonging to *Lactobacillus* and *Lactococcus* genera for their sensitivity to OTA and their ability to remove this toxin from liquid media. They added that the greatest adsorptions, more than 50 % of the initial OTA content, were obtained with *Lactobacillus acidophilus* CH-5, *Lactobacillus rhamnosus* GG, *Lactobacillus plantarum* BS, *Lactobacillus brevis* and *Lactobacillus sanfranciscensis*. Piotrowska and Zakowska (2000) concluded that elimination of the toxin occurs through binding to the bacterial biomass, and also suggested that an unknown mechanism, in addition to binding to the biomass, is involved.

Fuchs et al. (2008) investigated the detoxification of OTA by LAB. They found that the strongest decline of OTA (97 %) was detected with a *Lactobacillus acidophilus*. In this context it is notable that OTA is detoxified by representatives of the

ruminal microflora via cleavage of the peptide bond which leads to the release of phenylalanine (Hult et al. 1976; Özpinar et al. 1999).

Patulin

Fuchs et al. (2008) studied the effect of LAB on patulin (PAT), and noticed that the strongest decline of PAT (39 %) was detected by *Lactobacillus plantarum*. Hateb et al. (2012a) reported that the maximum PAT uptake from aqueous solution was achieved by *Lactobacillus rhamnosus* 6149 strains (51.1 %) for viable and (52.0 %) for nonviable cells after 24-h incubation. The highest removal of PAT was at pH 4.0 and 37 °C and increased with decreasing of toxin levels. On investigating the removal of PAT contamination from apple juice using 10 different inactivated LAB, results revealed that *Lactobacillus rhamnosus* caused a decrease of PAT by 80.4 % (Hateb et al. 2012b).

Fusarium toxins

It is known that certain LAB strains are able to detoxify other mycotoxins such as fusarium toxins (DON, NIV, T-2 toxin, HT-2 toxin) (El-Nezami et al. 2002a; Niderkorn et al. 2006) and ZEN (El-Nezami et al. 2002c, 2004). Niderkorn et al. (2006) revealed that FB_2 was removed more efficiently than FB_1 in spite of their similarity in chemical structure. They concluded that binding, and not biodegradation, is the mode of action of removal of *Fusarium* mycotoxins by fermentative bacteria. Thus, questions about the toxicity of products originated from enzymatic degradation are not relevant for this process. According to the former authors, the strength of the mycotoxin-LAB interaction was influenced by the peptidoglycan structure and, more precisely, by its amino acid composition (Niderkorn et al. 2009).

The ability of *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC-705 to remove ZEN, and its derivative α-zearalenol, from a liquid medium was investigated by El-Nezami et al. (2002c), who noticed that a significant proportion of both toxins (38 and 46 %) were trapped by the bacterial pellet, and that no degradation products of either ZEN or α-zearalenol were observed after 3 days of incubation. The authors added that both heat-treated and acid-treated bacteria were able to remove ZEN and α-zearalenol, suggesting that binding, not metabolism, was the mechanism by which both toxins were removed from the media. The process was fast and depended on the bacterial cells and toxin concentrations.

Niderkorn et al. (2007) reported that eight *Lactobacillus* and three *Leuconstoc* bio-transformed ZEN into α-zearalenol, but DON and fumonisins were not bio-transformed. They added that this cannot be considered detoxification since α-zearalenol is three to four times more estrogenic than ZEN (Mirocha et al. 1979). The biotransformation of ZEN to α-zearalenol might explain the results of a study by Mokoena et al. (2005), who observed a significant decrease in the concentration of ZEN during the fermentation of corn meal by LAB but without a reduction in toxicity. It was suggested that ZEN predominantly binds to carbohydrate moieties of the cell wall of LAB by means of hydrophobic interactions (El-Nezami et al. 2004). As hydrophobic interactions are relatively weak, this bacterium-mycotoxin complex could be unstable. Niderkorn et al. (2007) also suggested that ZEN binding is not limited to hydrophobic links and that other types of interactions could be important.

Propionobacterium species Niderkorn et al. (2006) were the first to examine in vitro interactions between *Propionibacterium* species and fumonisins. The ability of three strains of *Propionibacterium* to remove FB_1 and FB_2 from acidified MRS broth samples (pH 4.0) was evaluated, and demonstrated that FB_1 was not as effectively removed as FB_2. Most of the strains were able to remove both toxins, but considerable differences were observed among these strains. The authors added that binding efficiency was affected by the pH, since at pH 7, LAB was unable to trap FB_1 and FB_2. Niderkorn (2007) suggested that peptidoglycans were the most plausible fumonisin binding sites.

Mechanism of mycotoxin in vitro removal

In order to investigate the mechanisms which account for the removal of mycotoxins by LAB, the effects of viable and heat-inactivated bacteria were compared in a number of studies (Haskard et al. 2001; El-Nezami et al. 1998b, 2002a). Additionally, the bacteria were treated with enzymes (such as pronase E and lipase) or periodate which caused alterations of the structure of the cell walls (Haskard et al. 2000; El-Nezami et al. 2004; Lahtinen et al. 2004). However, since a decrease of their toxic effects was also seen with cytosolic preparations of LAB, it was hypothesized that other mechanisms (e.g. interactions with short chain fatty acids) may also play a role (Knasmuller et al. 2001; Stidl et al. 2007; Stidl et al. 2008).

In LAB, the cell wall consists of (1) a peptidoglycan matrix, which forms the major structural component of cell walls housing other components such as teichoic and lipoteichoic acid, (2) a proteinaceous S layer, and (3) neutral polysaccharides (Delcour et al. 1999). These components have various functions including adhesion and macromolecular binding. The results of various experiments suggest the involvement of both peptidoglycan and polysaccharides in toxin binding (Zhang and Ohta 1991; Peltonen et al. 2001; Haskard et al. 2001; Lahtinen et al. 2004). Haskard et al. (2000) studied the mechanism of binding between AFs and *Lactobacillus rhamnosus* and found that binding occurs

predominantly with the carbohydrate and to some extend the protein components in the cell wall. These results were based on the inhibitory effects of periodate and pronase E on AFB_1 binding by *Lactobacillus rhamnosus*. Periodate and pronase E can be used to degrade relatively non-specifically both carbohydrates and proteins, respectively. They also described the major role of hydrophobic interactions in binding and found that electrostatic interactions play only a minor role.

Additionally, it has been suggested that AFB_1 is bound to bacteria by weak, non-covalent interactions, such as association with hydrophobic pockets on the bacterial surface (Peltonen et al. 2001). However, it is likely that multiple components are involved in AFB_1 binding (Turbic et al. 2002) and that this interaction can be affected by environmental conditions.

In vivo removal of mycotoxins

Several probiotic bacteria have been shown to bind AFB_1 efficiently in vitro (Lee et al. 2003; Shahin 2007), but ex vivo results are controversial (El-Nezami et al. 2000). It has been suggested that these differences may be caused by intestinal conditions, such as pH (Haskard et al. 2000, 2001) and intestinal mucus. Gratz et al. (2004) reported that pre-incubation of two probiotic preparations (*Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC-705 plus *Propionibacterium freudenreichii* subsp. *shermanii* JS (LC-705+JS) with either AFB_1 or mucus reduced the subsequent surface binding of mucus and AFB_1, respectively, suggesting that the probiotic mixture is less capable of binding AFB_1 in the presence of mucus and is more susceptible to interfering factors in the intestinal tract, which may explain poorer results in AFB_1 binding in animals (Gratz et al. 2004).

In 2005, Gratz et al. investigated the ability of the probiotic mixtures (*Lactobacillus rhamnosus* LC-705 plus *P. freudenreichii* subsp. *shermanii* JS) to bind AFB_1 ex vivo. Results revealed that the probiotic mixture decreased AFB_1 levels in duodenal tissue by 25 %. On the other hand, Tuomola et al. (2000) showed that heat treatment disturbs mucus adhesion by *Lactobacillus rhamnosus* GG and LC-705 and concluded that proteins must be involved in the binding of mucus, whereas carbohydrates are known to bind AFB_1 (Haskard et al. 2000).

There was also evidence that LAB possessing the most efficient AFB_1-removing capacities could reduce AFB_1 absorption from the gastrointestinal tracts of chickens (El-Nezami et al. 2000). According to El-Nezami et al. (2006), the administration of a combination of two probiotic strains reduced the absorption of AFB_1 in young Chinese men.

Hernandez-Mendoza et al. (2011) assessed the ability of *Lactobacillus reuteri* to bind AFB_1 in the intestinal tract and established that the strain was able to bind AFB_1 in the intestinal tract, mostly in the duodenum, and that the AFB_1-lysine adducts were present in significantly lower levels in animals receiving AFB_1 plus bacteria than in those receiving only AFB_1, thus demonstrating the ability of the bacteria to act as a biological barrier in normal intestinal conditions, and thereby reducing the bioavailability of AFB_1 ingested orally. Hathout et al. (2011) evaluated the protective role of *Lactobacillus casei* and *Lactobacillus reuteri* against AF-induced oxidative stress in rats, and reported that treatments with the bacteria succeeded to induce a significant improvement in all the biochemical parameters and histological picture of the liver.

Yeast and yeast extract *Saccharomyces cerevisiae* has been utilized in food fermentation for several centuries. In African and Asian countries, fermentation is used as a predominant mode of food processing and preservation. Many yeast species, especially *S. cerevisiae*, play a predominant role in food fermentation (Jespersen 2003). *Saccharomyces cerevisiae* is one of the most widely commercialized species, rich in protein (40–45 %), and vitamin B complex (Çel k et al. 2003). Shetty et al. (2007) reported that *S. cerevisiae* cells were capable of binding high amounts of AFB_1 even at the highest concentration tested (20 $\mu g\ mL^{-1}$), and added that the ability of *S. cerevisiae* to bind AFB_1 was strain-specific with seven strains binding 10–20 %, eight strains binding 20–40 % and three strains binding more than 40 % of the added AFB_1. Raju and Devegowda (2000) attributed the aflatoxin-binding by yeast cell walls to mannan oligosaccharides, which is a product designed to influence microbial ecology, is derived from yeast cell walls, and consists primarily of phosphorylated glucomannans.

Bejaoui et al. (2004) stated that *Saccharomyces* heat-treated cells showed higher adsorption of OTA (90 %) than viable cells (35 %), indicating the physical nature of binding, whereas cell density played an important role in adsorption efficiency. It was indicated that the oenological strains of *Saccharomyces* yeasts can be used for the decontamination of OTA in synthetic and natural grape juice. On the other hand, strains of *S. cerevisiae* have also been shown to bind ZEN (Yiannikouris et al. 2004a, c), and the binding has been attributed to glucan components in both cases.

Esterified glucomannan polymer, extracted from the yeast cell wall, was shown to bind with AFB_1, OTA (Bejaoui et al. 2004; Cecchini et al. 2006; Angioni et al. 2007) and T-2 toxin (Freimund et al. 2003), individually and in combination (Raju and Devegowda 2000; Aravind et al. 2003; Karaman et al. 2005). Yiannikouris et al. (2004a, b, c, d) demonstrated the mechanism of binding yeast-modified glucan with ZEN, and indicated that those non-covalent bonds are involved in the interactions between β-D-glucans and ZEN, making them more of "adsorption type" than of "binding type". Freimund et al. (2003) made some speculations on the nature of the complexes formed between yeast cell walls and certain mycotoxins, suggesting that possible hydrophobic interactions could occur between ZEN and yeast cell wall components.

In contrast to previous studies, several authors reported that mycotoxins were transformed to less-toxic compounds by yeast strains. Böswald et al. (1995) reported that ZEN was reduced stereoselectively by cultures of seven *Saccharomyces* strains to both ά- ZOL and β-ZOL, meanwhile trichothecene mycotoxin DON was not metabolized by any of the yeast strains that were used for analysis.

Recently, Moss and Long (2002) reported that commercial yeast *S. cerevisiae* transformed PAT into ascladiol. The acute toxicity of ascladiol amounted to only one-fourth of the strength of PAT (Moss 1998). Coelho et al. (2008) evaluated the effectiveness of *S. cerevisiae*, in the biodegradation of PAT in vitro and results revealed that PAT levels were reduced by over 90 % after incubation at 25 °C under static conditions.

Industrial applications of biological detoxifying microorganisms

Application in food

There are microorganisms that can be applied in the fermentation of different food chains such as grains, apple juice, beer and wine. Fermentation is one of the oldest forms of food processing and preservation in the world going back as much as 7,000 years in Babylon (Battcock and Azam-Ali 1998). The use of yeast and LAB in fermentation is an ancient method that nowadays could be considered as an organic or natural procedure for food and feed elaboration. Detoxification of mycotoxins in maize through LAB fermentation has been demonstrated over the years (Mokoena et al. 2005; Mokoena et al. 2006). Using LAB fermentation for detoxification is more advantageous because it is a milder method which preserves the nutritive value and flavor of decontaminated food. In addition, LAB fermentation irreversibly degrades mycotoxins without leaving any toxic residues. The detoxifying effect is believed to be through a toxin binding effect (El-Nezami et al. 2002c). Other authors allude to the possibility of an enzymatic interaction, although this was not thoroughly investigated (Zinedine et al. 2005). Similarly, Oluwafemi et al. (2010) investigated the ability of LAB to reduce AFB_1 contamination in maize grains inoculated with an aflatoxigenic strain of *A. flavus*, and the AFB_1 produced by *A. flavus* was reduced by the different *Lactobacillus* species to varying degrees. Despite the relatively high abundance of LAB in food, only a low percentage of these bacteria had binding properties for mycotoxins, which could be considered a characteristic for the selection of LAB used as starter cultures in food and feed instead of chemical preservatives to enable the food industry to produce organic food.

On the other hand, Stinton et al. (1979) used yeast for the fermentation of apple juice contaminated with PAT and observed a rapid decrease of mycotoxin content during alcoholic fermentation. However, they were not able to characterize chemically the products of degradation. Meanwhile, Bennett and Richard (1996) revealed that 49 % of ZEN was destroyed, while 69 % was converted to a less toxic form, β-zearalenol, during traditional beer fermentation by *S. cerevisieae*.

During wine making, remarkable differences among wine yeasts, both *Saccharomyces* (Caridi et al. 2006a, b) and non-*Saccharomyces* (Cecchini et al. 2006), have been reported in OTA sequestering activity. The ability of wine yeast (*Saccharomyces* sensu stricto) to remove OTA from synthetic medium was evaluated by Caridi et al. (2006a) who reported that seven out of 20 yeast strains showed high levels of OTA removal ranging from 66 to 100 %, whereas the unitary removing activity ranged from 14.31 to 27.24 pg mg^{-1} of biomass. Caridi et al. (2006b) demonstrated that expressly selected wine yeast was used like a sponge to reduce the OTA during wine making. Var et al. (2009) described the abilities of 21 yeast strains isolated from six different wine-grapes to bind OTA in white wine. Ringot et al. (2005) reported that yeast mannoproteins play an important role in OTA removal from contaminated grape must. Recently, Caridi et al. (2012) explored the OTA adsorption phenotypes of the parental strains and their progeny, and results revealed that OTA adsorption is genetically controlled and is a polygenic inheritable trait of wine yeast. In the same trend, Del Prete et al. (2007) reported that OTA could be removed by wine LAB in a cell-binding phenomenon. Strains of yeasts and LAB with major OTA-binding abilities could be used as starter cultures for alcoholic and malolactic fermentation, respectively, to obtain wines containing lower concentrations of OTA than those found in the initial grape must, as recommended by the International Organisation of Vine and Wine.

On the other hand, the safe use of *A. niger* in the food industry comes primarily from its use for the production of many enzymes such as amylase, amyloglucosidase, cellulases, lactase, invertase, pectinases, and acid proteases (Ward 1989). In addition to the annual production of citric acid by fermentation using *A. niger*, this procedure is carried out commercially in both surface culture and in submerged processes (Kubicek and Rohr 1985; Ward 1989). Since ancient times, enzymes found in nature have been used in the production of food products such as cheese, beer, wine and vinegar (Kirk et al. 2002).

Application in animal feed

Microorganisms, able to detoxify mycotoxins and that can be used as feed additives, are pursued by industry. A proof of concept for the decontamination of grain containing trichothecenes was provided long before pure cultures of bacteria that de-epoxidize trichothecenes became available. He et al.

(1993) reported that more than 50 % of the DON in moldy maize was degraded when the maize was incubated with chicken intestinal contents under anaerobic conditions. Since 1998, a new detoxifying strain, *Eubacterium* BBSH 797, has been incorporated into the product in some countries (Biomin 2011). The strain was able to de-epoxidize DON in dissected pig intestine under anaerobic conditions (Schatzmayr et al. 2006). *Eubacterium* sp. strain BBSH 797 has been developed into a commercial product, Mycofix by BIOMIN GmbH, which is a commercial formulation designed for poultry and swine diets in which the encapsulated bacterium is used. The results of animal trials showed that Mycofix products significantly reduced the adverse effects of DON on dairy cows, and T-2 toxin on growing broilers. The positive effect of Mycofix on dairy cows has shown the enhanced activity of rumen flora (Hochsteiner et al. 2000). Ingested with feed, the strain should unfold its activity in the digestive systems of animals. Because it is a strict anaerobe, formulation securing the survival of the strain during storage and transportation of the product is a prerequisite for the success of the strategy. Karlovsky (2011) reported that other microorganisms (*Curtobacterium* sp. 114–2, *Pseudotaphrina kochii*, *Agrobacterium-Rhizobium* E3-39, *Nocardioides* sp. WSN05-2, etc.) that can detoxify DON can be used also as feed additives, and the genes encoding detoxification activities can be used in the genetic engineering of crops.

Conclusion

The field of mycotoxin detoxification by microorganisms (bacteria, yeast and fungi) is very promising, and most of the research has been focused on the biological control of mycotoxins in simple systems. More attention should be dedicated to complete evaluation of the practical use of these organisms or their enzymes in food and feeds. It is also very important to assess the potential of specific strains of LAB and yeast for binding and/or removal of mycotoxins as well as the factors that affect the stability of the toxin sequestration. The present reviews special microorganisms which are able to biochemically degrade mycotoxins and convert them into physiologically safe substances for the food and animal feed industries.

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