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REVIEW

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Detoxification approaches of mycotoxins: by microorganisms, biofilms and enzymes

Sahar Nahle[1,2,3], André El Khoury[3], Ioannis Savvaidis[4,5], Ali Chokr[1,2], Nicolas Louka[3] and Ali Atoui[1*]

Abstract

Mycotoxins are generally found in food, feed, dairy products, and beverages, subsequently presenting serious human and animal health problems. Not surprisingly, mycotoxin contamination has been a worldwide concern for many research studies. In this regard, many biological, chemical, and physical approaches were investigated to reduce and/or remove contamination from food and feed products. Biological detoxification processes seem to be the most promising approaches for mycotoxins removal from food. The current review details the newest progress in biological detoxification (adsorption and metabolization) through microorganisms, their biofilms, and enzymatic degradation, finally describing the detoxification mechanism of many mycotoxins by some microorganisms. This review also reports the possible usage of microorganisms as mycotoxins' binders in various food commodities, which may help produce mycotoxins-free food and feed.

Keywords: Detoxification, Mycotoxins, Microorganisms, Biofilm, Enzymatic degradation

Introduction

Mycotoxins are secondary toxic metabolites produced mainly by some fungal species belonging to *Aspergillus*, *Penicillium*, and *Fusarium* genera (Greeff-Laubscher et al. 2020). Mycotoxin production can occur either in the pre-harvest stage or in the post-harvest and storage ones under favorable environmental conditions (Waliyar et al. 2014). The most important conditions for fungal growth and mycotoxin production are temperature and water activity (Peraica et al. 1999; Darwish et al. 2014). More than 400 different types of mycotoxins have been identified with different levels of toxicity. Among all mycotoxins, aflatoxins (AFs), Ochratoxin A (OTA), patulin (PAT), Zearalenone (ZEN), and Trichothecenes (TCT) have received particular attention due to their severe health outcomes on both humans and animals that can range from acute to severe and chronic intoxications in both humans and animals (Milićević et al. 2010; Cwalina-Ambroziak et al. 2017; Vargas et al. 2001; El Khoury and Atoui 2010; El Khoury et al. 2011; Battilani et al. 2016; Nahle et al. 2020). Exposure to mycotoxins can occur directly through the ingestion of contaminated food or indirectly through bi-products of animals consuming contaminated feed (Bullerman 1979). Each year mycotoxin contamination causes severe losses worldwide at the level of humans, animals, agriculture, and industries (WHO 2016). Therefore, with such negative impacts, regulatory guidelines and limits for mycotoxin in foods and feed have been set by various countries to control contamination levels in the food markets. Moreover, researchers have been working on establishing several other ways to control mycotoxins in food and feed.

Over the last years, physical, chemical, and biological detoxification processes have been developed and they intended to mitigate mycotoxins in food and feed through destroying, modifying, or adsorbing them (El-Nezami et al. 1998; Karlovsky 1999; Scudamore 2005; Varga and Tóth 2005; Leong et al. 2006; Shetty et al. 2007; Dao and Dantigny 2011; El Khoury et al. 2011; Karlovsky et al. 2016; Assaf et al. 2017; Muhialdin et al. 2020). Mainly,

*Correspondence: aatoui@ul.edu.lb
[1] Research Laboratory of Microbiology (RLM), Department of Life and Earth Sciences, Faculty of Sciences I, Lebanese University, Hadat Campus, Beirut, Lebanon
Full list of author information is available at the end of the article

Physical methods comprise quick-drying, UV treatment, and floating which help reduce mycotoxins during post-harvest applications (Brandt and Klebaum 2009). Moreover, various organic, inorganic, or mineral mycotoxins' binders were studied for their ability to adsorb mycotoxins, (Fandohan et al. 2005; Scudamore et al. 2007; Khatibi et al. 2014; Assaf et al. 2017; Assaf et al. 2018a, b, c; Assaf et al. 2019a; Assaf et al. 2019b). Although they have been shown to reduce their bioavailability, however, they still cannot adsorb them completely. Moreover, their application as a detoxification method showed many drawbacks such as limited implementation, insignificant efficacy, and low potential as a detoxification approach when applied to foods (Assaf et al. 2019a). On the other hand, chemical processes, including ammoniation, ozonation, peroxidation, and others have been reported to destroy mycotoxins from foodstuffs (Norred et al. 1991) while however, failing to fulfill the criteria of a successful detoxification process due to the negative outcomes on food nutritional value, efficacy, and safety. Additionally, chemical methods are expensive and require complicated specifications to accomplish the detoxification process (Karlovsky 1999; Li et al. 2020). Thus, both physical and chemical methods are not considered as adequately effective in removing mycotoxins from food and feed.

Therefore, this issue has directed researchers to find alternative mycotoxin detoxification methods that would rather be highly efficient and safe. Recently, many studies have focused on the usage of some microorganisms including lactic acid bacteria (LAB), yeast, and fungi to remove mycotoxins from food (Abrunhosa et al. 2002; Assaf et al. 2018a, b, c; Assaf et al. 2019a; El-Nezami et al. 2004a, b, El-Nezami et al. 1998). In addition, the use of microorganisms' enzymes and their metabolites also showed efficiency in the mycotoxin degradation processes (Karlovsky 1999; Li et al. 2020). The biological methods used in the removal and degradation of mycotoxins are attractive and environmentally friendly and therefore may offer better substitutes to the chemical and physical methods (Assaf et al. 2018a, b, c; Assaf et al. 2019a).

This review aims to discuss the biological decontamination of mycotoxins and to review the mechanisms of detoxification by yeasts and bacteria, in addition to bacterial biofilms and their enzymes. Moreover, this review presents the contribution of biological detoxification methods to food safety and consumers' health.

Biological detoxification of mycotoxins using microorganisms

The biological detoxification of mycotoxins is defined as the usage of microorganisms, as well as their microbial enzymes and metabolites for mycotoxins binding and potential degradation (Muhialdin et al. 2020). The microorganisms implicated in biological degradation should follow certain standards such as being safe, non-pathogenic, possess mycotoxins degrading ability, pertain activity during packing, do not form improper odors or taste, and preserve the nutrient value of food (Varga and Tóth 2005). The benefits of the biological detoxification process include its easiness, cost-effectiveness, applicability over broad range of target mycotoxins, efficacy in a wide range of fluid and foodstuffs, and its insignificant effects against nutrients naturally found in food (Varga and Tóth 2005; Muhialdin et al. 2020).

Biological and organic binders

Various biological assays using either biological binders (bacteria, biofilm, and yeast) or organic binders (chitin, shrimp shells) have been established to remove different mycotoxins from laboratory liquid media or commercial beverages by adsorption mechanism (Hatab et al. 2012; Taheur et al. 2017; Chlebicz and Śliżewska 2020). Briefly, adsorption is an interaction mechanism between a special structure on the surface of the binder and the mycotoxin through non-covalent bonds such as Van der Waals interactions, that reduces the bioavailability of the mycotoxin found in the intended food commodity. On the other hand, biosorption is the use of biological binders for the detoxification process (Kolosova and Stroka 2011). The biosorption pathway is quick and direct in sequestering mycotoxins as compared to the biodegradation pathway. However, the toxins might be easily released back and this depends on the stability of the complex formed between the bacterial surface and the toxins (Solis-Cruz et al. 2019).

Bacteria

Many studies have reported that bacteria are useful biological agents for mycotoxin detoxification. Bacteria can remove mycotoxin by biosorption or biodegradation mechanisms. A summary of the literature reporting bacterial strains for mycotoxins detoxification in various media is presented in Table 1. The removal percentage of many mycotoxins involving various bacterial strains reached a high efficiency of up to 94%.

It was demonstrated that the use of Generally Recognized as Safe (GRAS) probiotic bacteria such as LAB is very promising in mycotoxins detoxification (Abdelmotilib et al. 2018; El-Nezami et al. 1998; Fuchs et al. 2008; Assaf et al. 2019a; Assaf et al. 2018a, b, c; Ben Taheur et al. 2019; Wang et al. 2018a, b). Haskard et al. (2001) revealed that several LAB has been established to detoxify AFB_1 which is the most potent human carcinogen. Haskard and his team have assessed the ability of five *Lactobacillus* strains: *L. rhamnosus* GG, *L. rhamnosus*

Table 1 Mycotoxins removal percentage of different bacterial strains

Strain	Mycotoxins	Sample	Removal (%)	References
Lactobacillus acidophilus VM20	OTA	Liquid medium	97	(Fuchs et al. 2008)
Lactobacillus bulgaricus	OTA	PBS	94	(Varga et al. 2005)
Lactobacillus plantarum	PAT	Apple juice	91.2	(Zoghi et al. 2017)
Lactobacillus rhamnosus LC705	AFB_1	PBS	87.8	(Haskard et al. 2001)
Lactobacillus rhamnosus GG	AFB_1	PBS	84.1	(Haskard et al. 2001)
Lactobacillus kefiri KFLM3	OTA	Milk	81	(Taheur et al. 2017)
Lactobacillus rhamnosus 6224	PAT	Apple juice	80.4	(Hatab et al. 2012)
Bifidobacterium animalis VM 13	PAT	Liquid medium	80	(Fuchs et al. 2008)
Lactobacillus rhamnosus 1088	AFB_1	PBS	79	(Chlebicz and Śliżewska 2020)
Lactobacillus rhamnosus GG	ZEN	PBS	70	(El-Nezami et al. 2002)
Enterococcus faecium 21,605	PAT	Apple juice	64.5	(Hatab et al. 2012)
Lactobacillus rhamnosus GG	AFM_1	PBS	63.1	(Assaf et al. 2017)
Lactobacillus rhamnosus GG biofilm	AFM_1	Milk	60.7	(Assaf et al. 2019a)
Actinobacteria AT8	OTA	PBS	52.6	(El Khoury et al. 2017)
Lactobacillus plantarum 13 M5	PAT	Apple juice	43.8	(Wei et al. 2020)
Lactobacillus plantarum VM 37	OTA	Liquid medium	43	(Fuchs et al. 2008)
Lactobacillus plantarum VM 37	PAT	Liquid medium	39	(Fuchs et al. 2008)

LC705, *L. acidophilus*, *L. gasseri*, and *L. casei* to remove AFB_1 from liquid media and have demonstrated that the probiotic strains *L. rhamnosus* GG and *L. rhamnosus* LC705 were highly effective in detoxifying of up to 80% AFB_1 (Haskard et al. 2001). Additionally, El-Nezami et al. (2002) have shown that *L. rhamnosus* GG was able to remove up to 70% of ZEN from liquid media. Fuchs et al. (2008) showed that *Bifidobacterium animalis* strain VM 12 was able to remove approximately 80% of PAT and *L. acidophilus* strain (VM 20) reduced the OTA levels by more than 90% among all tested strains.

Other strains of bacteria have been investigated for their binding capability to mycotoxins. El Khoury et al. (2017) have investigated the ability of actinobacteria, which are found in soil habitat and comprise the largest bacterial genera "*Streptomyces*", to bind and detoxify OTA. El Khoury et al. (2017) have established that seven different strains of actinobacteria were able to bind OTA to different extents. Among the tested strains, AT8 strain was remarkably able to detoxify OTA to up to 52.61%. Also, Verheecke et al. (2014) have found that the mutual interaction between *Streptomyces* spp. (27 strains) and *Aspergillus flavus* (NRRL 62477) can reduce the level of AFB_1 and AFB_2 in vitro to up to 73%. In 2018, Wang et al. (2018a, b) have shown that *Lysinibacillus* sp. strain, isolated from chicken large intestine digesta, demonstrated high ability in removing ZEN. Additionally, Taheur et al. (2017) have proved that *Lactobacillus kefiri*, *Kazachstania servazzii*, and *Acetobacter syzygii* were able to remove up to 100% of AFB_1.

Mycotoxins binding in several studies was reported to be rapid, and the binding percentages were generally affected by many factors such as incubation time, type of bacteria, bacterial concentration, pH, type of medium, and temperature (El-Nezami et al. 2004a). The use of bacterial adsorbents in beverages presents several advantages over chemical and physical detoxification methods. The bacterial detoxification assay is considered more effective and highly specific, especially since the binding affinity of mycotoxins varies not only among different species but also among different strains within the same species. Furthermore, the use of several probiotic bacteria has made the bacterial detoxification process safer.

Yeasts

Studies showed that probiotic yeasts or products containing yeast cell walls can remove mycotoxins from beverages (Pizzolitto et al. 2012). A summary of the literature in which yeast strains were used for mycotoxins detoxification is presented in Table 2. Generally, it is well established that *S. cerevisiae* is extensively used in biotechnology processes such as baking and distilling industries. Some studies shown that *S. cerevisiae* can remove OTA from microbiological media and other beverages (Piotrowska and Masek 2015). Other studies have demonstrated that *S. cerevisiae* is most effective in AFB_1 binding (Corassin et al. 2013). Corassin et al. (2013) have demonstrated that heat-killed *S. cerevisiae* cells have the potential to reduce AFM_1 levels in milk. Other yeast strains such as *Kluyveromyces Lactis* and *Kazachstania*

Table 2 Mycotoxins removal percentage of different yeast strains

Strain	Mycotoxins	Sample	Removal (%)	References
S. cerevisiae	AFM_1	UHT skim milk	90.3	(Corassin et al. 2013)
S. cerevisiae RC008	OTA	YPD broth	82.3	(Armando et al. 2012)
S. cerevisiae	AFM_1	PBS	78.7	(Abdelmotilib et al. 2018)
S. cerevisiae YS3	PAT	Apple juice	72.6	(Yue et al. 2011)
Kluyveromyces Lactis	AFM_1	PBS	69.1	(Abdelmotilib et al. 2018)
Kazachstania servazzii KFGY7	OTA	Milk	62	(Taheur et al. 2017)
S. cerevisiae 0068	AFB_1	PBS	46.7	(Chlebicz and Śliżewska 2020)

servazzii KFGY7 also showed removal ability of mycotoxins from milk reaching to up to 69.14%.

Chitin and shrimp shells

Both polysaccharides and peptidoglycans are found in the cell wall of different microorganisms and have been shown to be involved in mycotoxin binding (Kim et al. 2017). The amino sugar N-acetyl-D-glucosamine (GlcNAc) is the key component of the cell wall of microorganisms including fungi, bacteria, and yeast (Chen et al. 2010), and this has been mainly responsible for the detoxification of mycotoxins (Assaf et al. 2018a, b, c). This sugar is also found in the exoskeleton of crustaceans in the form of a polymer known as "chitin", specifically in shrimp shells (Xu et al. 2008; Iqbal et al. 2017) that contain primarily 30–40% of chitin (Venugopal 2016). In that sense, attention has been given to chitinous polymers and shrimp shells and their ability to remove mycotoxins. Assaf et al. (2018a, b, c) have evaluated the ability of chitin (as a natural biopolymer) and ground shrimp shells (which contain 30–40% of chitin) to detoxify AFM_1. They showed that chitin and shrimp shells were able to bind AFM_1 in milk at varied binding percentages in the range of 14.29 and 94.74%. Yearly, up to 8 million tons of crab, shrimp, and lobster shells are wasted worldwide, (Yan and Chen 2015) thus, valorisation of these wastes, to be used as potential biosorbents in mycotoxin detoxification would contribute to decreasing food waste and eliminating toxic effects on humans. They would also be of great interest as an added benefit to the food industry.

Mechanisms of action involved through microbial binding

To date, there are two theories by which LAB eliminates toxins: one through physical adsorption and the other through biodegradation of mycotoxins. Researchers have conducted several detoxification experiments to test which theory is more adequate. Experiments with thermally inactivated bacteria provoked higher detoxification of mycotoxins as compared to activated cells. Studies showed that the binding of mycotoxins by microorganisms is a rapid process, which forms a reversible complex between the toxin and the bacterial surface without altering the mycotoxins' structure (Bueno et al. 2007). Shetty and Jespersen's investigations revealed that the detoxification process is related to a physical union between the mycotoxins and the bacterial cell components, instead of covalent binding or biodegradation by bacterial metabolism (Shetty and Jespersen 2006). Yiannikouris et al. (2006) reported that hydrogen bonds and Van der Waals interactions may be implicated in this binding mechanism. On the other hand, according to Hernandez-Mendoza et al. (2009), the differences in the mycotoxins' binding ability of various *Lactobacillus* strains could be explained by the differences in the cell wall components specifically teichoic acid and peptidoglycan contents. Different structures in the cell wall of microorganisms are responsible for the mycotoxin binding capacity. Cell walls comprise carbohydrates (peptidoglycan, mannose, and glucan), proteins, and lipids, which may offer different binding sites (Wang et al. 2019a, b). However, there are arguments among different researches on the specific cell wall components implicated in the binding processes, such as glucogalactans and β-glucans (Taheur et al. 2017), mannoproteins (Caridi et al. 2012), β- glucans and mannans (Pereyra et al. 2015). Therefore, in the interaction of bacterial cells and mycotoxins, it appears that various binding mechanisms may be implicated involving non-covalent bonding, hydrophobic interactions, ionic interactions, or hydrogen bonds, (Huwig et al. 2001; Ringot et al. 2007).

The cell wall portion of *S. cerevisiae* is mostly composed of polysaccharides with an inner layer of β-D-glucans chains, which constitute 50 to 60% of the wall's dry weight (Jouany et al. 2005). Jouany et al. (2005) have shown that β-D-glucans are the components mainly responsible for the complexation of ZEN. Also, they showed that the chemical interaction is more of an adsorption type than binding, where both weak hydrogen bonds and Van der Waals interactions are involved in this adsorption. Moreover, it has been demonstrated

that the binding process of OTA to the surface of living or dead yeast cells, is achieved, in general, by adsorption mechanisms (Bejaoul et al. 2004). These researchers have concluded that the yeast cell wall and its charge are involved in the adsorption process (Bejaoul et al. 2004). These observations were similar to those of Piotrowska (2014) who found that bacteria, with partially removed cell walls, had less ability to bind OTA, as compared to the bacteria with intact cell walls (Piotrowska 2014), highlighting, therefore, the importance of the cell wall in the adsorption mechanisms (Piotrowska 2014).

The properties of the bacterial cell surface play a vital role in the binding mechanism. In another study, involving *Escherichia coli*, a Gram-negative bacterium, it was established that it was incapable of removing mycotoxins due to its moderately hydrophilic nature and its present surface components (lipopolysaccharides) (Pierides et al. 2000). On the other hand, LAB bacteria having hydrophobic sites on their cell surface were confirmed to have the ability to bind OTA (El-Nezami et al. 1998). Assaf et al. (2019a) hypothesized that the biofilm matrix may be involved in AFM_1 binding rather than the bacterial cells themselves. Additionally, this study observed that washing the biofilm had released some fraction of the weakly bound AFM_1. This confirms that binding is reversible due to the disruption of some electrostatic bonds as hydrogen bonds and Van der Waals interactions (Hernandez-Mendoza et al. 2009).

Moreover, higher mycotoxins' elimination was shown by inactivated bacterial cells. For example, Haskard et al. (2001) found that high temperature leads to protein denaturation in the bacterial cell wall, and this results in the generation of pores, that facilitate further aflatoxins' adsorption and mycotoxins elimination.

Additionally, the same authors suggested that protein denaturation might increase the hydrophobic nature of the surface or form Maillard reaction products and such alterations allow aflatoxins to bind to the bacterial cell wall and plasmatic membrane components, which were masked when the cell wall was intact (Haskard et al. 2001). Chlebicz and Śliżewska (2020) recently have shown that treatment of yeasts by heat and acidity improved significantly OTA detoxification by up to 75% from liquid medium compared to untreated cells. Expecting that both polysaccharides and peptidoglycans are affected by heat and acid treatments, these researchers postulated that acidic treatments could disturb polysaccharides, by releasing monomers that are broken down into aldehydes, which could lead to more adsorption sites than viable cells (Chlebicz and Śliżewska 2020).

Detoxification approaches of mycotoxins by biofilms

Bacteria exist in nature under two forms: either as freely swimming planktonic bacteria, or as sessile in attached colonies of microorganisms forming a biofilm. Thus, the biofilm may be considered as "a three-dimensional structure of sessile microorganisms that are irreversibly attached to biotic or abiotic surfaces, embedded in self-formed extracellular polymeric substances (EPS) (Lewandowski and Boltz, 2010).

In the last decades, biofilm formation has been implicated in many industrial and domestic domains (Roger et al. 2008). Salas-Jara et al. (2016) have studied the ability of some strains of LAB to form a biofilm and Lebeer et al. (2007) have shown the ability of *L. rhamnosus* GG to form biofilm on polystyrene support. Recently, a new method was developed for the detoxification of milk from AFM_1 by using *L. rhamnosus* GG biofilm (Assaf et al. 2019a). The biofilm was formed on polystyrene petri plates and in polyethylene tubes. The detoxification process was carried out using the biofilm formed on day 3, which is considered a highly attached biofilm and an efficient binding agent. The percentages of bound AFM_1 by *L. rhamnosus* GG biofilm reached to up to 60.7%. Moreover, the quality of milk after AFM_1 detoxification had no significant changes in the protein content, but some changes in fat and total dry matter contents ratio. Additionally, Assaf et al. (2019a) have studied the stability of the AFM_1-biofilm complex using different AFM_1 concentrations. The study has shown that the binding was reversible and a portion of the bound AFM_1 was released after successive washings. To date, this technique is a modern technique that relies on the removal of mycotoxins by a biofilm, making it of great challenge and appeal, especially to those that are interested in removing mycotoxins from foods. However, the use of biofilms as a technique of detoxification of mycotoxins is still in its early stages and additional studies that might be of a potential usefulness to food industries should be conducted. Hence, on a general note, using biofilms as biological adsorbents for mycotoxins may be considered as a solid base for the progress of biological detoxification methods.

Detoxification approaches of mycotoxins by microbial enzymes

Enzymatic detoxification is one of the most promising methods of mycotoxins control, especially that it is devoid of some significant disadvantages that chemical methods employ such as chemical contamination of raw materials, nutrient loss, time-consuming and expensive (Wang et al. 2019a, b). A wide range of microorganisms including bacteria, molds, and yeasts are involved in the enzymatic detoxification of mycotoxins (Hathout

and Aly 2014). Generally, microbial enzymes are able to metabolize, destroy or inactivate mycotoxins into less or nontoxic metabolites (Lyagin and Efremenko 2019). Degradation is a process by which most of the microorganisms use during their life activities to degrade certain substances converting them into less or even non-toxic products. During the degradation process, the vital active molecules secreted by microorganisms are enzymes (Guan et al. 2021).

Most of the scientific studies to date are dedicated to enzymatic degradation for detoxifying the most notable mycotoxins, including AF, OTA, ZEN, TCT, and PAT (Tang et al. 2013; Loi et al. 2018; Zhang et al. 2020). Several microbial enzymes from bacteria, yeast, and fungi that are capable of performing various modifications or transformations of mycotoxins have been isolated and investigated (Ben Taheur et al. 2019).

Examples of microbial enzymes, reported in the literature, involved in mycotoxins degradation are presented in Table 3. Studies have shown that some enzymes from yeasts have important characteristics that enable them to transform toxins into less toxic ones (Schatzmayr et al. 2006; Halász et al. 2009). A number of enzymes belonging to *Aspergillus* species are found to be involved in aflatoxin degradation. Furthermore, *Aspergillus niger*, an isolate from feed samples, was shown to biodegrade AFB_1 (Zhang et al. 2014). Other fungal strains that may contribute to the detoxification of aflatoxins, by secreting oxidative enzymes such as laccase and manganese peroxidase have been reported (Alberts et al. 2009). Interestingly, manganese peroxidase extracted from *Phanerochaete sordida* was responsible for eliminating 86% of AFB_1 in vitro, increasing to 100% with successive additions of the enzyme (Wang et al. 2011). Moreover, laccase enzyme purified from *Trametes versicolor* was able to remove 67% of AFB_1 (Zeinvand-Lorestania et al. 2015).

Recently, Zhang et al. (2018) showed that the intracellular enzymes of *Y. lipolytica* Y-2 were able to degrade OTA more rapidly than its viable cells (Zhang et al. 2018). Also, Chang et al. (2015) have reported that carboxypeptidase, an enzyme purified from *B. amyloliquefaciens* ASAG1 was capable of reducing OTA by 72%.

Additionally, the enzymatic extracts from *Rhodococcus erythropolis*, *N. corynebacterioides* DSM 20151, and *M. fluoranthenivorans* sp. nov. DSM 44556 T have revealed more than 90% degradation capability of AFB_1 (Hackbart et al. 2014). Recently, Shu et al. (2018) established that heat-treated supernatant from *Bacillus velezensis* DY3108 was able to degrade 94.7% of AFB_1 into less toxic metabolites. Recently, it was found that *Bacillus pumilus* was able to degrade 88% of AFB_1 (Sangi et al. 2018).

The mycotoxins detoxification efficacy of different enzymes produced as part of the yeasts/fungi metabolic activity are summarized in Table 3.

In one of the first studies to be conducted on microbial enzymes, manganese peroxidase can serve as a good candidate for detoxifying various types of mycotoxins (Wang et al. 2011). This peroxidase, purified from different lignocellulose-degrading fungi such as *Irpex lacteus*, *Phanerochaete chrysosporium, Ceriporiopsis subvermispora*, and *Nematoloma frowardii*, can degrade AFB_1, ZEN, and other mycotoxins (Tang et al. 2013). Loi et al. (2018) have described the ability of laccase purified from *Pleurotus eryngii* to simultaneously degrade AFB_1 and ZEN by 86% and 100% respectively.

Interestingly, *Bacillus pumilus* ES-21 was able to degrade 95.7% of ZEN into 1-(3,5-dihydroxyphenyl)-6-hydroxy-l-undecen-l0-one (Wang et al. 2017). Overall, three kinds of enzymes have been identified to degrade ZEN: Lactonase (Bi et al. 2018; Wang et al. 2018a, b), peroxidase (Tang et al. 2013), and laccase (Loi et al. 2018). Lactonase ZHD101 was mostly proposed for its high efficiency in ZEN degrading ability, found in *S. cerevisiae* (Takahashi-Ando et al. 2002) and *L. reuteri* (Yang et al. 2017). Even though lactonase has high degrading ability, however, it is not highly thermostable which restricts its applications (Zhang et al. 2020). Recently, Wang et al. (2018a, b) identified a lactonohydrolase enzyme, Zhd518, which exhibited high degrading activity against ZEN. The same authors reported that Zhd518 could be an excellent candidate for ZEN detoxifying due to its high specific activity against ZEN and its derivatives (Wang et al. 2018a, b). Recently, Zhang et al. (2020) have identified an effective ZEN-degrading lactonase from *Gliocladium roseum*, named ZENG for the first time (Zhang et al. 2020). This recombinant enzyme has great activity and stability at a pH of 7.0., characterized by its great detoxification ability of ZEN and its derivatives, α-zearalenol (α-ZOL), and α-zearalanol (α-ZAL) (Zhang et al. 2020).

Mechanisms of action involved in the enzymatic transformation of mycotoxins

Many biochemical transformation reactions of mycotoxins by microorganisms and their enzymes have been described such as acetylation, glucosylation, ring cleavage, hydrolysis, sulfation, deamination, and decarboxylation (Li et al. 2020). Some of the biotransformation reactions of mycotoxins by some microbial enzymes are shown in Table 4.

Application of biological detoxification processes in the food industry

Many microorganisms have been proposed for use as detoxifying agents in food and feed, but only few have

Table 3 Examples of some types of biotransformation reactions and the involved microorganisms or enzymes

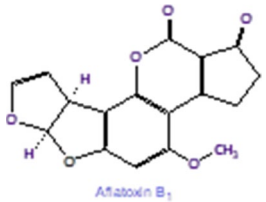
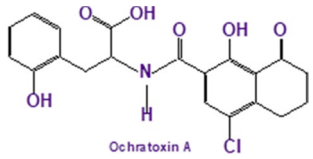
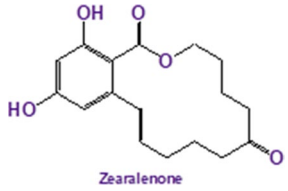
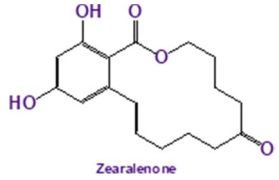
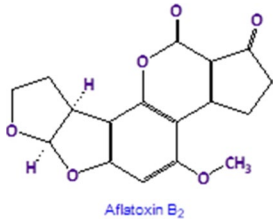
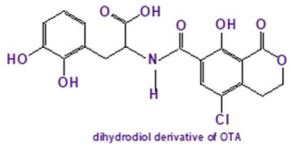
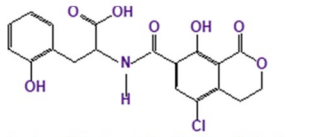
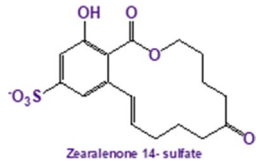
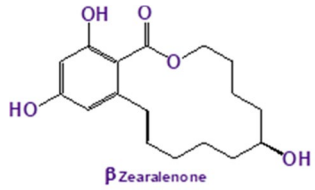
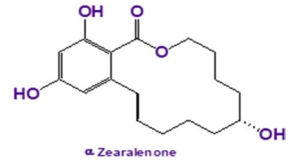
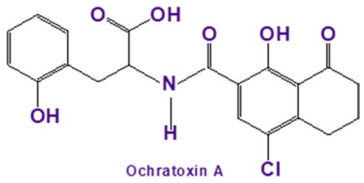
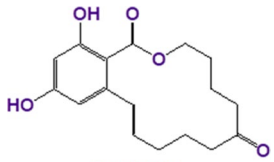
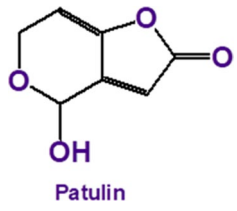
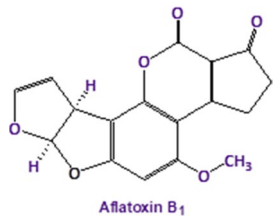
Type of Bio-Transformation Reaction	Hydrogenation	Hydroxylation	Sulfation	Reduction of the ketonic carbonyl group
Substrate	 Aflatoxin B_1	 Ochratoxin A	 Zearalenone	 Zearalenone
Product	 Aflatoxin B_2	 dihydrodiol derivative of OTA  7-carboxy-(20-hydroxy-1-phenylalanine- mide)-5-chloro-8-hydroxy-3,4-dihydro-3R-methylisocoumarin	 Zearalenone 14- sulfate	 β Zearalenone  α Zearalenone
Microorganism or Enzyme	*Penicillium raistrickii*	*Phenylobacterium*	*Sphaerodes mycoparasitica*	*Rhizopus* sp., *Aspergillus* sp
References	(Wu et al. 2009)	(Wegst and Lingens 1983)	(Kim and Vujanovic 2017)	(Brodehl et al. 2014)
Type of Bio-Transformation Reaction	Hydrolysis		Oxido-Reduction	Reduction of Carbonyl group
Substrate	 Ochratoxin A	 Zearalenone	 Patulin	 Aflatoxin B_1

Table 3 (continued)

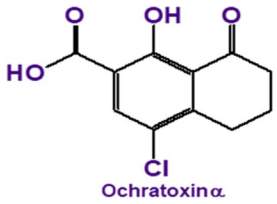
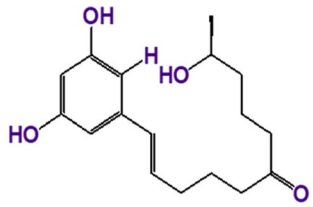
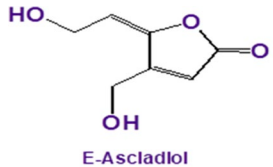
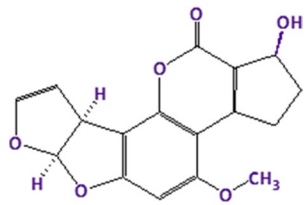
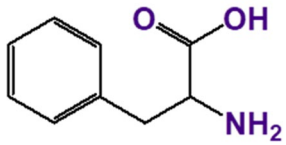
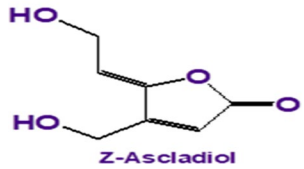
Type of Bio-Transformation Reaction	Hydrogenation	Hydroxylation	Sulfation	Reduction of the ketonic carbonyl group
Product	 Ochratoxin α	 1-(3,5-dihydroxyphenyl)- 60-hydroxy-l'- undecen-l0'- one	 E-Ascladiol	 Aflatoxicol
	 L-β-Phenylalanine		 Z-Ascladiol	
Microorganism or Enzyme	*Aspergillus niger*/ Lipase	*Bacillus pumilus* ES-21 /purified lactonase	*Lactobacillus plantarum*	*Aspergillus niger*, *Eurotium herbariorum*, *Rhizopus* sp.
References	(Stander et al. 2000)	(Xu et al. 2016; Wang et al. 2017)	(Soukup et al. 2016)	(Nakazato et al. 1991)

Table 4 Examples of enzymes and their microorganism origin that are involved in the degradation of mycotoxins

Microbial Origin	Name of enzyme Or Type of enzyme	Mycotoxins	Degradation (%)	References
Aspergillus niger MUM 03.58	Carboxypeptidase	OTA	99	(Abrunhosa et al. 2006)
Bacillus amyloliquefaciens ASAG1	Carboxypeptidase	OTA	72	(Chang et al. 2015)
Aspergillus tubingensis M036	–	OTA	97.5	(Cho et al. 2016)
Aspergillus tubingensis M074	–	OTA	91.3	(Cho et al. 2016)
Aspergillus niger	Ochratoxinase	OTA	ND	(Dobritzsch et al. 2014)
Yarrowia lipolytica Y-2	Carboxypeptidases	OTA	ND	(Zhang et al. 2018)
Phanerochaete sordida	Manganese peroxidase	AFB_1	86	(Wang et al. 2011)
Trametes versicolor	Laccase	AFB_1	67	(Zeinvand-Lorestania et al. 2015)
Pleurotus eryngii	Laccase	AFB_1	86	(Loi et al. 2018)
Pleurotus eryngii	Laccase	ZEN	100	(Loi et al. 2018)
Escherichia coli	Lactonohydrolase Zhd518	ZEN	ND	(Wang et al. 2018b)
lignocellulose-degrading fungi	Manganese Peroxidase	ZEN	34.0	(Tang et al. 2013)
lignocellulose-degrading fungi	Manganese Peroxidase	AFB_1	84.9	(Tang et al. 2013)
Rhodococcus erythropolis	–	AFB_1	90	(Hackbart et al. 2014)
Bacillus velezensis DY3108	–	AFB_1	94.7	(Alberts et al. 2009)
Bacillus pumilus	–	AFB_1	88	(Alberts et al. 2009)
Bacillus pumilus ES-21	–	ZEN	95.7	(Wang et al. 2017)
Gliocladium roseum	ZENG	ZEN	60	(Zhang et al. 2020)
Aspergillus niger	–	AFB_1	23.6	(Zhang et al. 2014)
Peniophora sp. SCC0152	–	AFB_1	40.45	(Alberts et al. 2009)

been further investigated for uses in the food industry. Many factors should be considered while selecting a binder for mycotoxins, particularly in the food sector, including non-pathogenicity, specificity, effective sequestering and the absence of adverse effects.

Assaf et al. (2018a, b, c) have proposed a novel machine that may be employed in the detoxification of mycotoxin from liquid beverages. This machine has probiotic LAB biofilms fixed to a customized cartridge; accordingly, it allows liquid food to pass through these adsorbents which results in detoxified liquid. The prototype of the invested detoxifying machine is presented in Fig. 1. Many aspects, such as the flow rate of the pump, the number of cartridges utilized, the kind of biological adsorbents are proposed to be explored throughout the development of this machine prototype. This machine has the potential to be a promising application in liquid food purification, particularly because the creation of biofilms is cost-effective, however, it is also very important to assess the effect of this treatment on the organoleptic characteristics of the beverages after detoxification process.

Lately, Foroughi et al. (2018) have suggested a method to detoxify AFM_1-contaminated milk by immobilizing yeast such as *S. cerevisiae* on perlite support. The results revealed a high significant removal of AFM_1 from milk samples where the highest detoxification percentage reached was 81.3% without altering the physicochemical properties of milk. Further optimization of the bio-filters may subsequently lead to different practical detoxification applications of various mycotoxins from dairy products and beverages.

In a previous study, Hatab et al. (2012) showed that *L. rhamnosus* 6224 and *Enterococcus faecium* 21,605 were able to remove 80.4 and 64.5% of patulin (PAT) from apple juice, respectively. These researchers have proved that LAB could be used as a novel and promising adsorbent to bind PAT without altering the quality of the juice.

Yue et al. (2011) have shown that inactivated *S. cerevisiae* YS3 powder was able to remove up to 72.61% of PAT from apple juice, and additionally, there was no negative impact on the quality of apple juice, based on the quality parameters measured, such as degrees Brix, total sugar, titratable acidity, color value, and clarity. This study suggests that the inactivated *S. cerevisiae* YS3 powder could be a hopeful binder of PAT in apple juice, especially since yeast has low cost, high biomass properties, and can be simply separated from apple juice (Yue et al. 2011).

Although many articles on mycotoxin elimination by adsorption and transformation have been published, their applicability in the food industry has been restricted. This might be attributed to a lack of knowledge

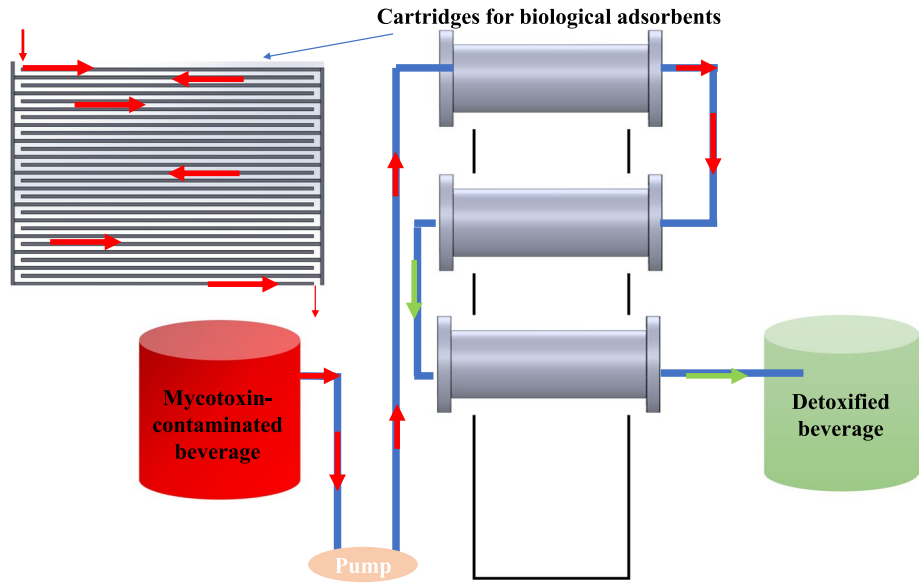


Fig. 1 Prototype machine for detoxification of mycotoxins from beverages using biological adsorbents

regarding transformation processes, the toxicity of transformation products, and the influence of transformation reactions on the nutritional content of food and feed. Degraded products have not been recognized in certain circumstances and so cannot be employed in industrial operations.

Recently, Zhang et al. (2020) have identified a potent enzyme, ZENG, that is efficient in detoxifying up to 60% of ZEN and its toxic derivatives. However, most enzymes do not meet the requirements of industrial applications due to their low ability to withstand high temperatures (Atalah et al. 2019). So thermostability is an important characteristic of industrial enzymes and to meet the industrial demands, efforts should also seek to provide highly thermostable enzymes, which can reduce the cost of production, develop its efficiency, and help in lowering microbial contamination in industrial approaches.

Although, an enzymatic catalysis is a promising approach that can be used to detoxify mycotoxins (Ben Taheur et al. 2019). However, not all mycotoxins' modifications and transformations can lead to detoxification products. For example, Hahn et al. (2015) showed that the α- and/or β-ZOL, the reduced products of ZEN, have similar estrogenic activity compared to ZEN. Karabulut et al. (2014) deduced that aflatoxicol, the reduced product of AFB_1, has a similar ability to form an exo-epoxide analog that binds to DNA.

Enzymatic detoxification, in our opinion, might be a viable technique for the majority of mycotoxins if applied properly. The efficacy of the biodegradation process can be validated by using two criteria: (i) discovering and identifying the transformed products, and (ii) performing toxicity assays on the resultant intermediate metabolites and products. Meanwhile, because the enzyme's genetic sequence is known, molecular engineers might improve the enzyme's selectivity and catalytic performance by making changes to the active region. All of these perceptions should be taken into account in order to aid in the mitigation of mycotoxins. Biological degradation of mycotoxins is a promising method that can be further developed by focusing on the isolation of specific microorganisms, optimizing their growth, and creating conditions that favor massive production of enzymes.

Conclusion

Mycotoxins have received much attention due to their severe impact on human and animal health. Their detoxification has been always the goal of research. While biological detoxification of mycotoxins has been widely studied, little is still known about the potential applications in the food, feed, and beverages industries. So practically, scientists should focus on the potential use of microorganisms in the detoxification of mycotoxin mixtures from food and beverages. In addition, it is of utmost importance to analyze the physicochemical profile, sensory properties, and organoleptic characteristics of the juices and food after detoxification of mycotoxins. Further, it is necessary to isolate and select suitable enzymes and microorganisms for efficient detoxification and to analyze the enzymatic properties and their catalytic processes in food and beverages. In brief, what makes a good binder is its adsorption

capacity, specificity, safety, and stability. Therefore, after the future development of these promising approaches, enzymes and microorganisms can be used as food and feed additives or as detoxifying agents during food processing, while preserving the nutritional and organoleptic quality of food commodities and beverages.

Abbreviations

AFs: Aflatoxins; OTA: Ochratoxin A; PAT: Patulin; ZEN: Zearalenone; TCT: Trichothecenes; LAB: Lactic Acid Bacteria; AFB_1: Aflatoxin B_1; AFM_1: Aflatoxin M_1; PBS: Phosphate Buffer saline; AFB_2: Aflatoxin B_2; GRAS: Generally Recognized as Safe; EPS: Extracellular Polymeric Substances; α-ZOL: *α*-zearalenol; α-ZAL: *α*-zearalanol.

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Authors' contributions

Sahar Nahle: Original draft preparation-Writing-Reviewing and Editing. Andre El Khoury: Reviewing-Editing. Ali Chokr: Reviewing-Editing. Nicolas Louka: Reviewing-Editing. Ioannis Savvaidis: Reviewing-Editing and Ali Atoui: Structuring/Formulation, Reviewing-Editing. The author(s) read and approved the final manuscript.

Authors' information

SN is a Ph.D. student between the Lebanese University and the Université Saint-Joseph de Beyrouth.
AK is a Professor at the Faculté des Sciences, Université Saint-Joseph de Beyrouth.
IS is a Professor at the Department of Environmental Health Sciences, University of Sharjah.
AC is a Professor at the Department of Life and Earth Sciences, Faculty of Sciences, Lebanese University.
NL is a Professor at the Faculté des Sciences, Université Saint-Joseph de Beyrouth.
AA is a Professor at the Department of Life and Earth Sciences, Faculty of Sciences, Lebanese University.

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Availability of data and materials

Not applicable.

Declarations

Competing interests

Authors have no financial and non-financial competing interests to declare.

Author details

[1]Research Laboratory of Microbiology (RLM), Department of Life and Earth Sciences, Faculty of Sciences I, Lebanese University, Hadat Campus, Beirut, Lebanon. [2]Platform of Research and Analysis in Environmental Sciences (PRASE), Doctoral School of Sciences and Technologies, Lebanese University, Hadat Campus, Beirut, Lebanon. [3]Centre d'Analyses et de Recherche (CAR), Unité de Recherche Technologies et Valorisation Agro-Alimentaire (UR-TVA), Faculté des Sciences, Université Saint-Joseph de Beyrouth, Campus des Sciences et Technologies, Mar Roukos, Matn, Lebanon. [4]Department of Environmental Health Sciences, College of Health Sciences, University of Sharjah, P.O. Box 27272, Sharjah, United Arab Emirates. [5]Department of Chemistry, School of Natural Sciences, University of Ioannina, 45110 Ioannina, Greece.

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