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Recent Trends in Microbiological Decontamination of Aflatoxins in Foodstuffs

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1. Introduction

Nowadays, about 100,000 fungi have already been identified. From these, more than 400 may be considered potentially toxigenic, and about 5% are known to produce toxic compounds or classes of compounds that cause adverse effects in animals and humans in several parts of the world [1]. These compounds, called mycotoxins, are secondary metabolites of low molecular weight produced by mycelia or spores of filamentous fungi [2]. It is suggested that mycotoxin production is generally limited to a relatively small number of mold species, and that toxin may be produced by the whole species or just one specific strain [3]. The more complex the synthesis pathway of a mycotoxin, the lesser the number of mold species that produce it.

The term "mycotoxin" originates from the Greek word "Mykes", meaning fungus, and from the Latin word "Toxicum", meaning poison or toxin [2]. Mycotoxins are classified as the most important chronic and noninfectious foodborne risk factor, more important than synthetic contaminants, plant toxins, food additives, and pesticide residues. Both humans and animals may show acute or chronic intoxication caused by mycotoxin ingestion, and the pathological condition that results from this ingestion is called mycotoxicosis [4]. Some factors affect the magnitude of toxicity in humans or animals, including the animal species, mechanism of action, metabolism and defense mechanisms [5].

About 400 types of mycotoxins have been already discovered, and they are generally divided into groups based on structural similarities and most important toxic effects [6]. From all

mycotoxins that have been isolated, aflatoxin is one of the most well-known and widely distributed in foodstuffs, with proven and marked toxic properties. Aflatoxins are predominantly produced by *Aspergillus flavus* and *A. parasiticus*, but may also be produced by other strains, such as *A. nomius*, *A. tamari*, and *A. pseudotamarii* [7]. Contamination of foodstuff with aflatoxigenic fungi may occur at any moment during production, harvesting, processing, transportation, and storage [8]. The most different kinds of foods may be affected, such as corn, peanuts, cotton seeds, rice, pistachio, almonds, chestnuts, Brazil nuts, and pumpkin seeds, as well as other oily seeds, such as sunflower and coconut [9].

Aflatoxins are distributed worldwide. *Aspergillus* species are able to grow in a wide variety of substrates and under different environmental conditions. Toxin formation in agricultural products occurs in hot and humid weather, and in inadequate or deficient storage facilities. The most important factors that influence growth and aflatoxin production are relative humidity, ranging from 88 to 95% in most of the cases [8], and temperature, ranging from 36 to 38 C for mold growth, and 25 to 27 C for maximum toxin production [10].

Other factors may also influence aflatoxin production: substrate composition, water activity, pH, atmosphere (concentration of oxygen and carbon dioxide), microbial competition, mechanical damage to the seeds, mold lineage, strain specificity and variation, instability of toxigenic properties, plant stress, insect infestation, and use of fungicides or fertilizers [2, 5, 11]. It is important to remember that aflatoxin contamination is cumulative, and the moment of harvesting and drying, and storage conditions may also play an important role in aflatoxin production [12].

Concerns related to the negative impacts of aflatoxins on health led to the study of strategies to prevent toxin formation in foodstuffs, as well as to eliminate, inactivate or reduce toxin bioavailability in contaminated products [13]. Contamination may be prevented by improved agricultural practices, antifungal agents, genetic engineering, and control of storage conditions [2]. Bioavailability may be reduced by enterosorption, which is done by adding nutritionally inert adsorbent compounds to the diet. These compounds are mycotoxin sequestrants, and prevent the toxin from being absorbed in the gastrointestinal tract of the animals, making its distribution to the target organs impossible [14]. This method has limited practical use, due to the safety of the adsorbent agents used, and the difficulty in applying them to human foods [15]. Elimination or inactivation, that is, decontamination, may be achieved by physical, chemical, and biological methods, which have to present the following characteristics: complete inactivation; destruction or removal of the toxin; no production or toxic residues in foods or no remainders of them; preservation of nutritional value and palatability of the food; destruction of fungal spores and mycelia to prevent production or reappearance of the toxin; no significant changes in the physical properties of the food; low cost and ease of use [1,11].

Physical methods for mycotoxin decontamination involve procedures such as thermal inactivation, ultraviolet light, ionizing radiation, or extraction with solvents. Chemical methods are based on agents that break mycotoxin structure, such as chlorine treatment (sodium hypochlorite or chlorine gas), oxidizing agents (hydrogen peroxide, ozone and sodium disulfide), or hydrolytic agents (acids, alkalis and ammonia). However, both chemical and

physical methods have disadvantages, either because removal is not efficient, or because of high costs or nutritional losses to the product [16,17]. Biological methods are based on the action of microorganisms on mycotoxins. These microorganisms may be yeasts, filamentous fungi, bacteria, algae, among others, and their mechanisms of action is based on competition by nutrients and space, interactions, and antibiosis, among others [18].

Biodegradation of aflatoxins by microorganisms offers an attractive alternative for the control or elimination of aflatoxins in foods and animal feed, preserving their quality and safety [19]. Besides, their use have a more "natural" appeal, given the ever-growing resistance of the consumer to chemical treatments [1]. Biological decontamination methods are being widely studied and may be a very promising choice, provided they show to be efficient, specific, cost-effective, and are environmentally friendly [20]. Among the types of microorganisms available and that may be used to remove aflatoxins from a contaminated medium, lactic acid bacteria (LAB) and yeasts are the most studied ones, showing the most promising results.

Therefore, the objective of this chapter was to present results of studies on microbiological methods for aflatoxin decontamination, more specifically on the ability of LAB and yeasts to degrade or sequestrate this mycotoxin.

2. Toxicological Properties of Aflatoxins

Nowadays, there are 18 similar compounds called aflatoxins. However, the most important types in terms of health and medical interest are identified based on their fluorescence under ultraviolet light (B = Blue and G = Green), such as aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2). From these compounds, AFB_1 is the most prevalent and toxic one [21]. When AFB_1 is ingested by domestic animals in contaminated feed or foodstuffs, such as by dairy cows, the toxin undergoes liver biotransformation and is converted into aflatoxin M_1 (AFM_1), becoming the hydroxilated form of AFB_1, which is excreted in milk, tissues and biological fluids of these animals [22-24]. It was reported that of all AFB_1 ingested in feed, about 0.3% to 6.2% is transformed in AFM_1 in milk and that there is a linear relationship between the concentration of AFM_1 in milk and the concentration of AFB_1 in contaminated feeds consumed by the animals [25,26].

Chronic exposure to low levels of aflatoxins represents a serious risk to economy, and mainly to health [21]. Economic losses are related to decreased efficiency in industrial or agricultural production, with loss in quality, lower yield, and defective product [27]. It was also reported that in some states of the USA, economic losses to agriculture amount to 100 million dollars [19]. On the other hand, these losses caused by mold contamination and mycotoxins are greater than 1.6 billion dollars in the US, and African feeds lose about 670 billion dollars a year due to barriers to the trade of aflatoxin-contaminated foodstuffs [28].

As for human and animal health, biological effects of aflatoxins may be carcinogenic, mutagenic, teratogenic, hepatotoxic, and immunosuppressive [29]. The International Agency for

Research on Cancer classifies AFB_1 and AFM_1 as Group 1 human carcinogens, even though AFM_1 is about 10 times less carcinogenic than AFB_1 [30]. All these aflatoxin effects are influenced by variations according to the animal species, sex, age, nutritional status, and effects of other chemical products, besides the dose of toxin and the length of exposure of the organism to it [31].

Aflatoxicosis is the poisoning caused by the ingestion of moderate to high levels of alfatoxin in contaminated foods. Acute aflatoxicosis causes quick and progressive jaundice, edema of the limbs, pain, vomiting, necrosis, cirrhosis and, in severe cases, acute liver failure and death, caused by the ingestion of about 10 to 20 mg of aflatoxin in adults. Aflatoxin LD50 shows the following order of toxicity: $AFB_1 > AFM_1 > AFG_1 > AFB_2 > AFG_2$ [4, 32]. Chronic aflatoxicosis causes cancer, immunosuppression and other pathological conditions, having the liver as the primary target organ [4].

The greatest risk presented by aflatoxins for human beings is chronic exposure causing hepatocellular carcinoma, which may be made worse by hepatitis A virus [5]. It was also report that aflatoxins were found in the tissues of children affected by Reye syndrome (encephalopathy with serious lesions in liver and kidneys after influenza or chickenpox), and Kwashiorkor (protein-energy malnutrition). Aflatoxicosis is considered, then, a contributing factor to these diseases.

AFB_1 is metabolized in the liver by the cytochrome P450 system, generating its most carcinogenic metabolite, AFB_1-8,9-epoxide (AFBO), or other less mutagenic forms, such as AFM_1, Q_1 or P_1. There are several pathways for AFBO after it is metabolized, with one of them leading to cancer, another to toxicity and another one, to excretion. AFBO exo-form easily binds to cell macromolecules, including genetic material such as DNA proteins, producing adducts. Formation of these DNA adducts leads to genetic mutations and cancer, and their excretion in the urine of infected people is not only a proof that humans have the necessary biochemical pathways for carcinogenesis, but also offers a reliable biomarker for AFB_1 exposure [24].

Potential risk to human health caused by aflatoxins has led to surveillance programs for the toxin in different raw materials, as well as regulations determined by almost every country in the world [9]. A study carried out by the Food and Agriculture Organization of the United Nations (FAO) in 2002 pointed out that about 100 countries had specific regulations for the presence of aflatoxin in foods, dairy products and animal feed, and that the total population of these countries amounted to 90% of the world population. The same study showed that regulations for aflatoxin are getting more diverse and detailed, including sampling methods and methods of analysis [33].

In countries where a regulation for aflatoxin exists, tolerance levels for the total aflatoxin (sum of aflatoxins B_1, B_2, G_1 and G_2) ranges from 1 to 35 µg/kg for foods, with an average of 10 g/kg; and from zero to 50 µg/kg for animal feed, with an average of 20 µg/kg. For AFM_1 in milk, tolerance levels are between 0.05 and 0.5 µg/kg, with most countries adopting a threshold of 0.05 µg/kg [10].

3. Decontamination of Aflatoxins by Lactic Acid Bacteria

LAB is a large group of genetically different bacteria that, besides producing lactic acid as the main product of their metabolism, have similar characteristics: they are all gram-positive, non-sporoformers, non-motile, and catalase, and oxidase negative. They are, therefore, aerotolerant anaerobes. Besides, they mandatorily ferment sugars and tend to be nutritionally fastidious, frequently requiring specific amino acids and B-complex vitamins as growth factors [34]. Several LAB genera, such as *Lactobacillus*, *Bifidobacterium* and *Lactococcus* are known for they ability to act as preserving agents in fermented foods, such as vegetables, cereals, dairy and meat products, actively inhibiting spoilage and growth of pathogenic bacteria, besides increasing shelf life and sensory properties of these foods [23].

Fermentation enables longer shelf life and improves sensory and nutritional properties of the product, as sugar fermentation lowers pH and inhibits growth of spoilage and pathogenic microorganisms. Fermentation is also responsible for other reactions, such as proteins hydrolysis, improving texture and flavor; synthesis of aromatic components and texturizers, affecting the consistency of the product; and production of inhibitory components [35,36]. This inhibition is, in part, caused by the final products of fermentation, such as lactic acid, diacetyl, acetaldehyde and acetic acid, which may accumulate in inhibitory concentrations in certain foods and drinks. In other cases, inhibition may also be caused by secondary by-products of metabolism, such as hydrogen peroxide or bacteriocins [37].

Therefore, two aspects may be considered when LAB are used: fermentation and antibiosis ability. In the first case, the starter culture added to the food acts on the substrate, causing advantages to the food. In the second case, the starter culture has to inhibit the development of undesirable microorganisms that may spoil the product or be hazardous to human health. In reference [38], authors state that one of the effects that were identified in LAB was protection against toxins found in foods, such as heterocyclic amines, polycyclic aromatic hydrocarbons, reactive oxygen species, and mycotoxins. In the latter case, studies have demonstrated that LAB have the ability to inhibit aflatoxin biosynthesis, or that they have the ability to remove mycotoxins from the medium, reducing their effects.

It should be emphasized that with increased interest in probiotic food production all over the world, selection of LAB cultures with probiotic characteristics and greater ability to remove mycotoxins may help to reduce risk of exposure to these toxins in foodstuffs, which is a very promising line of research in mycotoxicology. Yeast and LAB strains have great ability to remove mycotoxins, and may be used as part of starter cultures in the fermentation of foods and drinks [39]. These microorganisms have, thus, ability to ferment and decontaminate the medium, and purified components of these strains may be used in small amounts as food additives without compromising the characteristics of the final product.

One of the first studies in this area was carried out in the 1960s, when these authors evaluated the ability of about 1,000 types of microorganisms to degrade aflatoxins [40]. Yeasts, filamentous fungi, bacteria, actinomycetes, algae, and fungal spores were among the organisms studied. From these, only the bacterium *Flavobacterium aurantiacum* B-184 (known today as *Nocardia corynebacterioides*) was able to irreversibly remove aflatoxins from the solution.

After this study, many others followed. However, the most significant ones started to appear after the 1990s. Table 1 presents the most relevant studies carried out with bacteria for aflatoxin decontamination. The action of 7 different types of bacteria on AFB_1 was evaluated and it was found that some strains of *Lactobacillus* (*L. rhamnosus* GG and *L. rhamnosus* LC-705) were able to efficiently remove most mycotoxin from the medium, up to about 80% [17]. In reference [27] authors analyzed 9 strains of *Lactobacillus* and achieved the same result, that *L. rhamnosus* GG and *L. rhamnosus* LC-705 were the most efficient strains in removing AFB_1, with removal rates of 78.9% and 76.5%, respectively. Fifteen types of LAB, among them *Lactobacillus* and *Lactococcus*, and 5 types of bifidobacteria, were studied and it was observed that removal of AFB_1 ranged from 5.6% to 59.7% [23]. Strains of *Lactobacillus amylovorus* (CSCC 5160 and CSCC 5197) and *L. rhamnosus* LC 1/3 showed the best results: 59.7%, 57.8%, and 54.6%, respectively. It was also observed that different strains of bifidobacteria removed from 37% to 46% AFB_1, and that *Staphylococcus aureus* and *Escherichia coli* removed 46% and 37%, respectively [22]. It may be observed that among a given genus, and even a given species, not all the strains show equivalent toxin removal rates. On the contrary, the ability to remove aflatoxin is a characteristic of specific lineages, and efficiency varies widely [41].

Most assays on aflatoxin removal in the studies cited above were carried out in phosphate-buffered saline (PBS). In reference [42], besides testing the ability of 27 strains of *Lactococcus* spp. and 15 strains of *Streptococcus* spp. isolated from yogurt, raw milk, and Karish cheese to remove AFB_1 in buffered solution, observed that *Lactococcus L. lactis* and *Streptococcus thermophilus* presented the greatest rates of toxin removal (54.85% and 81.0%, respectively). They also tested the ability of viable and non-viable cells to remove AFB_1 in different vegetable oils, and observed that viable *L. lactis* cells removed from 71% to 86.7% AFB_1, whereas non-viable cells removed 100% of the toxin in all the oils. Moreover, viable *S. thermophilus* cells removed from 66.5% to 91.5% of the toxin, and non-viable ones, from 81.7% to 96.8%.

AFB_1 was added to yogurt and acidified milk in concentrations ranging from 1,000 to 1,400 g/kg, and a reduction of AFB_1 in yogurt (pH 4.0), ranging from 97.8% to 90% was obtained [43]. Maximum decrease in AFB_1 was observed during milk fermentation. As for milk acidified with citric, lactic, and acetic acid (pH 4.0) AFB_1 reduction (concentration of 1,000 µg/Kg) was 90%, 84% and 73%, respectively. The ability of probiotic bacteria (*L. paracasei, L. casei, L. brevis* and *L. plantarum*) and the yeast *Saccharomyces cerevisiae* to remove a sum of aflatoxins (B_1, B_2, G_1 and G_2) during fermentation of dough made up of 50% barley flour, 45% wheat flour, and 5% corn flour was evaluated [44]. They observed that after 6 hours of fermentation, the amount of aflatoxin had decreased 18% and 33% for dough added of 4 and 40 µg of aflatoxin, respectively, and after 24 hours, the amount of aflatoxin decreased 27% and 50%, respectively.

Toxin polarity has an important role in the binding mechanism. The percentage of aflatoxin removed by LAB decreases in the following order: $AFB_1 > AFB_2 > AFG_1 > AFG_2$. This observation correlates with the decrease in the polarity of these toxins, and is consistent with hydrophobic reactions, which may also have a role in the binding mechanism [45]. AFM_1 is less efficiently removed than AFB_1. However, scientific literature has few studies on the ability of LAB to remove AFM_1.

In reference [46], authors examined the ability of 4 strains of *Lactobacillus* spp. and 2 strains of *Bifidobacterium* spp. to remove AFM_1 in PBS and reconstituted skim milk. In PBS, viable cells of 6 strains were able to remove from 10.22 to 26.65% AFM_1 in solution, depending on the level of contamination and the length of incubation, whereas non-viable cells removed from 14.04 to 28.97% of the toxin. In reconstituted skim milk incubated for 4 hours, 7.85 to 25.94% AFM_1 were removed by viable cells, and 12.85 to 27.31% for cells rendered non-viable by heat treatment. These researchers concluded that the removal process was fast, with no differences between 0, 4, and 24 hours of contact, different from what was observed in [47] for strains of *Lactobacillus* spp., *Lactobacillus* spp. and *Bifidobacterium* spp., which showed removal rates ranging from 0 to 14.6% after 24 hours of contact, and from 4.5 to 73.1% after 96 hours of contact.

The ability of *L. rhamnosus* GG to remove AFM_1 from reconstituted skim and whole milk was investigated and it was observed rates of 18.8% and 26.0%, respectively [29]. The authors concluded that the decrease in removal efficiency may be explained by the fact that AFM_1 is possibly not accessible in milk, that is, it is associated with casein, and the interference of proteins in toxin removal may be the greatest responsible factor for the difference between skim milk and whole milk (approximately 10% lower), once powdered skim milk used in the study contained 37g of protein / 100 g, whereas protein content in powdered whole milk was 25g /100g. In the same study, AFM_1 removal in buffered solution (50.7%) was compared with AFB_1 removal by the same bacterial strain in the same solution (75.3%). It was concluded that AFM_1 removal was less effective possibly due to the presence of an -OH group in the molecule, increasing its polarity and making it less hydrophilic, what increases the tendency of the molecule to be retained in aqueous solutions.

Some physical, chemical, and enzymatic treatments may increase the ability of LAB to bind to aflatoxin in the medium. In reference [48] authors studied the ability of *L. rhamnosus* GG to bind to AFB_1, observing little difference between aflatoxin removal by heat-treated and acid-treated cells (85% and 91%, respectively), compared with viable bacterial cells (86%). The use of physical and chemical treatments (chloric acid, and heat treatment in autoclave or boiling at 100 °C) on *L. rhamnosus* GG and LC-705 caused a significant increase in AFB_1 removal, showing that metabolic degradation caused by viable bacterial cells may be ruled out as a possible mechanism of action [15-17].

Comparing the ability of viable and heat-treated bifidobacteria cells, it was observed that viable cells removed 4 to 56% AFB_1 from the medium, whereas non-viable cells removed 12 to 82% [23]. Evaluating the influence of the inactivation treatment on the ability of 4 types of *Lactobacillus* spp. to remove AFB_1, it was observed that acid treatment (58.6 to 87.0%) and heat treatment (33.5 to 71.9%) increased the ability to remove the toxin, compared with viable cells in PBS (16.3 to 56.6%) [49]. On the other hand, alkali treatment (8.3 to 27.4%) and ethanol treatment (15.9 to 46.5%) decreased the amount of aflatoxin removed from the medium.

Removal of AFM_1 with 8 LAB strains showed that heat-treated cells bound more efficiently (25.5 to 61.5%) to the toxin than viable bacterial cells (18.1 to 53.8%) [29]. In reference [50] it was observed that heat-treated cells removed greater percentages of AFM_1 (12.4% to 45.7%) in PBS compared with viable cells (5.6% to 33.5%), with no significant differences between 15

minutes or 24 hours of contact. Similar results were found in [51], because viable cells of *Lactobacillus delbrueckii* spp. *bulgaricus* CH-2 removed 29.42% AFM_1 in PBS after 4 hours of contact at 37 C. These authors also analyzed the ability of *Streptococcus thermophilus* ST-36, observing that 18.70% AFM_1 was removed from the medium. Until today, only one bacterium, *Flavobacterium aurantiacum* NRRL B-184, was able to remove 100% of AFM_1 from contaminated liquid medium, at a cell concentration of 5×10^{10} CFU/mL and 4 hours of contact [52].

In [53] authors observed that *B. subtilis* UTBSP1 presented significant removal of AFB_1 from a medium contaminated with 2.5 μg/g (52.67% and 80.53%, after 24 and 48 hours, respectively). After 72 and 96 hours, there was no significant increase in the amount of toxin removed from the medium. Strains of *B. subtilis* were analyzed and it was concluded that strain ANSB060 was the one that best removed AFB_1, AFM_1, and AFG_1 from the medium (81.5%, 60%, and 80.7%, respectively) [54]. Results of this study also demonstrated that aflatoxin degradation is mainly observed in the supernatant culture, compared with cells or cell extracts. Besides, in assays that simulated the gastrointestinal environment (pH 2.0, and 0.3% of biliary salts), viable cells of the same strain were able to survive for 24 hours of incubation, and presented antimicrobial activity against *E. coli*, *S. typhimurium*, and *S. aureus*.

These examples show that both viable and non-viable cells are able to remove aflatoxin from aqueous solutions. As non-viable cells are also able to remove the toxin, it is supposed that cells are physically bound to the toxin, that is, components of the bacterial cell wall adhere to it, mainly polysaccharides and peptidoglycans, taking into account the possibility of a covalent bond or degradation caused by bacterial metabolism [1, 55, 56].

Both polysaccharides and peptidoglycans of the bacterial cell wall may be extremely affected by heat and acid treatment, once heat may denature proteins or form Maillard reaction products. Besides, acid treatment may break glycosidic bonds of polysaccharides, releasing monomers that may be further broken into aldehydes, also degrading proteins to smaller components, such as peptides and amino acids. Thus, acid treatment may break the peptidoglycan structure, compromising its structural integrity, that is, decreasing the thickness of this layer, reducing cross links and increasing the size of the pores. These changes caused by the treatments cited above enable AFB_1 to bind to the bacterial cell wall and to the components of the plasmatic membrane that were not available when the bacterial cell was intact [27].

In reference [57] authors explained that the integrity of the bacterial cell wall is important in the process of toxin removal by both viable and non-viable cells. In their study of AFB_1, they observed that both the bacterial cell wall and its purified fragments were able to remove aflatoxin from the medium. However, when the cell wall was lost or destroyed (totally or partially) by enzymatic treatment, there was a significant decrease in the ability to remove the toxin. It was observed, using atomic force microscopy, that the bond between AFB_1 and *Lactobacillus casei* Shirota produced structural changes that modified the surface of the bacterial cell [58]. Before the toxin was bound to it, the surface was well-defined, smooth and homogenous, and after AFB_1 adsorption, there were changes in shape. These changes were probably caused by the bond between the toxin and the surface of the cell wall, which became very irregular and rough, with undefined edges. The authors suggest that changes in the shape of teichoic acids are responsible for these alterations, once these molecules are

found inside the cell wall in such a way that they produce no differences in the texture of the surface before the toxin was bound to it.

The ability of *L. rhamnosus* GG to bind to AFB_1 was studied, observing that the addition of urea - an anti-hydrophobic agent - to the medium, significantly decreased removal of the toxin by non-viable cells, from 85-91% to 50-60%, showing that hydrophobic interactions have a relevant role in the process [48]. Besides, addition of different concentrations of NaCl and $CaCl_2$ (from 0.01 to 1 M), and pH variations from 2.5 to 8.5 had practically no effect on AFB_1 removal by the bacterium, suggesting that hydrogen bonds and electrostatic interactions are not important in this process.

In the use of pronase E, lipase and periodate, treatment with periodate led to significant reduction in the ability to remove the toxin, both by viable and non-viable cells, once it oxidizes the -OH cis groups in aldehyde and carboxylic acid groups, suggesting that the bonds involve predominantly bacterial polysaccharides. Treatment with pronase E caused the same significant reduction in AFB_1 removal, evidencing that proteins may also be involved in the process. Thus, the fact that pronase E and periodate both have a significant reduction on AFB_1 removal indicates that binding sites are made of protein. Treatment with lipase, on its turn, did not cause any significant reduction in AFB_1, showing that lipids, such as lypoteichoic acid probably do not have a role in the process. Although the treatments decreased AFB_1 removal, it was still substantial in all cases, possibly showing the involvement of multiple components in the bond with mycotoxin [48].

However, not only the type of bacterial strain and the inactivation treatment used may influence formation and stability of the LAB/aflatoxin complex, but also of other factors, such as bacterial counts, specificity of the bacteria, pH, incubation temperature, addition of nutrients, and the solvents used, among others [23, 27, 48].

As for the number of bacterial cells in the medium, it has been concluded that there was a significant decrease in the amount of AFM_1 removed when cell counts changed from 10^7 CFU/mL (0 to 5.02%) to 10^8 CFU/mL (10.22 to 26.65%), indicating that bacterial counts are critical factors in the removal of AFM_1 by LAB [46]. In reference [59] authors observed that no less than 5×10^9 CFU/mL of *Lactobacillus acidophilus* or *Bifidobacterium longum* are necessary to remove only 13% AFB_1 in about one hour.

In reference [17] authors reported that, for *Lactobacillus rhamnosus* (strains GG and LC705), minimum counts of 2×10^9 CFU/mL were required to remove 50% AFB_1, and greater removal rates were obtained when LAB concentration was increased to 10^{10} CFU/mL. In this same study, the authors observed that the process depended on the temperature, once the efficiency in aflatoxin removal was greater at 37 °C than at 4 and 25 °C. Besides, the authors observed that Gram-positive bacteria are better aflatoxin sequestrants than Gram-negative bacteria, with removal rates of 80% and 20%, respectively, suggesting the ability to remove the toxin depends on the structure of the cell wall. It has also been stated that aflatoxin concentration in the medium also influences adsorption rates, leading to the conclusion that the greater its concentration in the medium, the greater the removal rate, both for viable and non-viable cells [60].

Assays with AFB_1 and *L. rhamnosus* GG and LC-705 at different incubation temperatures was also carried out, but it was not observed significant differences in the stability of the LAB/AFB_1 complex formed in the temperatures range between 4 °C and 37 °C [27]. When pH of the medium was changed from 2 to 10, a range that includes the pH switch in the gastrointestinal tract, only 10% AFB_1 removed was released back into the solution, different from what happened when organic solvents were used. In this case, almost all AFB_1 that was removed by the bacterial strains was released back into the medium, providing extra evidence that the process is based on a non-covalent bond. In this study, the release efficiency by solvents presented the following order: methanol < acetonitrile = benzene < chloroform, which does not coincide with the order of decreasing polarity. This may be explained by the fact that AFB_1 hydrophobicity is similar to that of the chloroform molecule. These results show once more that hydrophobic interactions have an important role in the binding mechanism between LAB and the toxin.

The effect of washing on the stability of the LAB/aflatoxin complex was analyzed [47]. They observed that after the first washing of bacterial pellets with PBS, the proportion of AFM_1 released by the bacteria was 87.3% for *Lactobacillus* spp. strains; 85.7% for *Lactococcus* spp. strains, and 85.7% for strains of *Bifidobacterium* spp. They also observed that after the third washing, practically all bacteria had released adsorbed AFM_1 back into the medium (92.0 a 100%). In reference [46] they concluded that AFM_1 removal by bacteria was reversible, and that small amounts of toxin were released back to the PBS solution (5.62 to 8.54%). This finding is consistent with those observations of reference [27], who reported that *L. rhamnosus* GG, *L. rhamnosus* LC-705, and *Lactobacillus casei* Shirota released, respectively, 3.7%, 3.0% and 2.4% AFB_1 back into the solution. Differently, in [23] authors showed that release of AFB_1 back into the solution in the first washing was 48.6%, 30.7% and 26.5% for *L. amylovorus* (strains CSCC 5160 and CSCC 5197) and *L. rhamnosus* Lc 1/3, respectively. After 5 washings, AFB_1 adsorbed by *L. amylovorus* CSCC 5160 was almost completely released (94.4%), whereas *L. amylovorus* CSCC 5197 and *L. rhamnosus* Lc 1/3 retained, respectively, only 17.4% and 32.2% AFB_1 found in the original solution.

Thus, the LAB/aflatoxin complex seems to be unstable, once part of the aflatoxin, both for AFB_1 and AFM_1, is released from the complex after washing, and gradually returns to the aqueous solution. Therefore, the greater the number of washings, the greater the amount of aflatoxin released back into the solution. This shows that the bond is not a strong one, suggesting it is a weak non-covalent bond and an association with hydrophobic sites on the surface of the bacteria [23, 48].

Different from this hypothesis, in reference [61], performing the same washings on a complex between *Flavobacterium aurantiacum* and AFB_1, authors observed that aflatoxin was not released into the aqueous solution. Analyzing the stability of the complex formed between AFB_1 and 8 strains of *Lactobacillus casei* after the washings, it was demonstrated that the amount of aflatoxin released ranged from practically zero and 9.2% [13]. Possible explanations for this variation in aflatoxin release include the differences in binding sites found in the different strains, or more probably, that these biding sites are similar, but that they present minimal differences depending on the strain. Authors explained that lower rate of toxin release into

the medium after the washings may be attributed to the interactions between aflatoxin molecules retained on the cell wall of a bacterium and molecules retained on the cell wall of the adjacent bacterium, forming a kind of reticulated matrix that prevents aflatoxin release. It has also been suggested that the greater the number of molecules that are removed by the bacterial cells, the longer these molecules remain adsorbed on the cell surface [60].

The stability of the LAB/aflatoxin complex in a wide range of pH is an important factor in the use of these microorganisms to remove aflatoxin from foods, once gastric release of the toxin would have negative health implications. Therefore, the complex formed has to resist environmental stress caused by the gastrointestinal tract, such as low pH and presence of bile. When the influence of the presence of bile on the LAB/aflatoxin complex was analyzed, it was observed that *Lactobacillus casei* removed more AFB_1 when exposed to bile, suggesting that this exposure causes changes in the structure and composition of the bacterial cell wall, probably inducing the formation of new biding sites for aflatoxin, or increasing the size of the sites available [13].

The ability of *L. rhamnosus* (strains GG and LC705) and *Propionibacterium freudenreichii* spp. *shermanii* JS to remove AFB_1 from intestinal liquid medium extracted from the duodenum of chicks was investigated, and it was observed that AFB_1 concentration was reduced in 54% in only 1 minute in the presence of *L. rhamnosus* GG, whereas it was reduced in only 44% in the presence of *L. rhamnosus* LC705, and 36% in the presence of *P. freudenreichii* spp. *shermanii* JS [62]. The authors observed that the accumulation of AFB_1 in the intestinal tissue was reduced in 74%, 63%, and 37%, respectively, for *L. rhamnosus* (strains GG and LC705) and *P. freudenreichii* spp. *shermanii* JS, showing that these bacteria may affect aflatoxin bioavailability and be used to reduce its toxicity to humans and animals.

Rats treated with feed added of aflatoxin (3 mg/kg of feed) presented a significant decrease in the feed intake compared with the control group, different from the animals fed diets containing *Lactobacillus casei* and *Lactobacillus reuteri* (10 mL/kg of feed, with 1×10^{11} CFU/mL) and aflatoxin [63]. The second group did not show reduced feed intake. Consequently, animals treated with the diet containing only aflatoxin presented lower body weight, significant increase in serum levels of transaminase, alkaline phosphatase, cholesterol, triglycerides, total lipids, creatinine, uric acid, and nitric oxide; and in lipid peroxidation in the liver and kidneys, followed by a significant decrease in total antioxidant capacity. Treatment with bacteria was able to induce a significant improvement in all biochemical parameters and in the histological condition of the liver, with *L. reuteri* being more efficient than *L. casei*.

In Egypt, a pilot study investigated the effect of the addition of *L. rhamnosus* LC-705 and *P. freudenreichii* spp. *shermanii* JS in human diet on the levels of aflatoxin in feces samples. It was observed that from 11 of 20 volunteers, AFB_1 ranged from 1.8 to 6 µg AFB_1/kg feces, and after two weeks of supplementation with probiotic bacteria, there was a significant reduction in the excretion rate, showing that these strains have the ability to influence the concentration of AFB_1 in feces [64].

Microorganism	AF	Bound (%)	Conditions	Ref.
L. rhamnosus GG	B1			
Viable cells	5 µg/mL	78.4	2 x 10^10 cfu/mL, 0h, 37 °C, PBS	[17]
Freeze-dried cells		65	4h, 37 C, PBS	
Heat-treated cells		81	4h, 37 °C, PBS	
L. rhamnosus LC-705				
Viable cells		78.8	2 x 10^10 cfu/mL, 0h, 37 °C, PBS	
Freeze-dried cells		50	4h, 37 °C, PBS	
Heat-treated cells		82	4h, 37 °C, PBS	
L. gasseri		58.1	2 x 1010 cfu/mL, 0h, 37 C, PBS	
L. acidophilus		67.4	7 x 109 cfu/mL, 0h, 37 °C, PBS	
L. casei Shirota		33.2	1 x 1010 cfu/mL, 0h, 37 C, PBS	
E. coli		16.3	5 x 1010 cfu/mL, 0h, 37 °C, PBS	
L. paracasei, L. casei, L. brevis, L. plantarum and *S. cerevisiae*	$B_1, B_2, G_1, G2$ 4 or 40 µg/kg	18-33	6h, 37 °C , barley flour (50%), wheat flour (45%) and corn flour (5%) mixed with water in 1:1.5 proportion	[44]
		27-50	24h, 37 °C , barley flour (50%), wheat flour (45%) and corn flour (5%) mixed with water in 1:1.5 proportion	
Lc. lactis ssp. cremoris	B_1	5.6	1 x 10^10 cfu/mL, 24h, 37 °C, PBS	[23]
Lactobacillus delbrueckii	5 µg/mL	17.3		
Lb. acidophilus		18.2		
Lb. rhamnosus		22.7		
Lb. plantarum		28.4		
Lc. lactis ssp. lactis		31.6		
Bifidobacterium lactis		18.0		
Lb. helveticus		34.2		
Lc. lactis ssp. cremoris		41.1		
Lb. rhamnosus Lc		54.6		
Lb. acidophilus		20.7		
Lb. fermentum		22.6		
Lb. johnsonii		30.1		
Lb. rhamnosus		33.1		
Lb. amylovorus		57.8		
Lb. amylovorus		59.7		
Bb. lactis		34.7		
Bb. longum		37.5		
Bb. animalis		45.7		
Bb. lactis		48.7		

Microorganism	AF	Bound (%)	Conditions	Ref.
L. rhamnosus GG	B1			[27]
Viable cells	5 µg/mL	78.9	1 x 10^{10} cfu/mL, 1h, 37 °C, PBS	
Heat-treated cells		84.1		
Acid-treated cells		86.7		
L. rhamnosus LC-705				
Viable cells		76.5	1 x 10^{10} cfu/mL, 1h, 37°C, PBS	
Heat-treated cells		87.8		
Acid-treated cells		88.3		
L. acidophilus LC1				
Viable cells		59.7	1 x 10^{10} cfu/mL, 1h, 37 °C, PBS	
Heat-treated cells		74.7		
Acid-treated cells		84.2		
L. lactis subsp. lactis				
Viable cells		59.0	1 x 10^{10} cfu/mL, 1h, 37 °C, PBS	
Heat-treated cells		58.1		
Acid-treated cells		69.5		
L. acidophilus ATCC 4356				
Viable cells		48.3	1 x 10^{10} cfu/mL, 1h, 37 °C, PBS	
Heat-treated cells		69.7		
Acid-treated cells		81.3		
L. plantarum				
Viable cells		29.9	1 x 10^{10} cfu/mL, 1h, 37 °C, PBS	
Heat-treated cells		35.5		
Acid-treated cells		62.7		
L. casei Shirota				
Viable cells		21.8	1 x 10^{10} cfu/mL, 1h, 37 °C, PBS	
Heat-treated cells		41.5		
Acid-treated cells		32.3		
L. delbrueckii subsp. bulgaricus			1 x 10^{10} cfu/mL, 1h, 37 °C, PBS	
Viable cells		15.6		
Heat-treated cells		33.7		
Acid-treated cells		75.8		
L. helveticus				
Viable cells		17.5	1 x 10^{10} cfu/mL, 1h, 37 °C, PBS	
Heat-treated cells		29.8		
Acid-treated cells		58.1		
P. freudenreichii subsp. shermanii JS				
Viable cells		22.3	1 x 10^{10} cfu/mL, 1h, 37 °C, PBS	
Heat-treated cells		67.3		

Microorganism	AF	Bound (%)	Conditions	Ref.
Acid-treated cells		82.5		
Lc. lactis subsp. *cremoris*				
Viable cells		26.9	1 x 10^{10} cfu/mL, 1h, 37 °C, PBS	
Heat-treated cells		40.1		
Acid-treated cells		43.7		
S. thermophilus				
Viable cells		32.7	1 x 10^{10} cfu/mL, 1h, 37 °C, PBS	
Heat-treated cells		42.0		
Acid-treated cells		63.8		
E. Coli	B1	37	30 min, 37 °C, PBS	[22]
L. rhamnosus GG	2 μg/mL	37		
S. aureus		46		
Bifidobacterium sp. Bf6		25		
B. adolescentis 14		31		
B. bifidum BGN4		46		
Bifidobacterium sp. CH4		37		
B. longum JR20		37		
Bifidobacterium sp. JO3		41		
Lc. lactis	B1		10^7-10^8 cfu/mL,30 min, 37 °C, in:	[42]
Living cells	0.5	54.8	PBS	
	μg/mL	86.7	maize oil	
		82.3	sunflower oil	
		71.0	soybean oil	
Dead cells by boiling		81.0	PBS	
		100	maize, sunflower or soybean oil	
Dead cells by autoclaving		80.0		
			PBS	
S. thermophilus				
Living cells		81.0	PBS	
		91.5	maize oil	
		90.7	sunflower oil	
		66.5	soybean oil	
Dead cells by boiling		100.0	PBS	
		96.8	maize oil	
		81.7	sunflower oil	
		96.0	soybean oil	
Dead cells by autoclaving		83.0	PBS	

Microorganism	AF	Bound (%)	Conditions	Ref.
Yoghurt Culture	B1			[43]
	0.6mg/kg	97	42 °C/3h, pH 4.0, overnight, milk	
	1 mg/kg	91		
	1.4mg/kg	90		
	1 mg/kg	90	milk acidified with citric acid	
		84	milk acidified with latic acid	
		73	milk acidified with acetic acid	
L. acidophilus NCC12	M1			[46]
Living cells	5, 10 and	14.9-20.2	10^8 cfu/mL, 0, 4, 24 h, 37 °C, PBS	
	20	14.4-15.4	10^8 cfu/mL, 4h, 37 °C, milk	
Heated cells	ng/mL	17.0-24.9	0, 4 , 24 h, 37 °C, PBS	
		16.6-19.0	4h, 37 °C, milk	
L. acidophilus NCC36				
Living cells		20.4-25.3	10^8 cfu/mL, 0, 4, 24 h, 37 °C, PBS	
		21.8-22.7	10^8 cfu/mL, 4h, 37 °C, milk	
Heated cells		22.1-26.8	0, 4, 24 h, 37 °C, PBS	
		23.7-25.1	4h, 37 °C, milk	
L. acidophilus NCC68				
Living cells		10.2-16.0	10^8 cfu/mL, 0, 4, 24 h, 37 °C, PBS	
		7.8-10.5	10^8 cfu/mL, 4h, 37 °C, milk	
Heated cells		14.0-21.8	0, 4, 24 h, 37 °C, PBS	
		12.8-15.9	4h, 37 °C, milk	
B. bifidum Bb13				
Living cells		23.5-26.6	10^8 cfu/mL, 0, 4, 24 h, 37 °C, PBS	
		24.0-25.9	10^8 cfu/mL, 4h, 37 °C, milk	
Heated cells		24.3-28.9	0, 4,24 h, 37 °C, PBS	
		25.4-27.4	4h, 37 °C, milk	
B. bifidum NCC 381				
Living cells		16.6-22.1	10^8 cfu/mL, 0, 4,24 h, 37 °C, PBS	
		15.5-18.3	10^8 cfu/mL, 4h, 37 °C, milk	
Heated cells		17.4-23.5	0, 4 and 24 h, 37 °C, PBS	
		17.1-22.2	4h, 37 °C, milk	
L. rhamnosus		20.1-24.0	10^8 cfu/mL, 0, 4, 24 h, 37 °C, PBS	
Living cells		20.4-22.2	10^8 cfu/mL, 4h, 37 °C, milk	
Heated cells		23.4-27.8	0, 4 and 24 h, 37 °C, PBS	
		22.9-26.3	4h, 37 °C, milk	
Lactobacillus strains	AFM1	9.4-73.1	96 h, 37 °C , PBS	[47]

Microorganism	AF	Bound (%)	Conditions	Ref.
Lactococcus strains		4.5-38.3	96 h, 37 °C , PBS	
Bifidobacterium strains		7.8-41.6	96 h, 37 °C , PBS	
L. plantarum		73	96 h, 37 °C , PBS	
B. adolescentes		41.6	96 h, 37 °C , PBS	
Lactobacillus strains		64-80.5	96 h, 37 °C ,milk	
Lactococcus strains		46.0-68.5	96 h, 37 °C , milk	
Bifidobacterium strains		67.0-72.5	96 h, 37 °C , milk	
L. bulgaricus		80.5	96 h, 37 °C , milk	
B. adolescentes		73	96 h, 37 °C , milk	
L. rhamnosus strain GG (pre-cultured)	M1 0.15		5.3×10^8, 15 - 16h, , 37 °C, in:	[29]
Viable cells	µg/ml	50.7	PBS	
		18.8	skim milk	
		26.0	full cream milk	
Heat-killed cells		57.8	PBS	
		26.6	skim milk	
		36.6	full cream milk	
*L. rhamnosus*strain LC-705 (pre-cultured)				
Viable cells		46.3	PBS	
		69.6	skim milk	
		27.4	full cream milk	
Heat-killed cells		51.6	PBS	
		63.6	skim milk	
		30.1	full cream milk	
L. rhamnosus strain GG (lyophilized)				
Viable cells		53.8	1.0×10^{10}, 15-16h, 37 °C, PBS	
Heat-killed cells		56.2		
L. rhamnosus strain LC-705 (lyophilized)				
Viable cells		45.7	1.0×10^{10}, 15-16h, 37 °C, PBS	
Heat-killed cells		57.4		
L. lactis ssp. *cremoris* strain ARH74				
Viable cells		40.4	2.9×10^8, 15-16h, , 37 °C, PBS	

Microorganism	AF	Bound (%)	Conditions	Ref.
Heat-killed cells		38.9		
L. gasseri (ATCC 33323)				
Viable cells		30.8	3.9×10^8, 15-16h, , 37 °C, PBS	
Heat-killed cells		61,5		
L. acidophilus strain LA1				
Viable cells		18,3	1.7×10^9, 15-16h, , 37 °C, PBS	
Heat-killed cells		25,5		
L. rhamnosus strain 1/3				
Viable cells		18,1	3.9×10^8, 15-16h, , 37 °C, PBS	
Heat-killed cells		39,9		
L. rhamnosus strain GG	B_1			[48]
Pre-treatment:	5 µg/mL			
Pronase E				
Viable cells		66	1h, 37 °C, 5% CO_2, PBS	
Heat-treated cells		72	Boiled for 1h, PBS	
Acid-treated cells		85	2 mol/L HCl, 1h, 37 °C, 5% CO2	
Lipase				
Viable cells		76	1h, 37 °C, 5% CO2, PBS	
Heat-treated cells		74	Boiled for 1h, PBS	
Acid-treated cells		89	2 mol/L HCl, 1h, 37 °C, 5% CO2	
Phosphate Buffer				
Viable cells		86	1h, 37 °C, 5% CO2, PBS	
Heat-treated cells		85	Boiled for 1h, PBS	
Acid-treated cells		91	2 mol/L HCl, 1h, 37 °C, 5% CO2	
m-Periodater				
Viable cells		60	1h, 37 °C, 5% CO2, PBS	
Heat-treated cells		49	Boiled for 1h, PBS	
Acid-treated cells		36	2 mol/L HCl, 1h, 37 °C, 5% CO2	
Iodate				
Viable cells		83	1h, 37 °C, 5% CO2, PBS	
Heat-treated cells		84	Boiled for 1h, PBS	
Acid-treated cells		80	2 mol/L HCl, 1h, 37 °C, 5% CO2	
Urea				
Viable cells		64	1h, 37 °C, 5% CO2, PBS	
Heat-treated cells		60	Boiled for 1h, PBS	
Acid-treated cells		50	2 mol/L HCl, 1h, 37 °C, 5% CO2	
Water (Milli Q)				
Viable cells		76	1h, 37 °C, 5% CO2, PBS	
Heat-treated cells		83	Boiled for 1h, PBS	
Acid-treated cells		84	2 mol/L HCl, 1h, 37 °C, 5% CO2	

Microorganism	AF	Bound (%)	Conditions	Ref.
L. acidophilus	B1			[49]
Pre-treatment:	5 µg/mL			
None		56.6	4h, 37 °C, PBS	
Heat		71.9		
Ethanol		46.5		
Acid		87.0		
Alkaline		27.4		
L. casi				
None		22.4		
Heat		41.8		
Ethanol		21.8		
Acid		43.1		
Alkaline		12.0		
L. helveticus				
None		17.8		
Heat		28.5		
Ethanol		18.0		
Acid		56.3		
Alkaline		9.1		
L. bulgaricus				
None		16.3		
Heat		33.5		
Ethanol		15.9		
Acid		586		
Alkaline		8.3		
L. plantarum	M1		10^{10} cfu/mL, 15 min, 37°C, in:	[50]
Viable cells	0.15	5.6	PBS	
Heat-killed cells	µg/mL	8.1	PBS	
E. avium	PBS			
Viable cells	solution	7.4	PBS	
Heat-killed cells	0.5	6.6	PBS	
P. pentosaceus	µg/mL			
Viable cells	skimmed	8.7	PBS	
Heat-killed cells	milk	7.8	PBS	
L. gasseri				
Viable cells		21.4	PBS	
Heat-killed cells		22.8	PBS	
L. bulgaricus				
Viable cells		30.2	PBS	
Heat-killed cells		33.5	PBS	

Microorganism	AF	Bound (%)	Conditions	Ref.
		33.5	skimmed milk	
L. rhamnosus				
Viable cells		17.1		
Heat-killed cells		27.8	PBS	
		24.5	PBS	
			skimmed milk	
B. lactis				
Viable cells		16.9	PBS	
Heat-killed cells		23.6	PBS	
		32.5	skimmed milk	
L. delbrueckii subsp. *bulgaricus* CH-2	M1 10 ng/mL	18.7 27.6	4h, 37 °C, PBS 4h, 42 °C, milk	[51]
S. thermophilus ST-36		29.4 39.2 14.8	4h, 37 °C, PBS 4h, 42 °C, milk Yoghurt	
F. aurantiacum NRRL B-184	M1 10 µg/mL	100	5 x 10^{10} cfu/mL, 30 °C, 4h, PBS and milk	[52]
B. subtilis UTBSP1 Viable cells Cell Free Supernatant	B1 2.5 µg/mL	85.7 95 78.4	96h, 30 °C, nutrient broth culture 10^8 cfu/ml, 120 h, 30 °C, pistachio nuts 120 h, 35 °C, nutrient broth culture	[53]
B. subtilis ANSB060 "Inocula" suspension	B$_1$ G$_1$ M1 (0.5 µg/mL)	81.5 80.7 60	72h, 37 °C, Luria-Bertani medium	[54]
Cell Cell extract Culture Supernatant	B1	10.5 9.6 78.7	72h, 37 °C, PBS	
L. rhamnosus strain GG "In vivo" "In vitro" *L. rhamnosus* strain LC-705	B1 3 µg/mL	51 92 80	10^{10}cfu/mL: 1 min, duodenum of chicks 1 h, duodenum of chicks 37 °C, 1h , pH 7.3	[62]

Microorganism	AF	Bound (%)	Conditions	Ref.
"In vivo"		36	1 min, duodenum of chicks	
		71	1 h, duodenum of chicks	
"In vitro"		77	37 °C, 1h , pH 7.3	
P. freudenreichii subsp. *shermanii* JS				
"In vivo"		37	1 min, duodenum of chicks	
		82	1 h, duodenum of chicks	
"In vitro"		22	37 °C, 1h , pH 7.3	

Table 1. Aflatoxin binding / absorption by microorganisms. Note: PBS, Phosphate-Buffered Saline; cfu, colony formingunit.

4. Decontamination of Aflatoxins by Yeasts

Yeasts are non-photosynthetic organisms with a separate nucleus and complex life cycle. They are larger than bacteria, normally spherical, non-motile, and reproduce by budding. Although their main function is alcoholic fermentation, these organisms are also capable of producing enzymes and vitamins. The primary substrates for yeasts are fermentable sugars, which are mainly transformed in ethanol, carbon dioxide, and biomass under oxygen-limited conditions. Under adequate oxygen supply, yeast produces carbon dioxide, water, and biomass [65]. *Saccharomyces cerevisiae* (SC) is the most well-known and commercially important species of yeast, and SC strains are widely used in the production of alcoholic drinks and in the baking industry.

As it occurs with LAB, SC cells have been studied to evaluate their ability to remove aflatoxins from contaminated media. The most important results obtained until now are summarized in Table 2. Products based on SC (cell wall from baker and brewer yeasts, inactivated baker yeast, or alcohol yeast) was studied, and it was observed that in pH 3, 37 °C and 15 minutes of contact, AFB_1 removal ranged from 2.5% to 49.3%, depending on the concentration of the toxin in the medium, and on the yeast-based products used [66]. These authors also observed a decrease in toxin adsorption as the initial concentration increased, and concluded that adsorption is not a linear phenomenon. Similar results with a SC strain and AFB_1 concentration ranging from 1 to 20 µg/mL was also reported [56]. At the 1 µg/mL concentration, 69.1% AFB_1 was removed; at 5 g/mL, removal rate was 41%; and at 20 µg/mL, 34%. *S. cerevisiae* strains were isolated from animal feed, feces and intestines, and tested for their ability to tolerate gastrointestinal conditions and remove AFB_1 from a contaminated medium [67]. These researchers observed that all strains isolated were able to survive in gastrointestinal conditions, and that the percentage of toxin removed ranged among SC strains (10^7 CFU/mL), and with AFB_1 concentration used (16.4% to 82% of adsorption for 50 ng/mL AFB_1; 21.3% to 48.7% for 100 ng/mL AFB_1; and 20.2% to 65.5% for 500 ng/mL AFB_1).

The ability of SC (0.1%, 0.2%, and 0.3%) to adsorb AFB_1 in contaminated corn (150, 300, 450 and 800 µg/kg corn was analyzed [68]. The adsorption process showed an inversely proportional relationship with the concentration, that is, the greater the AFB_1 concentration in the medium, the lower the efficiency of AFB_1 removal by SC (16% to 66% for 800 µg/kg AFB_1 vs. 40% to 93% for 150 µg/kg AFB_1). The authors concluded, using densitogram analysis, that the adsorption process did not change the molecular structure of the mycotoxin, and that the decreased AFB_1 adsorption rates observed as the toxin concentration increased may possibly be caused by saturation of the adsorption sites on the SC cell. Other factors, such as length of incubation, pH, method of biomass purification, and methods of analysis, may also influence this process.

Immobilized SC cells (ATTC 9763) was investigated for their ability to remove AFB_1 from pistachio seeds, and it was observed that the amount of toxin removed was dependent on its concentration in the medium (40% and 70% of removal for concentrations of 10 ng/mL and 20 ng/mL AFB_1, respectively) [69]. The authors also concluded that this ability to remove the toxin was greater in SC exponential growth phase, and that the process was a quick one, being saturated after 3 hours of contact. Besides, the ability of SC cells to remove toxin was increased after treatment with acid (60% and 73% for 10 ng/mL and 20 ng/mL AFB_1, respectively) and heat (55% and 75%, respectively). In another study, authors also concluded that the treatment of SC cells with heat at 60 °C and 120 °C, and with chloric acid (2 mol/L) increased their ability to remove AFB_1 from the medium to 68.8%, 79.3%, and 72.1%, respectively, against 38.7% when viable yeast cells were used [56].

Heat treatment may increase the permeability of the external layer of the cell wall due to the suspension of some mannanes on the cell surface, leading to increased availability of previously hidden binding sites. Besides, countless physical-chemical changes take place on the cell wall during heat treatment, leading to more exposed binding sites. On the other hand, acid conditions may affect polysaccharides by releasing monomers, which are further fragmented in aldehydes after glycosidic bonds are broken. Continuous removal of aflatoxin, even after use of acid and heat treatments, confirms that yeast cell viability is not a significant factor for the removal of aflatoxin from the medium [69].

During the fermentation of broiler feed using LAB (3 strains of *Lactobacillus*) and SC strains resistant to gastric juices and bile, 55% AFB_1 was removed when AFB_1 concentration in the medium was 1 mg/kg, and 39% when concentration was 5 mg/kg AFB_1, after 6 hours [70]. This tendency for removal was maintained as incubation continued, and after 24 hours, the amount of AFB_1 removed was 73% and 53%, respectively, for the two concentrations of the toxin. The authors considered that, from a practical point of view, the most important factor was the 6-hour fermentation period, once the passage of feed through the gastrointestinal tract of broilers lasts from 4 to 8 hours. In reference [71], authors analyzed the ability of SC to remove AFB_1 from a contaminated medium at different pH values (3.0, 6.0, and 8.0), and observed that the three strains analyzed showed great ability to remove the toxin (41.6% to 94.5%), and that after washing, only a small amount of AFB_1 was released back into the medium. *In vitro* studies are not always good indications of the *in vivo* responses, as *in vivo*

studies are affected by physiological parameters, such as pH, peristaltic movements, and gastric and intestinal secretions.

In vivo studies using SC are not as rare as those with LAB, mainly in poultry science. Generally, SC is added to the feed as a growth promoter. However, the addition of yeasts has also presented beneficial effects against the exposure to AFB_1. It was observed that the addition of 1% SC to feed contaminated with 5 g/g of AFB_1 prevented loss of weight; liver and heart hyperplasia; and decreased serum albumin and total protein concentrations in broilers [72]. The addition of SC in feed containing aflatoxin decreased the deleterious effects on feed intake, weight gain, and feed conversion in Japanese quails [73]. Compared with control animals, weight gain was 37% lower in birds fed a diet added only of aflatoxin, and was 15% greater than the control in the group that received feed containing aflatoxin and SC. The authors concluded that the diet containing with only SC significantly improved all growth parameters investigated (about 40%), compared with the control group.

In a study with mice, it was observed that the addition of AFB_1 to the diet (0.4 and 0.8 mg/kg) caused a significant reduction in weight gain, and an increase of 85% (0.8 mg/kg) in the rate of micronucleated normochromatic erythrocytes (MNE) after 3 weeks of ingestion, compared with the control group [68]. When diets containing AFB_1 and SC (0.3%) were administered, weight gain was twice greater than in diets that contained only the toxin, and the rate of MNE increased only 46% (0.8 mg/kg) The authors stated that reduced body weight is one of the most common consequences of AFB_1 ingestion, because the toxin alters the activity of several digestive enzymes, giving rise to a malabsorption syndrome characterized by steatorrhea, hypovitaminosis A and a decrease in the levels of bile, pancreatic lipase, trypsin, and amylase. Besides, biotransformation of AFB_1 gives rise to several metabolites, particularly AFB_1-8,9-epoxide, which may bind covalently to DNA and proteins, changing enzymatic processes such as gluconeogenesis, Krebs cycle, and fatty acid synthesis [74]. MNE rate is used to determine the genotoxicity of AFB_1, because it quantifies broken chromosomes and whole chromosomes that are abnormally distributed to daughter cells, showing thus, that AFB_1 is a potent mutagenic agent.

A diet containing 5 g/g of aflatoxin (82.06% AFB_1, 12.98% AFB_2, 2.84% AFG_1, and 1.12% AFG_2) by female quails (49 to 84 days of age) led to decreased egg production, feed intake, and feed conversion (31%, 28%, and 47%, respectively) [75]. However, addition of SC (2 g/kg) significantly increased these parameters (16%, 4%, and 14%, respectively). They also observed that the diet with aflatoxins caused a marked decrease in weight gain and egg weight, besides increasing animal mortality (39%, 7%, and 50%, respectively), whereas addition of SC reverted the negative effect on these parameters (65%, 8%, and 50%, respectively). The authors stated that these negative effects of aflatoxins in egg production, feed intake, and feed conversion may have been caused by anorexia, apathy, and inhibition of protein synthesis and lipogenesis. Besides, affected liver function and mechanisms of use of protein and lipids may have affected performance criteria and the general health of the animals. In reference [76] authors reported that the components of the cells wall of SC are able to adsorb mycotoxins, stimulate the immune system, and compete for binding sites in the enterocytes, inhibiting intestinal colonization by pathogens.

SC cell wall is mainly made up of polysaccharides (80-90%), and its mechanical resistance is due to an inside layer composed of β-D-glucans, which are formed by a complex network of highly polymerized β-(1,3)-D-glucans, branched off as β-(1,6)-D-glucans, that have a low level of polymerization. This inside layer is firmly bound to the plasmatic membrane by linear chains of chitin, which have a significant role in the insolubility of the overall structure and packing of the branched β-D-glucans. Both chitin chains and β-D-glucans affect the plasticity of the cell wall. The external layer of the yeast cell wall is formed by mannoproteins, which have an important role in the exchanges with the external environment. This whole structure is highly dynamic and may vary according to the yeast strain, phase of the cell cycle, and culture conditions, such as pH, temperature, oxygenation rate, nature of the medium, concentration and nature of the carbon source. Thus, these differences in the composition of the cell wall among yeast strains are related with their ability to bind to the mycotoxin [77].

Studies have shown that the components of SC cell wall, called oligomannanes, after esterification, are able to bind more than 95% AFB_1 [78]. Addition of 0.05% glucomannanes in the basal diet improved broiler performance [79].

The possible binding mechanisms between yeast cell wall and mycotoxins were studied, and authors suggested that β-D-glucans are the components of the cell wall that are responsible for forming the complex with the toxin, and that the reticular organization of β-D-glucans and their distribution in β-(1,3)-D-glucans and β-(1,6)-D-glucans have an important role in the efficiency of the bond [77]. Besides, studies have shown that weak hydrogen and van der Waals bonds are involved in the complex chemical connection between the mycotoxins and β-D-glucans, a chemical interaction that is much more "adsorption" than "bond". As for AFB_1, they observed that the aromatic ring, the lactone and ketone groups of the polar form of AFB_1, or chemical bonds with glucose units in the single helix of the β-D-glucans, are what keep the toxin bound to the glucans.

It was demonstrated that yeast strains isolated from environments were animals are raised are able to bind to AFB_1 in saline solution (PBS, pH 7) [67]. These strains presented other properties that were beneficial to the host, such as the inhibition of pathogenic bacteria. Therefore, SC strains acted both as probiotics (co-aggregation and inhibition of pathogenic bacteria), and as mycotoxin adsorbents.

In reference [72], SC was able to reduce the deleterious effects of AFB_1 in the diet of broilers and in [68] authors replicated these findings in rats. Protective effect against aflatoxins produced by yeasts was confirmed in rats. However, when yeast cells were inactivated by heat, they were inefficient [80] but when glucomannanes extracted from the cell wall of yeasts were used, there was an increase in the efficiency of the bond with AFB_1, OTA and T-2 toxin [81-84], individually or in combination [75, 79, 85, 86]. The addition of SC in the diet reduced AFB_1 toxic effects in chickens [72, 87]. The ability of SC to reduce AFB_1 toxic effects in quails was demonstrated, and this effect was apparently more efficient with the increase in inclusion rates [88].

In [89] authors obtained a significant reduction in AFB_1 concentration during beer production, probably due to the bond between mycotoxins and SC cell. This hypothesis was supported by other studies [39, 90]. A 19% reduction in AFB_1 during dough fermentation in bread production was observed [91].

Microorganism	AF	Bound (%)	Conditions	Ref.
S. cerevisiae	B_1		15 min, 37 °C:	[66]
	0.0058-	7.6-49.3	YCW from brewer's yeast	
	6.35 µg/mL	7.6-29	YCW from brewer's yeast	
		10-24	Inactivated baker's yeast	
		4-29	YCW from baker's yeast	
		17-44	Inactivated baker's yeast	
		3-44	YCW from baker's yeast	
		23-35	YCW from baker's yeast	
		27-44	Alcohol yeast	
S. cerevisiae	B1			[56]
Strain A18	1 µg/mL	69.1	3h, 25 C, PBS	
	5 µg/mL	41		
	10 µg/mL	33		
	20 µg/mL	34.2		
Strain 26.1.11	1 µg/mL	65.1	3h, 25 C, PBS	
	5 µg/mL	37.2		
	10 µg/mL	31		
	20 µg/mL	32.6		
Pre-treatment:				
Heated cells 52°C	5 µg/mL	58.8	3h, 25 C, PBS	
Strain A18		56.5		
Strain 26.1.11				
Heated cells 55 °C		64.5		
Strain A18		64		
Strain 26.1.11				
Heated cells at 60 °C		68.8		
Strain A18		67		
Strain 26.1.11				
Heat cells at 120 °C		79.3		
Strain A18		77.7		
Strain 26.1.11				
2 mol/L HCl / 1h		72.1		
Strain A18		69.3		
Strain 26.1.11				
S. cerevisiae	B_1 (ng/mL)			[67]

Microorganism	AF	Bound (%)	Conditions	Ref.
	50 ng/mL			
Strain RC008	100	67.6	10^7 cells/mL, 1h, 37 °C, PBS	
	500	43.5		
	50	38.2		
Strain RC009	100	16.4		
	500	21.3		
	50	31.8		
Strain RC012	100	29.6		
	500	20.6		
	50	20.2		
Strain RC016	100	82.0		
	500	48.7		
		65.5		
S. cerevisiae	B1			[68]
Yeast concentration:	(µg/kg)			
0.1 %	150	40	37 °C, 24 h, corn	
	300	25		
	450	17		
	800	16		
0.2 %	150	88	37 °C, 24 h, corn	
	300	76		
	450	64		
	800	51		
0.3 %	150	93	37 °C, 24 h, corn	
	300	86		
	450	81		
	800	66		
S. cerevisiae ATTC 9763	B1			[69]
Pre-treatment:	(ng/mL)			
None	10	40	3 h, 25 °C, pistachio nuts	
	20	70		
Acid treated cells	10	60		
(2 mol/L / 90 min)	20	73		
Heat-treated cells	10	55		
(120 °C / 20 min)	20	75		
L. paracasei LOCK 0920,	B_1			[70]
L. brevis LOCK 0944, L.	1 mg/kg	55	37 °C, 6h fermentation in broiler	
plantarum LOCK 0945,	5 mg/kg	39	feed	
and S. cerevisiae LOCK				
0140				

Microorganism	AF	Bound (%)	Conditions	Ref.
S. cerevisiae	B$_1$			[89]
	1 µg /g	86	12 °C, 8 days, brewing process	
	10 µg /g	72		

Table 2. Aflatoxin binding by yeasts. YCW, Yeast Cell Wall

5. Concluding Remarks

Considering the data from several studies carried out until now, it may be observed that microorganisms, among them lactic acid bacteria and yeasts, have a huge potential application in aflatoxin degradation in foodstuffs. However, new studies are necessary to identify bacterial species with greater binding potential with aflatoxins, once there are differences in sensitivity and selectivity, besides the influence of factors that are intrinsic and extrinsic to the bacteria in the decontamination process. After this step of choosing species with greater efficiency has been overcome, new production technologies that are economically viable to be applied to human and animal foods may be developed.

Several studies have demonstrated that the cell wall of SC and LAB and their components are responsible for binding with aflatoxins. However, the mechanisms by which this bond occurs remain unclear. Cell walls with glucomannanes and manno-oligosaccharides have been pointed out as the responsible elements for AFB$_1$ bond with yeasts. The great advantage in the commercial use of these microorganisms as binding agents is that these strains are approved and already used in a wide range of fermented food products, being recognized as safe. However, aflatoxin may be released from the cell-aflatoxin complex with changes in the pH and temperature conditions. Therefore, further studies are necessary to determine the behavior of yeasts in the different environmental conditions before they are used commercially.

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