Invited review: Microbe-mediated aflatoxin decontamination of dairy products and feeds

Sejeong Kim,* Heeyoung Lee,* Soomin Lee,* Jeeyeon Lee,* Jimyeong Ha,* Yukyung Choi,* Yohan Yoon,*¹ and Kyoung-Hee Choi†¹

*Department of Food and Nutrition, Sookmyung Women's University, Seoul 140-742, Republic of Korea

†Department of Oral Microbiology, College of Dentistry, Wonkwang University, Iksan, Chonbuk 570-749, Republic of Korea

ABSTRACT

Aspergillus flavus, *Aspergillus parasiticus*, and *Aspergillus nomius* contaminate corn, sorghum, rice, peanuts, tree nuts, figs, ginger, nutmeg, and milk. They produce aflatoxins, especially aflatoxin B₁, which is classified as a Group 1 carcinogen by the International Agency for Research on Cancer. Many studies have focused on aflatoxin removal from food or feed, especially via microbe-mediated mechanisms—either adsorption or degradation. Of the lactic acid bacteria, *Lactobacillus rhamnosus* GG efficiently binds aflatoxin B₁, and a peptidoglycan in the bacterium cell wall plays an important role. This ability of *L. rhamnosus* GG should be applied to the removal of aflatoxin B₁. Aflatoxin can be removed using other aflatoxin-degrading microorganisms, including bacterial and fungal strains. This review explores microbe-associated aflatoxin decontamination, which may be used to produce aflatoxin-free food or feed.

Key words: aflatoxin, decontamination, *Lactobacillus rhamnosus* GG, adsorption, degradation

INTRODUCTION

Mycotoxins produced by fungi contaminate 25% of the cereals and grains marked for human consumption; of these, aflatoxins are among the most toxic types (Wild and Turner, 2002; CAST, 2003). *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*, which are known to contaminate corn, sorghum, rice, peanuts, tree nuts, figs, ginger, nutmeg, and milk, produce aflatoxins that are carcinogenic to the liver (Ellis et al., 1991; FDA, 2012). Aflatoxins are secondary metabolites of low molecular weight that are synthesized by some aspergilli. Four major aflatoxins are aflatoxin

B₁ (most carcinogenic), aflatoxin B₂, aflatoxin G₁, and aflatoxin G₂, and they have half-maximal lethal dose (LD₅₀) values varying from 0.3 mg/kg of BW in rabbits to 18 mg/kg of BW in rats (Moss, 1998; IARC, 2002; FDA, 2012). Aflatoxins are classified by the International Agency for Research on Cancer in 2012 as Group 1 carcinogens (i.e., carcinogenic to humans; IARC, 2014).

The occurrence of aflatoxins in foods and feeds has been frequently reported in many countries. For instance, many reports have shown that raw agricultural products—including nuts, cereals, fruits, vegetables, herbs and spices—were contaminated with aflatoxin B₁ at high levels, exceeding the maximum permissible limit (Chen et al., 2013; Guchi, 2015; Waliyar et al., 2015). In addition, contamination with aflatoxin M₁ has occurred in milk and milk products, including cheese, yogurt, and cream, and it remains even after milk pasteurization (Yitbarek and Tamir, 2013). Moreover, high levels of aflatoxin have been found in milk and dairy feed products, at contamination levels ranging from 0.028 to 4.98 µg/L and 7 to 419 µg/L, respectively, in a Greater Addis Ababa milk shed (Gizachew et al., 2016).

Many physicochemical technologies have been developed to decontaminate food or feed containing aflatoxin B₁, but most of them also cause unwanted alteration of food properties, such as decreases in safety and sensory quality, and unsatisfactory applicability and practicability. To prevent aflatoxin B₁ contamination in food, agricultural practices and storage conditions need to be improved (Wu et al., 2009; Gonçalves et al., 2015). Therefore, chemical, physical, and biological treatments have been suggested to minimize toxin production and eliminate mycotoxins in food and feed (Faucet-Marquis et al., 2014). Both chemical and physical approaches have drawbacks, including inefficient removal, lack of cost-effectiveness, or nutritional loss (El-Nezami et al., 1998a). Adsorbents as physical treatments have been widely used, and silicates, clays, and activated carbons are extensively available, but their efficacy depends on the chemical structure of the adsorbent: that is, the total charge and charge distribution, the size of the

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¹Corresponding authors: yoon@sookmyung.ac.kr and kheechoi@wku.ac.kr

pores, and the accessible surface area (Kabak et al., 2006; Di Natale et al., 2009). In addition, these nonedible materials need to be eliminated after aflatoxin decontamination from foods or feeds. Therefore, the use of probiotic strains has been suggested as a better technique for removing aflatoxin B₁ through adsorption, especially using *Lactobacillus rhamnosus* GG. Additionally, many other microorganisms have been reported to convert aflatoxin into less toxic substances. Therefore, the objective of this article was to review the published literature on aflatoxin B₁ decontamination by microbiological action, and to propose the applicability of microbes as additives for aflatoxin decontamination from dairy products and feeds.

BACTERIA-BASED PHYSICAL ADSORPTION

Yeast and a number of lactic acid bacteria can bind aflatoxins, decreasing aflatoxin bioavailability in feed or food. Because lactic acid bacteria prevent the growth of pathogenic bacteria by producing pathogen-inhibitory substances, and because most are used as probiotics and generally regarded as safe, they are considered a desirable method for aflatoxin removal (Hernandez-Mendoza et al., 2009). Among lactic acid bacteria, physical adsorption by *L. rhamnosus* GG has been extensively studied. Therefore, this review focuses more on describing the interaction between aflatoxin and *L. rhamnosus* GG.

Removal of Aflatoxin B₁ by *L. rhamnosus* GG

The application of lactic acid bacteria to remove aflatoxin B₁ is important for making food safer without changing its properties. Furthermore, lactic acid bacteria strains are known to be nonpathogenic and safe, and they function as natural agents and probiotics. El-Nezami et al. (1998a) examined the abilities of *L. rhamnosus* GG (ATCC53103), *L. rhamnosus* LC-705, *Lactobacillus acidophilus* ATCC4356, *Lactobacillus gasseri* ATCC33323, and *Lactobacillus casei* Shirota (YIT9018) to remove aflatoxin B₁. One of the strains, *L. rhamnosus* GG, was more efficient than *L. gasseri*, *L. acidophilus*, and *L. casei* (El-Nezami et al., 1998a; Oatley et al., 2000; Haskard et al., 2001). Indeed, *L. rhamnosus* GG was found to be capable of removing 80% of the aflatoxin B₁ from contaminated media (El-Nezami et al., 1998a). *Lactobacillus rhamnosus* GG is a gram-positive bacterium that was isolated in 1983 by Barry R. Goldin and Sherwood L. Gorbach (hence the letters GG; Silva et al., 1987). It has been used as a probiotic bacterium due to its resistance to gastric acid and bile and its great avidity for human intestinal mucosal cells, but it is a transient inhabitant (Conway et

al., 1987; Walter, 2008). It has powerful adhesive properties and can exclude or reduce pathogenic adherence, as well as produce substances antagonistic to foodborne pathogens (Gorbach, 2000). Many human trials have shown that *L. rhamnosus* GG reduced diarrhea in children and adults, including rotavirus diarrhea, traveler's diarrhea, and *Clostridium difficile* diarrhea (Oksanen et al., 1990; Oberhelman et al., 1999; Vanderhoof et al., 1999; Guandalini et al., 2000). For this reason, many in vitro studies have suggested the use of this strain as a mycotoxin-removal agent in food. Pierides et al. (2000) found that *L. rhamnosus* GG efficiently removed aflatoxin B₁ from PBS by 65 to 77%, and from skim milk and full-cream milk by 26.6 and 36.6%, respectively. A study by Vosough et al. (2014) also found that *L. rhamnosus* GG removed aflatoxin B₁ from de Man, Rogosa and Sharpe broth medium by 50%. The differences in removal efficiencies between these studies may have been due to the different matrices contaminated with aflatoxin. Bovo et al. (2014) found no difference in aflatoxin elimination between live and lyophilized *L. rhamnosus* GG cells. Therefore, lyophilized *L. rhamnosus* GG can be considered a practical alternative for aflatoxin B₁ decontamination in food.

The effect of *L. rhamnosus* GG on aflatoxin removal has also been confirmed in host cells and in animal models. Gratz et al. (2007) evaluated the potential of *L. rhamnosus* GG to reduce aflatoxin B₁ availability in vitro using Caco-2 cells, and found that treatment with the bacteria reduced aflatoxin B₁ uptake, resulting in the protection of Caco-2 cells from both membrane and DNA damage. This result suggested a beneficial role for *L. rhamnosus* GG upon dietary exposure to aflatoxin. Deabes et al. (2012) evaluated whether *L. rhamnosus* GG could remove aflatoxin in vivo and showed that oral administration of *L. rhamnosus* GG at 1×10^8 cfu for 7 d to male albino mice significantly decreased aflatoxin-induced toxicity (0.7 mg/kg of BW) by preventing oxidative stress, and by maintaining glutathione levels and superoxide dismutase activity. Another group assessed the activity of *L. rhamnosus* GG in vivo and demonstrated that rats fed aflatoxin B₁ (4.8 μ mol/kg of BW) along with *L. rhamnosus* GG were safer from the hazardous effects of aflatoxin B₁ (Gratz et al., 2006).

Taken together, these findings show that *L. rhamnosus* GG can be considered as a dietary supplement for effective aflatoxin removal from contaminated hosts, including humans and livestock.

Mechanism of Aflatoxin B₁ Decontamination by *L. rhamnosus* GG

To determine the mechanism of aflatoxin B₁ decontamination, El-Nezami et al. (1998b) evaluated the

binding of aflatoxin B₁ by both viable and nonviable (heat- and acid-treated) *L. rhamnosus* GG, revealing that even nonviable *L. rhamnosus* GG had the ability to bind aflatoxin B₁. Haskard et al. (2001) also showed that the binding of aflatoxin B₁ by viable and heat-treated *L. rhamnosus* GG strains appeared to be predominantly extracellular. Furthermore, the ability of *L. rhamnosus* GG to adsorb aflatoxin B₁ is enhanced by the physical and chemical conditions of the medium. When bacterial cells were treated with acid or heat, the ability to bind to aflatoxin was slightly increased (Haskard et al., 2000), and in a study from El-Nezami et al. (1998a), heat and acid treatments caused even more significant increases. Vosough et al. (2014) also showed that the aflatoxin B₁-binding capacity of viable (43%), heat-killed (49%), and acid-killed bacteria (50%) was not different. These results indicate that aflatoxin B₁ is not removed by metabolism, but because it becomes physically bound to molecular components of the bacterium.

Haskard et al. (2000) showed that the addition of protease or lipase to *L. rhamnosus* GG did not affect aflatoxin B₁ binding, suggesting that binding is related to the carbohydrate components of the bacterial species and is involved in the hydrophobic and electrostatic interactions between the bacterial component and the toxin. Carbohydrates exist in 3 major forms in the bacterial cell wall: exopolysaccharides, teichoic or lipoteichoic acids, and peptidoglycans (Lahtinen et al., 2004). Lahtinen et al. (2004) extracted exopolysaccharides and a cell wall isolate containing peptidoglycans from bacteria, to establish which components of the *L. rhamnosus* GG cell envelope are involved in aflatoxin B₁ binding. They suggested that peptidoglycans are associated with aflatoxin-B₁ binding and ruled out the involvement of exopolysaccharides. They also suggested that there was no evidence of the involvement of teichoic acids, citing a study by Knox and Wicken (1973) that showed no change in bacterial aflatoxin binding ability despite the removal of teichoic acid by trichloroacetic acid treatment.

Besides *L. rhamnosus* GG, other lactic acid bacteria have also displayed the ability to bind aflatoxin B₁. Lee (2005) showed that both heat- and acid-treated *Lactobacillus plantarum* KTCC3099, isolated from kimchi, also removed 49.8% of the aflatoxin B₁. Hernandez-Mendoza et al. (2009) recently examined the binding of aflatoxin B₁ by *Lactobacillus reuteri* NRRL14171, *L. casei* Shirota, *Bifidobacterium bifidum* NCFB2715, *Lactobacillus johnsonii* NCC533, and *L. casei* DN-114-001, finding that *L. reuteri* NRRL14171 and *L. casei* Shirota were the most efficient strains. The authors suggested that teichoic acids in the peptidoglycans may have been responsible for this binding, in contrast to the sugges-

tion provided by Lahtinen et al. (2004). In summary, aflatoxin B₁ binding by *L. rhamnosus* GG is clearly related to the bacterial peptidoglycans, but it has not yet been established which components are involved in the process.

Other Microbial Binders of Aflatoxin

Aflatoxin removal from foods or feeds can be accomplished with another lactic acid bacterium, *Enterococcus faecium*, which is naturally found in food and is added to dairy products such as cheese (Giraffa, 2003; Topcu et al., 2010). Furthermore, *E. faecium* strains isolated from the feces of healthy dogs have been shown to reduce the levels of aflatoxin B₁. The isolates were suggested as promising pet feed additives for aflatoxin decontamination. Similar to the findings of other studies on the aflatoxin-decontaminating capability of *L. rhamnosus*, nonviable and viable *E. faecium* strains show similar levels of decontamination, suggesting that the removal effect is again attributable not to metabolic transformation of the toxin, but to the bacterial physical adsorption (Fernández Juri et al., 2015).

Saccharomyces cerevisiae also has the ability to adsorb aflatoxin to its cell wall components, as well as resistance to salivary and gastrointestinal environmental conditions. These benefits may increase the possibility of its use as feed additive for livestock such as poultry and ruminants (Dogi et al., 2011; Pizzolitto et al., 2012a). Furthermore, its aflatoxin-binding capability was not altered by co-contamination with another mycotoxin, fumonisin, produced by *Fusarium* spp., whose removal is also achieved by binding to the cell wall components of the microorganism (Pizzolitto et al., 2012a).

Another aflatoxin-binding bacterial strain, *Brevibacillus lacterosporus*, has been isolated from the gastrointestinal tract of Japanese quails (Bagherzadeh Kasmani et al., 2012). Because many *Bacillus* species are usually regarded as excellent probiotics for animal feeds due to their long shelf life and resistance to a number of stress conditions (Shivaramaiah et al., 2011), these bacteria are also prospective beneficial additives for controlling aflatoxin contamination in feeds.

Aflatoxin M₁, which is found in milk, can be removed by *Lactobacillus* and *Bifidobacterium* strains through reversible binding, but a low level of aflatoxin M₁ is discharged back into the milk (Kabak and Var, 2008). Likewise, because adsorption-based aflatoxin removal is reversible, aflatoxin can be released into the gastrointestinal tracts of humans or animals that consume feeds or foods treated with aflatoxin-adsorbing microorganisms. Therefore, continuous administration of these microbial agents is needed to ensure that they reside

as gut commensal flora, and to decrease aflatoxin availability inside the host.

BIODEGRADATION

Physical and chemical methods lead to unwanted side effects, and adsorption-associated technologies still have limitations, such as aflatoxin residues in foods, feeds, and hosts. For this reason, aflatoxin would best be removed by detoxifying action for successful decontamination. Accordingly, to compensate for these drawbacks, microbe-mediated biodegradation methods have been suggested for effective control of aflatoxin contamination at a higher rate. Indeed, many studies have reported aflatoxin-degrading fungal and bacterial strains isolated from soil, feces, and crops (Tables 1 and 2). Many aflatoxin-degrading microorganisms cleave the lactone ring of coumarin, a key aflatoxin structure responsible for its toxicity (Lee et al., 1981; Guan et al., 2008). However, the use of living microorganisms as a food additive may raise safety issues and consumers may be reluctant to eat microbe-supplemented foods. The use of aflatoxin-degrading enzymes, produced by fungi and bacteria, may be able to overcome those drawbacks.

Enzymes of Fungal Origin

Aspergillus flavus secretes aflatoxin to thrive in the presence of other microorganisms in the same ecological niches (Shcherbakova et al., 2015). Some fungi such as *Phoma glomerata* PG41, a fungus pathogen causing fiber spoilage, survive and coexist with aflatoxigenic *A. flavus* by producing aflatoxin-degrading enzymes (Shcherbakova et al., 2015). Interestingly, other *Aspergillus* species are involved in aflatoxin degradation. For example, *Aspergillus niger*, an isolate from feed samples, can biodegrade aflatoxin B₁, potentially serving as a feed additive (Zhang et al., 2014).

Among fungal strains belonging to the phylum *Basidiomycota*, white-rot fungi such as *Peniophora*, *Pleurotus ostretus*, and *Trametes versicolor* convert aflatoxin B₁ into less toxic substances (Guan et al., 2008; Alberts et al., 2009). These fungal strains secrete oxidative enzymes such as laccase and manganese peroxidase, which contribute to detoxifying aflatoxin (Alberts et al., 2009; da Luz et al., 2012). Laccase, a copper-containing oxidase, is known to degrade various xenobiotics, including aflatoxin, due to its low substrate specificity (Alberts et al., 2009). The activity of these enzymes is attributed to cleavage of the aflatoxin lactone ring, abolishing or decreasing fluorescence (Lee et al., 1981; Motomura et al., 2003). Among these strains, the edible mushroom *Pleurotus ostretus* can use a variety of crop

wastes, including corn cobs, rice straw, rye, and sawdust as a nutrient for growth (Sánchez, 2010). Aflatoxin B₁ in contaminated rice straw was successfully degraded by *P. ostretus*, and the activity was enhanced in the presence of metal salts and surfactants, suggesting the usefulness of this fungus as an aflatoxin-biodegradable agent in agronomy and animal husbandry (Das et al., 2014). Manganese peroxidase purified from *Phanerochaete sordida* was capable of removing 70% of aflatoxin in vitro, increasing to 100% with multiple additions of the enzyme (Wang et al., 2011).

Producers of enzymes that are generally recognized as safe, such as *Rhizopus oryzae* and *Trichoderma reesei*, are also associated with detoxification of aflatoxins B₁, B₂, G₁, G₂, and M₁ (Hackbart et al., 2014). These strains reduced aflatoxin B₁ in contaminated defatted rice bran by 80% (Cacciamani et al., 2007). Other *Rhizopus* species, including *Rhizopus oligosporus*, *Rhizopus arrhizus*, and *Rhizopus stolonifer*, have displayed aflatoxin-degrading ability. Of the *Rhizopus* species, *R. oligosporus* had the most powerful activity, exhibiting the best ability in combination with *Saccharomyces cerevisiae*, an aflatoxin-biotransforming yeast (Kusumaningtyas et al., 2006). In fact, the aflatoxin B₁ contaminating the raw materials used for beer and wine can be converted into a less toxic substance, a hydrated form of aflatoxin B₁, during fermentation with *Saccharomyces* species, *Saccharomyces pastorianus* or *S. cerevisiae* (Inoue et al., 2013).

Armillariella tabescens, an edible mushroom, is a natural treatment agent for several diseases, including appendicitis, cholecystitis, hepatitis, and otitis media (JSNMC, 1977), and it is a powerful degrader of aflatoxin. Specifically, the fungal strain detoxifies aflatoxin by producing enzymes exerting dual reactions: epoxide formation and epoxide hydrolysis to dihydrodiol (Liu et al., 1998). The detoxifying enzyme is called aflatoxin-oxidase, unlike other aflatoxin-degrading enzymes, such as fungal laccase and horseradish peroxidase (Li et al., 2011). The dual reactions result in cleavage of the bisfuran ring, a primary toxic structure of the aflatoxin (Liu et al., 1998; McKean et al., 2006; Cao et al., 2011). Opening of the bisfuran ring does not cause a change in fluorescence, a physicochemical property of aflatoxin (Cao et al., 2011).

Enzymes of Bacterial Origin

Aflatoxin-detoxifying enzymes from fungi are usually more stable than those from bacteria, but enzymes of certain bacterial origin seem to work more rapidly than those of fungal origin (Praveen Rao et al., 1998).

Actinomycetales of the suborder *Corynebacterineae*, including *Mycobacterium*, *Rhodococcus*, and *Norcardia*

Table 1. Aflatoxin-degrading fungal strains

Name	Source	Enzyme	Activity ¹	Mechanism	Target type	Reference
<i>Armillariella tabescens</i>	NA ²	Aflatoxin-oxidase	3.72 U/mL	Opening of bisfuran ring	B ₁	Liu et al., 1998, 2001; Cao et al., 2011; Guan et al., 2015
<i>Aspergillus niger</i>	Grains	Laccase	118 U/L (55%)	Cleavage of lactone ring	B ₁	Alberts et al., 2009; Zhang et al., 2014
<i>Peniophora</i>	NA	Laccase	496 U/L (40.45%)	Cleavage of lactone ring	B ₁	Alberts et al., 2009
<i>Phanerochaete sordida</i>	NA	Manganese peroxidase	86%	Cleavage of lactone ring	B ₁	Wang et al., 2011
<i>Phoma glomerata</i>	Beans of <i>Phaseolus vulgaris</i> L.	NA	78%	NA	B ₁	Slishebakova et al., 2015
<i>Pleurotus ostreatus</i>	Decomposing tree trunk, laboratory collection	Manganese peroxidase, laccase	416.39 U/L (35.90%)	Cleavage of lactone ring	B ₁	Motomura et al., 2003; Alberts et al., 2009; Das et al., 2014; Yehia et al., 2014; Ginterová et al., 1980
<i>Rhizopus oligosporus</i>	NA	NA	64.50%	NA	B ₁	Kusumaningtyas et al., 2006
<i>Rhizopus oryzae</i>	Rice husk	Peroxidase	100%	Cleavage of lactone ring	B ₁ , B ₂ , G ₁ , G ₂ , M ₁	Hackbart et al., 2014
<i>Saccharomyces cerevisiae</i>	NA	NA	70%	Hydration	B ₁	Inoue et al., 2013
<i>Trametes versicolor</i>	NA	Laccase	1 U/mL (87.34%)	Cleavage of lactone ring	B ₁	Alberts et al., 2009
<i>Trichoderma reesei</i>	Culture collection	Peroxidase	100%	Cleavage of lactone ring	B ₁ , B ₂ , G ₁ , M ₁	Hackbart et al., 2014

¹Percentage of aflatoxin removal (%) or specific enzyme activity (units) indicate the activity of the fungal strain in aflatoxin degradation. Conditions of activity were different for each study.

²NA = not available.

Table 2. Aflatoxin-degrading bacterial strains

Name	Source	Enzyme	Activity ¹	Mechanism	Target type	Reference
<i>Bacillus licheniformis</i>	Thai fermented soybean	NA ²	74%	NA	B ₁	Petchkongkaew et al., 2008;
<i>Bacillus stearothermophilus</i>	NA	NA	87%	NA	B ₁	Smith and Harran, 1993
<i>Bacillus subtilis</i>	Fish gut, Thai fermented soybean	NA	85%	Cleavage of lactone ring	B ₁ , G ₁ , M ₁	Petchkongkaew et al., 2008;
						Ma et al., 2012; Fan et al., 2013; Gao et al., 2011
<i>Brachybacterium</i> sp.	Rabbit feces	NA	74.83%	Cleavage of lactone ring	B ₁	Guan et al., 2008
<i>Brevundimonas</i> sp.	Yellow cheek feces, goral feces	NA	78.10%	Cleavage of lactone ring	B ₁	Guan et al., 2008
<i>Cellulosimicrobium funkei</i>	Soil around coal factories	NA	97%	Cleavage of lactone ring	B ₁	Sun et al., 2015
<i>Enterobacter</i> sp.	Hog deer feces	NA	75.92%	Cleavage of lactone ring	B ₁	Guan et al., 2008
<i>Klebsiella</i> sp.	Rabbit feces	NA	77.57%	Cleavage of lactone ring	B ₁	Guan et al., 2008
<i>Mycobacterium fluoranthenorans</i> sp.	Soil of a former coal gas plant	NA	>90%	NA	B ₁	Teniola et al., 2005;
<i>Mycobacterium smegmatis</i>	ATCC 700084 (type strain)	F ₄₂₀ H ₂ -dependent reductase	83,000 nmol·min ⁻¹ ·μmol ⁻¹ enzyme	Reduction of α,β-unsaturated double bond C2-C6	B ₁ , B ₂ , G ₁ , G ₂	Hormisch et al., 2004
<i>Mycrococcus fulvus</i>	Deer feces	NA	96.96%	NA		Taylor et al., 2010; Lapalakar et al., 2012
<i>Flavobacterium aurantiacum</i>	NA	NA	>90%	NA	B ₁ , G ₁ , M ₁	Zhao et al., 2011
					B ₁	Ciegler et al., 1966; Smiley and Draughon, 2000;
						D'Souza and Brackett, 2001;
<i>Pseudomonas aeruginosa</i>	Grain kernels and soils	NA	82.80%	NA		Teniola et al., 2005
<i>Pseudomonas stutzeri</i>	<i>Budorcas taxicolor</i> feces	NA	90.03%	NA	B ₁ , B ₂ , M ₂	Sangare et al., 2014
<i>Pseudomonas putida</i>	NA	NA	100%	NA	B ₁	Li et al., 2012
<i>Rhodococcus erythropolis</i>	NA	NA	4.16 mU/mL	Cleavage of lactone ring	B ₁	Samuel et al., 2014
						Alberts et al., 2006; Teniola et al., 2005; Kong et al., 2012; Eshelli et al., 2015;
<i>Streptomyces aureofaciens</i>	Laboratory collection	NA	87.95%	NA	B ₁	Cserháti et al., 2013
<i>Streptomyces lividans</i>	Laboratory collection	NA	86.10%	NA	B ₁	Eshelli et al., 2015
<i>Stenotrophomonas maltophilia</i>	South American tapir feces	NA	84.80%	Cleavage of lactone ring	B ₁	Eshelli et al., 2015
						Guan et al., 2008

¹Percentage of aflatoxin removal (%) or specific enzyme activity (units) indicate the activity of the bacterial strain in aflatoxin degradation. Conditions of activity were different for each study.

²NA = not available.

genera, are known to biodegrade aflatoxin (Taylor et al., 2010). It has been reported that *Mycobacterium fluoranthenorans* sp. nov., which metabolized polycyclic aromatic hydrocarbons in the soil of a former coal gas plant, could biotransform aflatoxin B₁ into a less toxic substance, suggesting its use for decontamination of aflatoxin in foods and livestock feeds (Hormisch et al., 2004; Teniola et al., 2005). Furthermore, cofactor F₄₂₀-dependent reductase from *Mycobacterium smegmatis* has been shown to detoxify aflatoxin by catalyzing reduction of the α,β -unsaturated lactone moiety and its subsequent hydrolysis (Taylor et al., 2010; Lapalika et al., 2012). *Rhodococcus erythropolis*, a gram-positive bacterium closely related to *Mycobacterium*, has also been extensively studied for its effectiveness in aflatoxin B₁ degradation and optimization of the degradation conditions (Alberts et al., 2006; Kong et al., 2012). Extracellular extracts of *R. erythropolis* liquid culture were responsible for the bioconversion of aflatoxin by an enzymatic process (Alberts et al., 2006). The conditions showing the best degrading efficiency were 23°C and pH 7.0 (Kong et al., 2012). However, another research group had different findings for optimal conditions, suggesting they were 30°C and pH 6.0 (Esheli et al., 2015). Living cells of *Norcardia corynebacterioides* (formerly *Flavobacterium aurantiacum*) biodegraded aflatoxin B₁ in aqueous foods such as peanut milk, and the degradation was attributed to an enzyme that is maximally active at neutral pH (Hao and Brackett, 1988; Smiley and Draughon, 2000). *Streptomyces*, which belong to the family of *Actinomycetales*, are also involved in aflatoxin bioconversion (e.g., *Streptomyces lividans* and *Streptomyces aureofaciens*), and the activity of biodegradation was maximal in acidic conditions, at pH 5.0 (Esheli et al., 2015).

Interestingly, several bacteria harboring aflatoxin-degrading activity have been isolated from feces, with coumarin as their carbon source. The culture supernatant of *Stenotrophomonas maltophilia* was capable of degrading aflatoxin effectively at 37°C, pH 8.0 with the addition of ions Mg²⁺ and Cu²⁺, at the highest degradation efficiency (Guan et al., 2008). Unlike *S. maltophilia*, another *Stenotrophomonas* sp., an isolate from a soil sample, inhibited aflatoxin production and possibly prevented aflatoxin contamination in the presence of aflatoxin-producing *Aspergillus* strains (Jermak et al., 2013). Furthermore, other bacteria such as *Bacillus* spp., *Brachyбактерium* spp., *Enterobacter* spp., *Brevundimonas* spp., *Klebsiella* spp., and *Rhodococcus* spp., have been isolated from animal feces as metabolic degraders of aflatoxin (Guan et al., 2008).

Myxococcus fulvus, an isolate obtained from deer feces, possesses an extracellular enzyme that is responsible for the degradation-mediated removal of aflatoxins

B₁, G₁, and M₁ in solution (Zhao et al., 2011). Furthermore, the enzyme activity was maintained at a wide range of pH values (5–7) and temperatures (30–45°C), which may be beneficial for the efficient degradation of aflatoxin, especially in the animal digestive system (Zhao et al., 2011). Similar to enzymes from *Norcardia corynebacterioides* and *Stenotrophomonas maltophilia*, the activity of the enzyme was enhanced in the presence of Mg²⁺, although it was reduced in the presence of Zn²⁺, suggesting that these enzymes are from the same family (D'Souza and Brackett, 2000; Guan et al., 2008; Zhao et al., 2011). However, it seems difficult for bacteria to produce these enzymes on an industrial scale, because they can be easily contaminated with other bacteria during fermentation, and the production yield of the enzyme is low (Zhao et al., 2011).

Several studies have reported that *Bacillus subtilis* is also capable of detoxifying aflatoxins B₁, M₁, and G₁ (Petchkongkaew et al., 2008; Gao et al., 2011; Ma et al., 2012; Fan et al., 2013). The *B. subtilis* ANSB060 strain, an isolate from fish gut, inhibited the growth of *Aspergillus flavus*, degraded aflatoxin, and was resistant to adverse conditions such as simulated gastric and intestinal environments. More importantly, its detoxification effect was confirmed in a chicken broiler fed peanuts naturally contaminated with aflatoxins and in laying hens exposed to certain levels of aflatoxins, strengthening the case for its utility as a feed additive (Gao et al., 2011; Ma et al., 2012). Furthermore, *B. subtilis* and *Bacillus licheniformis* isolated from Thai fermented soybean biotransformed aflatoxins (Petchkongkaew et al., 2008).

It was recently reported that *Pseudomonas* strains also act as aflatoxin-degrading agents. *Pseudomonas stutzeri*, an isolate from *Budorcas taxicolor* feces, has been suggested as a good aflatoxin-degrader (Li et al., 2012). Furthermore, *Pseudomonas putida* has been shown to convert aflatoxin B₁ into the less toxic metabolite, aflatoxin D (Samuel et al., 2014). *Pseudomonas aeruginosa* N17-1, isolated from grain kernels and soils, displayed enzyme-based degradation of aflatoxins B₁, B₂, and M₁. The highest ratio of degradation was observed at 55°C and in the presence of metal ions such as Mn²⁺ and Cu²⁺, but not Mg²⁺, Li²⁺, Zn²⁺, Se²⁺, or Fe²⁺ (Sangare et al., 2014).

Cellulosimicrobium funkei, a novel strain known as an opportunistic pathogen (Petkar et al., 2011), reduced the amount of aflatoxin B₁ (97%) in ducklings by detoxifying the mycotoxin (Sun et al., 2015). More desirably, the administration of the bacterial strain to ducklings, even at high levels, was found to be non-toxicogenic, thus suggesting that *C. funkei* could be used as a feed additive, playing a role in diminishing aflatoxicosis in ducklings (Sun et al., 2015).

Many bacterial strains are associated with aflatoxin degradation and may be applicable as feed additives, especially for cows. However, because the effects of these bacterial strains vary according to environmental conditions such as temperature, pH, and the presence of metal ions as enzyme cofactors, further studies are needed to determine their practical use.

CONCLUSIONS

Live or dead microorganisms can decontaminate aflatoxin produced by *A. flavus* contamination in foods or feeds, either by binding the toxin to their cell wall components or by degrading the toxin into less toxic or nontoxic compounds. Yeast and lactic acid bacteria are involved in aflatoxin binding. Among these, *L. rhamnosus* GG efficiently binds aflatoxin B₁ in a process involving peptidoglycans, but further research is needed to establish which components of the peptidoglycan of *L. rhamnosus* GG take part in the binding of aflatoxin B₁. The aflatoxin-B₁-binding capability of *L. rhamnosus* GG should be applied to the practical decontamination of aflatoxin B₁. A variety of microorganisms, including bacterial and fungal species, contribute to aflatoxin detoxification by bioconversion. These microorganisms use degradation machinery to remove xenobiotics such as aflatoxin to survive in the same ecological niches as aflatoxigenic *Aspergillus* species. All of these strains execute bioconversion via an enzymatic process. Because many bacterial strains isolated from animal feces or guts are capable of degrading aflatoxin, they can be sustained for a long period in animal gastrointestinal tracts and continuously degrade aflatoxin in the gut, making them a promising feed additive for aflatoxin decontamination. These microorganisms should also be further researched for commercial application in dairy products and feeds.

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