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

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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 B_1, 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 B_1, 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 B_1. 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 B_1 (most carcinogenic), aflatoxin B_2, aflatoxin G_1, and aflatoxin G_2, and they have half-maximal lethal dose (LD_{50}) 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 B_1 at high levels, exceeding the maximum permissible limit (Chen et al., 2013; Guchi, 2015; Waliyar et al., 2015), In addition, contamination with aflatoxin M_1 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 7–419 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 B_1, 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 B_1 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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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 B_1 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 B_1 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, causing a decrease in 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 B_1 by L. rhamnosus GG

The application of lactic acid bacteria to remove aflatoxin B_1 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 B_1. 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 B_1 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 B_1 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 B_1 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 B_1 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 B_1 availability in vitro using Caco-2 cells, and found that treatment with the bacteria reduced aflatoxin B_1 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 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 B_1 (4.8 μmol/kg of BW) along with *L. rhamnosus* GG were safer from the hazardous effects of aflatoxin B_1 (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 B_1 Decontamination by L. rhamnosus GG

To determine the mechanism of aflatoxin B_1 decontamination, El-Nezami et al. (1998b) evaluated the