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Review paper

BIOLOGICAL CONTROL OF AFLATOXIN B1 BY PROBIOTIC BACTERIA

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ABSTRACT
Aflatoxins are large groups of mycotoxins that are produced by specific species of molds. Aflatoxin B1 (AFB1) has been known as the most potent toxin among various aflatoxins. Aflatoxins are highly toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic compounds. The main target organ for their toxicity and carcinogenicity is the liver. Probiotics are microbial food supplements that beneficially affect the health of the host. Probiotic bacteria are used to balance the intestinal flora and to prevent several gastrointestinal disorders. In this review, recent developments in biocontrol of AFB1 by probiotic bacteria have been investigated.

KEYWORDS: Probiotic, Biocontrol, Aflatoxin B1

INTRODUCTION

Aflatoxins are natural toxins produced as secondary metabolites by the filamentous fungi *Aspergillus flavus*, *Aspergillus nomius*, *Aspergillus parasiticus*, *Aspergillus ochraceoroseus*, *Aspergillus pseudotamarii*, *Aspergillus bombycis* and *Aspergillus tamari* (Chen et al., 2005; Corassin et al., 2013). Aflatoxins have sub-acute and chronic effects such as chronic hepatitis, liver cancer, hepatomegaly, jaundiceand cirrhosis in humans (Azizollahi Aliabadi et al., 2013). The major aflatoxins of concern are designated B_1, B_2, G_1 and G_2, also M_1 and M_2 as metabolic products of AFB1. AFB1 to G2 belong to Group 1, and M1 belongs to Group 2, according to IARC (Celik et al., 2005). Probiotics are usually defined as live microbial food ingredients beneficial to health which comprise of normal commensally bacteria as a part of the healthy human gut micro flora. Different species of microorganisms such as lactic acid bacteria or yeasts have been proposed for human use. Probiotics could be used for several conditions such as Diarrhea, Urinary Tract Infections, Irritable Bowel Syndrome, Cancer, Immune Disorders, Lactose Intolerance, hyper Cholesterolaemia, Inflammatory Bowel Disease and Allergy (Zerehpoosh and Darsanaki, 2013). The probiotic microorganisms consist mostly of the strains of the genera *Lactobacillus* and *Bifidobacterium*, but strains of *Bacillus*, *Pediococcus* and some yeasts have also been found as suitable candidates (Soccol *et al.*, 2010). Using microorganisms including bacteria, yeasts and nontoxigenic *Aspergillus* fungi are of the well-known strategies for the management of aflatoxins in foods and feeds (Yin *et al.*, 2008; Bata and Lasztity, 1999). In this paper, we review recent development in biological control of AFB1 by probiotic bacteria.

AFLATOXINS

Mycotoxins produced by *Aspergillus*, *Penicillium* and *Fusarium* spp. are natural contaminants in foods. Mycotoxins are well known to cause toxicities to humans and animals, any species of bacteria, fungi and yeasts have been shown to enzymatically degrade mycotoxins (Reddy et al., 2010; Yabe and Nakajima, 2004). Aflatoxins are a group of highly toxic secondary metabolites produced mainly by *Aspergillus* species fungi. Aflatoxins can be found mainly in cereals, oilseeds, tree nuts, spices, and milk. Among 18 different types of aflatoxins, such as B1, B2, G1, G2, P, Q, M1, M2, B2a, etc., were identified. The most commonly occurring ones in fungi cultures are aflatoxins B1, B2, G1, and G2, then aflatoxins M1 and M2 in milk. The AFB1 metabolite, 8, 9- epoxide, forms DNA adducts primary with N^7 of guanine (Fig 1) (Qinghua *et al.*, 2009).

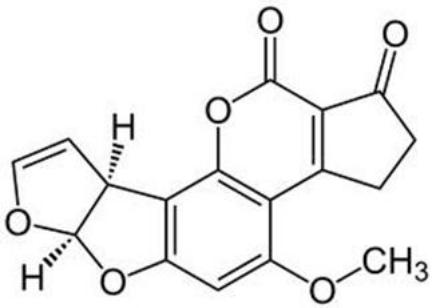

Figure 1. Chemical structure of AFB1 ($C_{17}H_{12}O_6$)

AFB1 has been known as the most potent toxin among various aflatoxins. This compound exerts its toxic effects upon the liver cells and it is responsible for inflicting damages on DNA, mutation, induction of abortion, cancer, birth deformity, suppression of immune system, and phytotoxic reactions (Hamidi et al., 2013). Toxic and especially carcinogenic effects of aflatoxins have been reported in humans and several different animals. Several outbreaks of aflatoxicosis have occurred in India and Africa, mostly in adults with poor nutritional status and maize as staple food. Recent outbreak of aflatoxicosis in May 2004 in Kenya has resulted in 125 deaths among 317 cases of poisoning. Numerous animal studies have shown that the liver is the main target organ and therefore the main symptoms of aflatoxin exposure in domestic and laboratory animals are hepatic injuries (Silvia, 2007).

PROBIOTIC BACTERIA

Probiotics are defined as the viable microorganisms that exhibit a beneficial effect on the health of the host by improving its intestinal microbial balance. Probiotic consumption is reported to exert a myriad of beneficial effects including: enhanced immune response, vaccine adjuvant effects, balancing of colonic microbiota, reduction of fecal enzymes implicated in cancer initiation, treatment of diarrhea associated with travel and antibiotic therapy, control of rotavirus and *Clostridium difficile*-induced colitis and prevention of ulcers related to *Helicobacter pylori*. Probiotics are also implicated in the reduction of serum cholesterol, the antagonism against food-borne pathogens and tooth decay organisms, the amelioration of lactose malabsorption symptoms as well as candidiasis and urinary tract infections (Zerehpoosh and Darsanaki, 2013; Kaur et al., 2002). A probiotic organism should be nonpathogenic and non-toxic, and also resistant to low pH and to bile salts to improve its chances of survival in the gastrointestinal tract (Suvarna and Boby, 2005). In Table 1 are listed some of the known probiotics available. The lactic acid bacteria group includes *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Bifidobacteria*. The non-lactic acid bacteria group includes *Bacillus* and the yeast *Saccharomyces.*

Table 1. Common probiotics for human use

Lactobacillus species	*Bifidobacterium* species	Other bacteria	Non-lactic acid producing bacteria
L. acidophilus	*B. adolescentis*	*E. faecalis*	*B. cereus*
L. casei	*B. animalis*	*E. faecium*	*B. subtilis*
L. crispatus	*B. bifidum*	*L. lactis*	*S. boulardii*
L. gasseri	*B. infantis*	*P. acidilactici*	*S. cerevisiae*
L. johnsonii	*B. lactis*	-	-
L. reuteri	-	-	-
L. rhamnosus	-	-	-

BIOCONTROL OF AFLATOXIN B1 BY PROBIOTIC BACTERIA

Microorganisms, especially bacteria, have been studied for their potential to either degrade mycotoxins or reduce their bioavailability. Among bacteria, lactic acid bacteria are the most important probiotic microorganisms typically associated with the human gastrointestinal tract. They are widely used in food industry because of their beneficial health effects in humans. One of the effects identified is the protection against toxins contained in foods such as

heterocyclic aromatic amines, amino acid pyrolysates, polycyclic aromatic hydrocarbons and mycotoxins (Topcu et al., 2010). LAB including some probiotic species have been searched and some strains have shown great ability to bind aflatoxin in contaminated medium, Although LAB mechanism of action on aflatoxin has not been clarified yet, it is suggested a physical union, an adhesion to bacterial cell wall components (polysaccharides and peptidoglycans), instead of covalent binding or degradation (Elsanhoty, 2014). El-Nezami *et al.* (1998) showed that probiotic lactobacilli have the ability to remove aflatoxins from contaminated liquid media. In addition, they showed that heat and acid treated (metabolically inactivated) cells performed aflatoxin removal. In study by Topcu *et al*. (2010) Both *Enterococcus faecium* M74 and *E. faecium* EF031 strains have the ability to remove AFB1 and patulin. While M74 removes19.3 to 30.5% of aflatoxin B1 and 15.8 to 41.6% of patulin, EF031 removes 23.4 to 37.5% of aflatoxin B1 and19.5 to 45.3% of patulin throughout a 48h incubation period. In study by Hamidi *et al*. (2013) two strains of *Lactobacillus pentosus* and *Lactobacillus beveris* exhibited the capability of absorbing and isolating aflatoxin B1 by respectively absorbing and discharging 17.4% and 34.7%. Peltonen *et al*. (2001) the ability of six probiotic bacteria to bind a common food carcinogen, AFB1, was assessed. The aflatoxin-binding capacity of the *Lactobacillus* and *Bifidobacterium* strains was found to range from 5.8 to 31.3%. In study by Khanafari *et al*. (2007) in 1h 45% and in 90h 100% AFB1 was removed from solution by *Lactobacillus plantarum* PTCC 1058. Haskard *et al*. (2001) reported that viable cells of *Lactobacillus rhamnosus* strain GG (ATCC 53103) and *L. rhamnosus* strain LC-705 (DSM 7061) removed AFB1 from solution. In study by Oluwafemi *et al*. (2010) a biological detoxification strategy was tested using bacteria of the *Lactobacillus* species collected from the biotechnology laboratory at University of Ibadan, Nigeria. Five different cultures consisting of *Lactobacillus acidophilus*, *L. brevis*, *L. casei*, *L. delbruekii*, and *L. plantarum* were used to inoculate the AFB1–contaminated maize samples at 37ºC. After 5 days, the residual AFB1 on maize was determined. All treatments showed significant reductions ($P< 0.05$) in AFB1. *L. plantarum* was the most efficient organism in degrading AFB1. Abdella *et al*. (2005) reported that *Lactobacillus* strains could remove more AFB1 than *Pediococcus* and *Leuconostoc* strains. In study by Slizewska *et al*. (2011) after 6h fermentation with the probiotic (*Lactobacillus paracasei* LOCK 0920, *Lactobacillus brevis* LOCK 0944, *Lactobacillus plantarum* LOCK 0945, *Saccharomyces cerevisiae* LOCK 0140), in feed mixture with a low concentration of AFB1 (1mg/kg), the amount of AFB1decreased by 55%.

CONCLUSION

AFB1 is a well-known carcinogen and is classified by the International Agency for Research in Cancer as a class 1 human carcinogen. Therefore, reducing its bioavailability is of great interest for human health. Several Probiotic bacteria have been found to be able to bind AFB1 in vitro and in vivo. Probiotic bacteria have antimutagenic and anticarcinogenic effects. The application of this phenomenon in the removal of mycotoxins from contaminated food and feed is urgently needed to improve the safety of food and feed. Additional studies are needed to investigate the mechanisms involved in the removal process of toxin by Probiotic bacteria aiming its application in dairy industry.

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