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Review

Potential of lactic acid bacteria in aflatoxin risk mitigation

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ABSTRACT

Aflatoxins (AF) are ubiquitous mycotoxins contaminating food and feed. Consumption of contaminated food and feed can cause a severe health risk to humans and animals. A novel biological method could reduce the health risks of aflatoxins through inhibiting mold growth and binding aflatoxins. Lactic acid bacteria (LAB) are commonly used in fermented food production. LAB are known to inhibit mold growth and, to some extent, to bind aflatoxins in different matrices. Reduced mold growth and aflatoxin production may be caused by competition for nutrients between bacterial cells and fungi. Most likely, binding of aflatoxins depends on environmental conditions and is strain-specific. Killed bacteria cells possess consistently better binding abilities for aflatoxin B_1 (AFB_1) than viable cells. Lactobacilli especially are relatively well studied and provide noticeable possibilities in binding of aflatoxin B_1 and M_1 in food. It seems that binding is reversible and that bound aflatoxins are released later on (Haskard et al., 2001; Peltonen et al., 2001). This literature review suggests that novel biological methods, such as lactic acid bacteria, show potential in mitigating toxic effects of aflatoxins in food and feed.

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Abbreviations: AF, aflatoxins; AFB_1, aflatoxin B_1; AFM_1, aflatoxin M_1; *A*, *Aspergillus*; *Bb*, *Bifidobacterium*; CYP, cytochrome P; CFU, colony forming units; DNA, deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; EU, European Commission; FAO, Food and Agricultural Organization; FB_1, fumonisin B_1; FB_2, fumonisin B_2; FDA, US Food and Drug Administration; HACCP, hazard analysis and critical control points; HCl, hydrochloric acid; LAB, lactic acid bacteria; *Lb*, *Lactobacillus*; *Lc*, *Lactococcus*; *L*, *Leuconostoc*; NaCl, sodium chloride; MIC, minimum inhibitory concentration; PBS, phosphate buffered saline; *P*, *Pediococcus*; *P*, *Propionibacterium*; *S*, *Streptococcus*; *W*, *Weissella*; WHO, World Health Organization.

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1. Introduction

Mycotoxins are a group of toxic secondary metabolites produced by fungi which are found ubiquitously in the soil. Elevated temperatures and humid environmental conditions promote the fungal growth and toxin production. Mycotoxins appear in six major classes: aflatoxins, fumonisins, ochratoxins, trichothecenes, zearalenone and ergot alkaloids.

Among mycotoxins, aflatoxins are the most toxic. AFB_1 is the most carcinogenic of the naturally occurring aflatoxins (IARC, 2002) and causes acute and chronic intoxication in humans and animals (Shetty et al., 2007). AFB_1 metabolizes to the 4-hydroxy derivate aflatoxin M_1 (AFM_1) in lactating animals including humans (IARC, 2002).

Aspergillus species produce aflatoxins and *Fusarium* species produce fumonisins (IARC, 2002). These two fungal genera frequently occur simultaneously and it is highly probable that fumonisin B_1 (FB_1) and AFB_1 are co-contaminants of foods (Pizzolitto et al., 2012). FB_1 and FB_2 constitute up to 70% of the fumonisins found in foods (Niderkorn et al., 2006).

Mycotoxins are present in foods as a result of raw material contamination, or deficiencies in harvesting, storage or processing. Mycotoxins in maize pose a notable health risk to humans and certain farm animals. In East and West African countries where maize is a staple food, dietary exposure to aflatoxins is frequent and may occur at high levels. Exposure to mycotoxins increases the prevalence of bacterial and parasitic infections (IARC, 2002).

Acute exposure to aflatoxins can cause aflatoxicosis, and in severe hepatotoxicity cases the mortality rate is approximately 25%. Chronic exposure to aflatoxins is associated with hepatocellular carcinoma, especially in the presence of hepatitis B infection. Other probable health impacts are immunological suppression, impaired growth and nutritional interference (Strosnider et al., 2006). These impacts have been demonstrated in various species of livestock and fish, and while they may have similar effects on humans causal evidence is still lacking.

Biological, chemical, and physical methods exist to eliminate, inactivate, and prevent fungal growth and toxin production. Most of these applications have not been adopted, especially in poor countries, due to high costs, loss of nutritional value, altered organoleptic characteristics of the products, practical difficulties, and (un)known effects to human health. On the other hand, traditionally used indigenous food microorganisms may have potential in prevention of mycotoxin-caused health impacts (Bhat et al., 2010; Di Natale et al., 2009).

Lactic acid bacteria (LAB) are commonly used in silage feed production and in fermented food product processes. LAB are known to inhibit fungal growth and extend the shelf-life of the product (Broberg et al., 2007). This literature review evaluates the potential of different LAB strains to prevent the growth of aflatoxin forming fungal strains, bind AFB_1 and AFM_1 *in vivo* and *in vitro* and examine the nature of aflatoxin-binding. The published studies will be discussed critically, and finally some conclusions will be drawn.

2. Aflatoxin in the feed — food chain

Aflatoxin-producing fungi are ubiquitous in the soil. The prevention of contamination and good crop handling practices play a key role in the control of fungal growth in foods and feeds. Maize and milk in different forms comprise a significant part of staple food diet in Kenya and some other parts of the East African region. Climate change is likely to worsen the challenges during harvesting and storage. Aflatoxin contamination is especially severe after a long-term storage in excessive heat and humid conditions. Damage-causing insects and rodents spread the fungal spores allowing proliferation. Even small exposure reductions from several sources in high-exposure areas can have important benefits (Turner et al., 2005).

Fungi live on the surface of the crop and *Aspergillus* species are dominant in favorable conditions of maize cultivation areas (Kpodo et al., 2000). In areas of seasonal food scarcity spoiled maize is occasionally used for human consumption, but even more often spoiled maize is fed to dairy animals and poultry. Aflatoxin does not accumulate in muscle meat, but is excreted in milk, urine, and feces and is found also in blood.

AFB_1 is metabolized predominantly by the cytochrome P450 enzyme system (involved in xenobiotic metabolism in humans) to produce a range of metabolites (Guengerich, 2001). The toxicity of AFB_1 is generally regarded to occur *via* CYP 3A4, but also by CYP 1A2 by the production of the highly reactive AFB_1 — 8,9-epoxide which forms covalent adducts with macromolecules, such as proteins and DNA. The 8,9-epoxide of AFB_1 is short-lived but highly reactive and is capable of causing damage to cells in the liver and at the intestinal interface. Direct damage caused by aflatoxin exposure within the intestine may alter nutrient uptake (Gratz et al., 2007).

According to a WHO estimation (Wu et al., 2011), 25,000 to 155,000 aflatoxin-induced liver cancer cases occur globally each year of which 40% are estimated to be in Africa. Co-occurrence of AFB_1 with hepatitis B increases the liver cancer risk 12-fold. Data from Wu and Tritscher (2011) suggests that children are more vulnerable to acute hepatotoxicity from ingested aflatoxins than adults.

Aflatoxins are genotoxic carcinogens and their combined and separate levels are subject to regulation in most countries. Accepted exposure levels are different due to the wide variety of standards, levels, and geographical area of consumption. The international levels are political compromises to promote trade. Little is known about specific threshold levels or health effects associated with consumption of aflatoxin concentrations between 20 μg/kg and 300 μg/kg (Strosnider et al., 2006).

US Food and Drug Administration (FDA) levels are set at 20 μg/kg for human foods, 0.5 μg/kg for milk, and 20–300 μg/kg for feed. The Codex Alimentarius limit for all foods worldwide is 15 μg/kg (FAO and WHO, 1999). In the European Union the limit for AFB_1 in maize products is 5 μg/kg and combined aflatoxins 10 μg/kg, other cereal products 2 μg/kg and combined aflatoxins 4 μg/kg, AFM_1 in raw milk 0.05 μg/kg, AFB_1 in infant cereal 0.10 μg/kg, and AFM_1 in infant milk 0.025 μg/kg (EU, 2006).

For feeds given to milk-producing animals, a level of 5 μg/kg AFB_1 is widely supported, as an AFM_1 level of 0.05 μg/kg is achievable with such a limit (FAO and WHO, 1993). The European Commission limit for AFB_1 in feeds is 20 μg/kg (EU, 2002). A summary of different AFB_1 and AFM_1 limits in different materials is presented in Fig. 1.

3. Potential of LAB to inhibit fungal growth and reduce toxin production

Aflatoxins are excreted by toxin-producing fungi, but fungal growth does not necessarily entail toxin production. Even if toxin production is

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