ROLE OF PROBIOTICS AND AFLATOXINS IN HUMAN HEALTH

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Abstract

Aflatoxins are a group of naturally occurring carcinogens that contaminate different human and animal food stuffs. The occurrence of aflatoxins in foods and food products vary with geographic location, agricultural and agronomic practices. People can be exposed to these toxins by consuming contaminated animal and plant products like peanuts, meat or dairy products. Agricultural workers and farmers are exposed by inhaling dust that is generated from contaminated crops and feed. Aflatoxin exposure is associated with an increased risk of liver cancer. According to the food and agriculture organization (FAO), the worldwide maximum tolerated levels of aflatoxin B1 was reported to be in the range of 1-20μg /kg in food and 5-50 μg in dietary cattle food. Probiotics are live bacteria and yeasts that are good for human beings, especially for digestive system. Different types of bacteria, some species of yeast are used as probiotics. Probiotics are used in fermentation and for the production of different foods. The probiotics and their metabolites like antimicrobial proteins, lactic acid, peptides, short chain fatty acids are used to control some pathogens, metabolic diseases and intestinal diseases like inflammatory bowel disease and cancer through multiple mechanisms. These aflatoxins are toxic to the probiotic microorganisms causing disorders in human health.

Keywords: Aflatoxins, Probiotics, Human health.

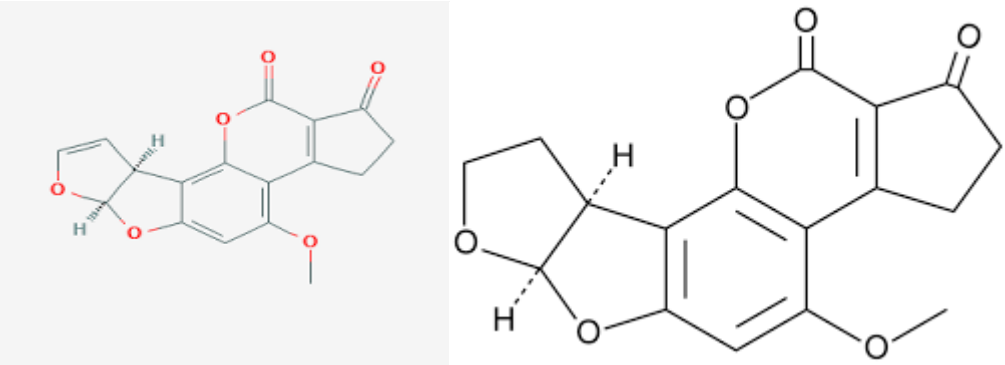
Introduction

Aflatoxins are secondary metabolites produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. Aflatoxin belongs to a group of fungal toxin known as mycotoxins [1,2]. The term mycotoxin combines the Greek word “mykes” which means fungus and the Latin word ‘toxicum” meaning poison [3]. Aflatoxins are white crystalline solids which are optically active and have a strong absorbance at 365 nm with a fluorescence emission of 415-450 nm. Aflatoxins are poisonous carcinogens and mutagens produced by fungi which grow in soil decaying vegetation and grains. They are regularly found in improperly stored staple commodities like chilli, pepper, peanuts, rice, cotton seed, millet, sorghum, sunflower seed, tree nuts, sweet corn, wheat and a variety of spices. Aflatoxins enter in to the body through contaminated food by transformation of products mainly eggs, milk products and meat. Aflatoxins are soluble in a range of organic solvents such as chloroform, methanol, acetone and ethanol. These are insoluble in lipophilic solvents like petroleum ether, diethyl ether and hexane. The major aflatoxins are B1, B2, G1, and G2, which can poisonous the body through respiratory, mucous or cutaneous routes, resulting in over activation of the inflammatory response [4].

The safe limit of aflatoxin lies in the range of 4-30 micro grams/kg for human consumption. Serious health complication occurs in humans and animals due to the consumption of aflatoxin contaminated food and feed [5, 6, 7]. Food processing techniques are not sufficient to eliminate aflatoxins from contaminated food and feed. This is due to the heat resistant nature of the aflatoxin.[8]. Twenty five percent of the world’s crop is affected by mycotoxins [9,10]

Chemistry of Aflatoxin

Aflatoxins are difurano coumarin derivatives which contains a bifuran group attached to the coumarin nucleus, and a lactone ring in case of AFGS or a pentanone ring in case of CAFBS. AFB1, AFB2, AFG1and AFG2 are the four major aflatoxins identified among 20. *A. flavus* produces B type toxins while G type toxins are produced by *A. Parasiticus.* [11] Eighteen enzymatic steps are involved in the biosynthesis of AFS. Regulation and biosynthesis of aflatoxin is controlled by 25 genes. [12,13].



Structure of aflatoxin B1 Structure of aflatoxin B2

Factors influencing *Aspergillus* Growth

Growth of *Aspergillus* and level of contamination of aflatoxin in food is influenced by various factors. These factors include climate of the region; type of the soil; temperatures; genotype of the crop and daily net evaporation [14]. Aflatoxin contamination of crop is also influenced by stress or damage due to drought prior to harvest and activity. Other factors like heavy rains at harvest and post harvest, poor timing of harvest, inadequate drying of the crop before storage, humidity and aeration during drying and storage also influenced [15].

Probiotics as food

To be recognized as functional food components, they should demonstrate the following properties: acid- and bile-stability, resistance to digestive enzymes, adhesion to intestine surface, antagonistic activity against human pathogens, anti carcinogenic and anti-mutagenic activity, cholesterol-lowering effects, stimulation of the immune system without inflammatory effects, enhancement of bowel motility, maintenance of mucosal integrity, improvement of bioavailability of food compounds and production of vitamins and enzymes. The technological properties of bacteria play a very significant role in the production of probiotics. They possess good sensorial properties, fermentative activity. Probiotics provide all sorts of powerful benefits for human body and brain. It can be consumed as food supplements and fermented foods. The following are important examples of probiotic foods.

The Gastro intestinal Tract of human contains various species of bacteria like *Bactroidetes, Firmicutes* and *Actinobacteria*. [16] The GIT of human is inhabited with 10^{th} to 14^{th} microorganism, which is tenfold greater than the human cell members. Consumption of sufficient concentration of probiotics can exert health benefits to the host. In addition to cellular function of probiotics, the extra cellular proteins help in bacterial colonization and establishment of gastrointestinal environment. The important health benefits due to the consumption of probiotics are improvement of gastro intestinal microflora, reduction of serum cholesterol, anti hypersensitivity effects, Cancer prevention, enhancement of immune system, enhanced lactose metabolism, atopic dermatitis. Certain evidences showed that nonviable bacteria as well as extract component from bacteria could also exert health potentials. Probiotics are also used to treat and prevent oral diseases.

General applications of probiotics

Foods act as carriers for probiotic delivery on to the human body. These food substances regulate the colonization and the bio active components of these foods alter the functionality and efficacy of the probiotics. [17] Originally probiotic delivery was consistently associated with foods mainly dairy foods, which gives good results. Now a day's probiotics are delivering as neutraceuticals such as in capsules which may lead to reduction in functional efficacy.

Maintenance of this ecological flora helps in preventing in infections. Various enteric pathogens like *Salmonella typhimurium, Shigella, Clostridium difficile, Escherichia coli,* and *Campylobacter jejuni* can be controlled by probiotic bacteria. Probiotic organisms also provide protection against urogenital pathogens like

Candida albican, Chlamydia trachomartis, Bacteroides bivius, and *Grandeneralla veginalis.* Probiotic bacteria are effective tools for controlling over growth of potentially pathogenic microorganisms.

Bifidobacterium with other probiotics are used to treat constipation, prevention and treatment for necrotizing enterocolitis in new born, and some food allergies. *Bacillus subtilis* spores are also used as probiotics for treating diarrhea. *Escherichia coli* is a common inhabitant of the lower intestine. *E coli* nissle 1917 is used as probiotic. This bacterium with other probiotics is used as treat constipation, ulcerative colitis, colon cancer, inflammatory bowel disease.

Effect of Aflatoxin on Human Health

Aflatoxin in foods is generally found as aflatoxin B1. It is responsible for aflatoxicosis and also has potent genotoxic and carcinogenic effect. The primary target of aflatoxins is the hepatic system. The effect of aflatoxin is dependent upon dosage and duration of exposure. Aflatoxin is linked with both toxicity and carcinogenicity in human and animal populations. Aflatoxicosis is a disease caused by aflatoxin consumption and acute aflatoxicosis resulted in death. Chronic aflatoxicosis is associated with immune supression cancer and other reproductive disorders. Anorexia, Malaise and low grade fever are the symptoms of early aflatoxicosis. Aflatoxin causes acute effects like haemorrhagic necrosis of the liver and bile duct proliferation abdominal pain, jaundice fulminate hepatic failure and death. Chronic effects include hepato cellular carcinoma (HCC) which is generally observed in association with hepatitis B virus or other risk factors. Aflatoxin exposure also causes stunted growth in children and immune suppression. Studies showed that aflatoxin plays a significant role in developing liver cancers in developing countries than developed countries. The carcinogenicity and mutagenicity of aflatoxin B1, G1 and M, are considered to arise as the result of the formation of a reactive epoxies at the 8, 9 positions of the terminal furan ring and its subsequent covalent binding to nucleic acids. The effect of aflatoxin B1 depends on the type of species, age, sex, weight, diet, exposure to infectious agents and the presence of other mycotoxins and toxic substances. The International Agency for Research on Cancer (IARC) has placed aflatoxin B1 as group, carcinogen. [18].

Toxins are poisonous substances produced by living organisms and are poisonous to other living organisms. Aflatoxins, ochratoxins are produced by fungi. In the liver it is metabolised to produce aflatoxin M1 and Q1. Aflatoxin B1 is a potent hepatotaxic; mutagenic, teratogenic and hepatocarcinogenic toxin produced by *Aspergillus flavus.* This toxin is produced from a wide range of crops like teratogenic mycotoxin produced by *Aspergillus flavus* and *parasiticus.* This toxin is produced from commodities like peanuts, corn, oilseeds and other cereals in small amounts. Aflatoxin B2 is a moderately potent toxin. This is hepatoxic, mutagenic, hepatocarcinogenic and tetragenic toxin that is produced by *Aspergillus parasiticus* and *flavus*. Aflatoxin B2 is metabolised to produce Aflatoxin M2. Aflatoxin G1 is similar to Aflatoxin B1. It is potent carcinogen and genotoxic mycotoxin produced by *Aspergillus parasiticus* and other *Aspergillus* species. This toxin is formed during the growth of crops like corn, peanuts, oilseeds, and other cereals. Aflatoxin G2 is a dihydroxy derivative of aflatoxin G1. This is mildly carcinogenic and genotoxic and produced by *Aspergillus parasiticus* and other species of *Aspergillus*. This toxin is present in a wide range of foods like nuts, seeds beans and spices. Aflatoxin M1 is a 4-hydroxy derivative of aflatoxin B1 which is found in the liver, kidneys, feaces, urine, blood and milk of mammals that have consumed aflatoxin B1 contaminated food and feed. Aflatoxin M1 is produced in small quantities by *Aspergillus flavus* and *parasiticus*. This toxin causes liver damage and cancers. U.V.radiation degrades aflatoxin M1. Aflatoxin M2 is less toxic than aflatoxin M1, it is produced in small amounts by *Aspergillus flavus* and *parasiticus.* Aflatoxin Q1 is less toxic than aflatoxin B1. This is 3-hydroxy derivative and a metabolite of aflatoxin B1 in humans and rats and primates.

Mechanism of probiotic activity

Probiotic microorganisms are considered to support the host health. However, the support mechanisms have not been explained. There are studies on how probiotics work. So, many mechanisms from these studies are trying to explain how probiotics could protect the host from the intestinal disorders. Production of some organic acids, hydrogen peroxide and bacteriocins which are inhibitory to both gram-positive and gram-negative bacteria.[19] Probiotics and pathogenic bacteria are in a competition. Probiotics inhibit the pathogens by adhering to the intestinal epithelial surfaces by blocking the adhesion sites. Despite of the lack of studies *in vivo*, probiotics inhibit the pathogens by consuming the nutrients which pathogens need.

Stimulation of specific and nonspecific immunity may be one possible mechanism of probiotics to protect the host from intestinal disease. This mechanism is not well documented, but it is thought that specific cell wall components or cell layers may act as adjuvants and increase humoral immune response.

Methods for the Reduction of Aflatoxin Exposure

Now a days, the physical and chemical techniques are used to eliminate aflatoxin from foods [20]. Removing mold-damaged kernels, seeds or nuts physically from commodities has been observed to reduce aflatoxins by 40-80% ([30]. The fate of aflatoxin depends on types of heat treatment (e.g., cooking, drying, pasteurization, sterilistion and spray drying [21]. Pasteurization of milk cannot protect against aflatoxin AFM1 [22]. By boiling corn can be reduced by 28%, frying after boiling reduced aflatoxin levels by 34-53%. Roasting nuts at above 90^{o}c reduce aflatoxin levels by 17-63% [23]. The decrease in aflatoxin content depends on the time and temperature. Alkaline cooking and steeping of corn reduces aflatoxin by 52% [24]. Addition of hydroxide (0.7 and 1%) or bicarbonate (0.4%) has enhanced the reduction of aflatoxin to 95%. Aflatoxin B1 and G1 are completely removed by ozone treatment at 8.5-40ppm. Biodegradation of aflatoxin by microbial and enzymatic degradation is preferred due to its eco-friendly nature [25]. *Flavobacterium aurantiacum* reportedly removes AFM1 from milk and *Nocardia asteroides* transforms AFB1 to fluorescent product. Other species that are able to degrade aflatoxins are *Ralstonia ruber, R. Globerulus, R. Gordoniae, R. Erythropolis, R. Coprophilus* [26]. The use of biocontrol agents like *Bacillus subtilis, Pseudomonas spp., Ralstonia spp., Lactobacillus spp* and *Burkholceria spp* are effective at controlling and management of aflatoxins [27]. Several strains of *B. subtilis and P. Solanacearum* isolated from the non-rhizosphere of maize soil have been reported to eliminate aflatoxin [28]. Pre and post harvest methods influences aflatoxin contamination. The important solution to control pre harvest aflatoxin contamination is through enhancing the ability of the crop to resist fungal infection or prevent production of afltoxins by the invading fungus which can be achieved through plant breeding or through genetic engineering crops. Post harvest measures to control aflatoxin contamination include usage of adequate storage conditions (moisture, temperature, aeration and mechanical or insect damage).

Degradation of toxin receptor:

Because of the degradation of toxin receptor on the intestinal mucosa, it was shown that *S. boulardii* protects the host against *C.difficile* intestinal disease. Some other offered mechanisms are suppression of toxin production, reduction of gut pH, attenuation of virulence.

Probiotics exhibit various mechanisms of action, but the exact manner in which they exert their effects is still not fully elucidated. Probiotics exert their effect by producing bacteriocins and short chain fatty acids , nutrient competition, immune modulation and lowering of gut pH and by producing certain interleukins. Certain lactic acid bacteria produce bio active peptides called bacteriocins and exert anti microbial activity against pathogenic bacteria. Probiotics provide therapeutic solution for anxiety and depression. Probiotic *Lactobacillus salivarius* exert anti inflammatory effect by producing IL 10/ IL12. According to Zhang *et al.*, [29]. probiotics exert hypo cholesterolemic effects through the assimilation of cholesterol by the secretion of bile salt hydrolase (BSH) Gut brain access is the bidirectional interactions between the GIT and Brain. Evidences explain that the alterations in the Gut microbiota greatly influence the interaction between Gut and brain and intern affect brain function as well as modulate behavior.

Detection of Aflatoxin

Present day needs the detection and quantification of aflatoxin in food and feed is an important aspect for the safety. Aflatoxins are detected and identified according to their absorption and emission spectra. Most frequently used methods for the detection of aflatoxins are thin layer chromatography (TLC), high performance liquid chromatography (HPLC), liquid chromatography and enzyme linked immune sorbent assay (ELISA) and Mass spectroscopy (LCMS).

Conclusion

The review has focused on different types of aflatoxins and sources of contamination of food with aflatoxin. The susceptibility of food product to fungal attack occurs during pre harvest transportation, storage, and processing of the foods. In tropical and sub tropical regions particularly in developing countries, aflatoxin contamination of food products is a common problem. Aflatoxins are associated with liver cancer, stunted growth in children and immuno suppression. Some of the evidences proved that aflatoxins are causing severe health problems and some other probiotic microorganisms are disease curing factors of chronic diseases. So, further studies of research is evidenced by Supplementation of Probiotics may be reducing the toxicity of aflatoxins and measured by using various physic- chemical techniques and mass spectroscopy studies.

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