Absorption of Aflatoxins by Lactic Acid Bacteria

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List of abbreviation

Aflatoxin B_1	AFB_1
Aflatoxin B_2	AFB_2
Aflatoxin G_2	AFG_2
Aflatoxins	AFs
Aspergillus flavus	*Asp. flavus*
Aspergillus parasiticus	*Asp. parasiticus*
De man, Rogosa and Sharpe agar	MRS agar
Enterococcus	*Ec*
Grey	Gy
Lactic acid Bacteria	LAB
Lactobacillus	*Lb*
Lactococcus	*Lc*
Optical density	OD
Phosphate Buffer solution	PBS

INTRODUCTION

Mycotoxins are toxic fungal metabolites found as contaminants in many agricultural products. Feeds and foodstocks contaminated with mycotoxins have a health risk to animals, human and cause big economical losses **(CAST, 2003 and Richard , 2007).** Aflatoxins (AFs) are secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* . AFs represent a group of closely related difuranocoumarin compounds **(Mishra and Das, 2003)**, they are potent hepatocarcinogens in human **(IARC, 1993)**. Aflatoxin B_1 (AFB_1) is considered one of the most potent carcinogenic agents in nature **(Zinedine *et al.*, 2005)**. Its ingestion is associated with hepatic and renal necrosis, mutagenic, carcinogenic, tetratogenic and immunosupptessive effects, as well as Reye syndrome ***(*Bennett and Klich 2003; Ruiqian *et al.*, 2004).**

Aflatoxins have been found in many foods and animal feeds and their production can be influenced by several factors including; temperature, water activity, pH, available nutrients and competitive growth of other microorganisms **(Ellis *et al.*, 1991).** The incidence of AFs is worldwide but is typically much higher in tropical and subtropical regions, where warmer climates provide optimal conditions for mould growth. *Aspergillus* genus grow on grains (corn, sorghum, millet) ,beans ,peanuts and tree nuts (almonds, pistachios, etc.) during growth, harvest, storage and transportation

so fungal contamination occure easily, make its prevention difficult **(Pereyra *et al.*, 2010*).***

Numerous pre- and post harvest methods have been used for detoxification or inactivation of aflatoxins, including physical separation, thermal inactivation, irradiation and chemical treatments **(Rustom, 1997).** Once food is contaminated with aflatoxin , there are only two options if the food is to be used : either the toxin is removed or the toxin is degraded into less toxic or non-toxic compounds . The first option is allowed when aflatoxin is present in identifiable pieces of food which can be removed from the remainder of the lot, or when solvents can be used to extract aflatoxin without leaving unwanted residues or markedly altering the composition of the food. As for the second option, a variety of methods have been developed. Aflatoxin may be degraded by physical, chemical, biological methods **(Park, 1993).** Physical methods to aflatoxin destruction involve treating with heat, UV light or ionizing radiation. These current methods are not very effective. Chemical degradation of aflatoxin is usually carried out by the addition of chlorinating, oxidizing or hydrolytic agents. Chemical treatments require expensive equipment and may result in losses of nutritional quality of treated commodities. In addition, the undesirable health effects of such treatments have not been fully evaluated **(Samarajeewa *et al.*, 1990 and Phillips *et al.*, 1994).**

New strategies involving use of microorganisms for biological detoxification of aflatoxin have been investigated. Specific strains of lactic acid bacteria have the ability to remove mutagenic contaminants from food **(Turbic *et al.*, 2002; Corthier, 2004)**. The studies reported that certain *Lactobacilli* and *Bifidobacteria* can bound AFB_1 from liquid solution **(El-Nezami *et al.*, 1998a; Peltonen *et al.*, 2000, 2001; Haskard *et al.*, 2001*)***.* Also, LAB are known to inhibit mold growth and bind aflatoxins in different matrices. Reduced mold growth and aflatoxin production may be caused by competition for nutrients between bacterial cells and fungi or due to antifungal compounds produced by LAB. Binding of aflatoxins depends on environmental conditions and is strain-specific **(Haskard *et al.*, 2001; Peltonen *et al.*, 2001 and Ahlberg *et al.*, 2015)**. The present study aimed to isolate local strains of LAB that can bind AFB_1 with high efficiency.

REVIEW OF LITERATURE

1. Aflatoxins in Foods

Aflatoxinxs (AFs) are poisonous carcinogenic compounds produced by *Aspergillus flavus* and *Aspergillus parasiticus* which grow in soil, decaying vegetation, hay, and grains. They are regularly found in improperly stored commodities such as chili peppers, corn, cotton seeds, peanuts, rice, sesame seeds, sorghum, sunflower seeds, tree nuts, wheat, and a variety of spices. When contaminated food is processed, aflatoxins enter the general food supply where they have been found in both pet and human foods, as well as in feed stocks for agricultural animals. Aflatoxins have an undeniable role in the deterioration of the marketable quality and hygiene of foodstuffs. So these toxins have been identified and responsible for significant problems in foodstuffs **(Azizollahi *et al.*, 2013)**

Among mycotoxins, aflatoxins are the most toxic. There are different types of aflatoxins B_1, B_2, G_1 and G_2, also M_1 and M_2 as metabolic products of AFB_1. AFB_1 to G_2 belong to Group 1 carcinogen, M_1 and M_2 belongs to Group 2 carcinogen, AFB_1 is the most carcinogenic type of aflatoxins **(IARC, 2002)** and causes acute and chronic intoxication in humans and animals **(Shetty *et al.*, 2007).**The metabolized aflatoxin M_1 (AFM_1) and M_2 are

excreted into the tissues, biological fluids and milk of lactating animals including humans **(IARC, 2002).**

2. Physicochemical properties of Aflatoxins

The aflatoxins compounds are colorless to pale yellow crystals. Fluorescence in ultraviolet (UV) light is blue by aflatoxins B_1 and B_2, and green fluorescence by aflatoxins G_1 and G_2. Afs optically active and have a strong absorbance at about 365 nm with a fluorescence emission of 415–450 nm, depending on the solvent or physical status. The aflatoxins are soluble in a range of organic solvents such as chloroform, ethanol, methanol, and acetone, and insoluble in lipophylic solvents such as hexane, petroleum ether, and diethyl ether. The molecular formula of aflatoxin B_1 is $C_{17}H_{12}0_6$, and of aflatoxin G_1 is $C_{17}H_{12}0_7$; aflatoxins B_2 and G_2 were found to be the dihydro derivatives of the parent compounds, $C_{17}H_{14}0_6$ and $C_{17}H_{14}0_7$ **(Moss, 2003)**.

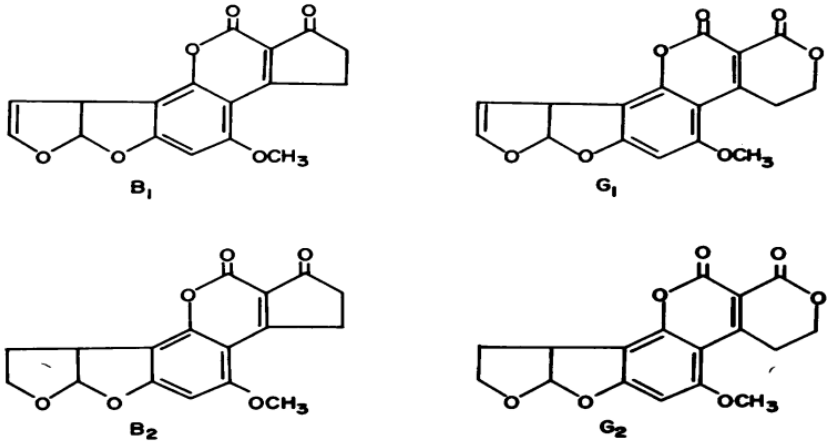

Figure (1): Structures of aflatoxins

3. Biological effect of aflatoxins

Exposure to aflatoxins can cause aflatoxicosis and severe hepatotoxicity and hepatocellular carcinoma, resulting in mortality rate reaching to 25%. Moreover AFs side effects are immunological suppression, impaired growth, nutritional interference, teratogenic, neurotoxic, nephrotoxic and hepatotoxic **(Williams *et al.*, 2004 and Strosnider *et al.*, 2006).** 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)** and **Wild, (2007)** reported that children are more susceptible to acute hepatotoxicity from ingested aflatoxins than adults.

AFB_1 is metabolized predominantly by the cytochrome P450 enzyme system to produce highly reactive AFB1-8,9-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).** Aflatoxin does not accumulate in muscle meat, but is excreted in milk, urine, and feces and is found also in blood. Accepted exposure levels are different due to the wide