Binding of Aflatoxin G_1, G_2 and B_2 by Probiotic *Lactobacillus* spp.

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ABSTRACT : The ability of ten probiotic bacteria to bind a common food carcinogen aflatoxin G_1,G_2 and B_2 was assessed. The strains were incubated *in vitro* with aflatoxins and the toxin residues in the supernatant were measured using high performance liquid chromatography. The aflatoxin G_1 binding capacity of the strains was found to strain dependent, most efficient binding of AFG_1 was observed by *L. acidophilus* CU028 and *L. brevis* CU06 which bound approximately 50%. *L. acidophilus* CU028 was capable of bind approximately 67% of AFG_2, difference in their binding ability showed statistical significance ($p>0.05$). *L. acidophilus* CU028 and *L. helveticus* CU 631 were the best binders and the strains were observed to possess variable AFB_2-binding ability in the range was from 38.0% to 55.9%. *Lactobacillus acidophilus* CU028 was the best common binders of the three types of food carcinogen aflatoxins. The application of binding phenomenon in the removal of mycotoxins from contaminated feeds is urgently needed to improve the safety of feeds. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 11 : 1686-1689)***

Key Words : Aflatoxin (G_1, G_2, B_2), Binding Activities, *Lactobacillus* spp.

INTRODUCTION

Aflatoxins represent a group of closely related difuranocoumarin compounds produced by the common fungal molds *Aspergillus flavus* and *A. parasiticus*. They are a group from fungal secondary metabolites that are recognized as being of economic and health importance and are potent hepatocarcinogens in several species of animals (Eaton and Callinger, 1994). The link between aflatoxin exposure and both hepatoxicity (aflatoxicosis) and liver cancer are well established. Aflatoxicosis characterized by jaundice, ascites and other signs of hepatic failure has been described in human, and immunosuppression effects caused by aflatoxins has been demonstrated in laboratory animals.

They are found in many foods and feeds and considered as a major public health problem especially in developing coutries where long term food storage is often inadequate for high heat and humidity, which encourage the growth of mold. 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). Once foods are contaminated with aflatoxins, there are only two options, either the toxin is removed or the toxin is degraded into less toxic or non toxic compounds. It has been observed that many microorganisms are able to remove or degrade aflatoxins in foods and feeds (Marth and Doyle, 1979), which has been known as probiotic bacteria, and they are used to balance the intestinal flora and to prevent several gastrointestinal disorders (Yoon and Won, 2002). Biological detoxification of aflatoxin has not been established in practice therefore the aim of this study was to elucidate and compare the effects of lactobacilli on the removal and binding activities of three types of alfatoxins G_1, G_2 and B_2.

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Received May 29, 2003; Accepted July 16, 2003

MATERIALS AND METHODS

Bacterial strains and media

The strain and sources of bacteria used in this study were given in Table 1. *Lactobacillus* spp. were cultured in MRS broth (Difco, USA) at 37°C and maintained in 11% skim milk containing 0.75 M adonitol at -70°C. The estimation of bacterial concentration was performed using standard plate count method using standard plate count agar (Difco USA) employing Accucount 1.000 (Biologics, USA).

Aflatoxin binding assay

Solid aflatoxin G_1, G_2 and B_2 (Sigma, St Lousis, MO, USA) was suspended in benzene/acetonitrile (97:3 v/v) to obtain an AF G_1, G_2 and B_2 concentration of approximately 2 mg ml^{-1}. A working solution of 5 μl ml^{-1} of AF G_1, G_2 and B_2 were prepared in phosphate-buffered saline (PBS, pH 7.3) and the benzene/acetonitrile was evaporated by heating in a water bath. For each strain a concentration of 1-1.5×10^{10} bacteria ml^{-1} was adjusted to avoid the effect of bacterial concentration on alfatoxin binding. A volume of the culture broth conrresponding to 1-1.5×10^{10} bacteria based on SPC estimation was centrifuged (3,000×g, 10-15 min) and the bacterial pellets were washed with 5ml of water. Bacterial pellets were resuspended in 1.5 ml of an AF G_1, G_2 and B_2 solution and incubated for 24hr at 37°C. The bacteria were pelleted (3,000×g, 10-15 min) and samples of the supernatant fluid were collected and stored frozen.

Quantitation of aflatoxin an AF G_1, G_2 and B_2 by HPLC

The supernatant samples were analysed with a reverse phase high-performance liquid chromatography (HPLC)

Table 1. Sources of strains of *Lactobacillus* spp. used in this investigation

Spieces	Strains	Source
L. casei	CU 001	Lab. of Dairy Microbiol. Chung-Ang University
L. helveticus	CU 631	〃
L. rhamnosus	CU 02	Isolate from Bio-deodorizing agent (EasyFix) 〃
L. plantarum	CU 03	〃
L. laflfinolactis	CU 04	〃
L. brevis	CU 05	〃
L. brevis	CU 06	〃
L. fermentum	CU 07	〃
L. salivarius	CU 041	〃
L. acidophilus	ATCC 4356	Food Research Institute of Canada
L. rhamnosus GG	ATCC 5310	〃
L. acidophilus	CU028	Lab. of Dairy Microbiol. Chung-Ang University
L. casei	YIT 9018	〃

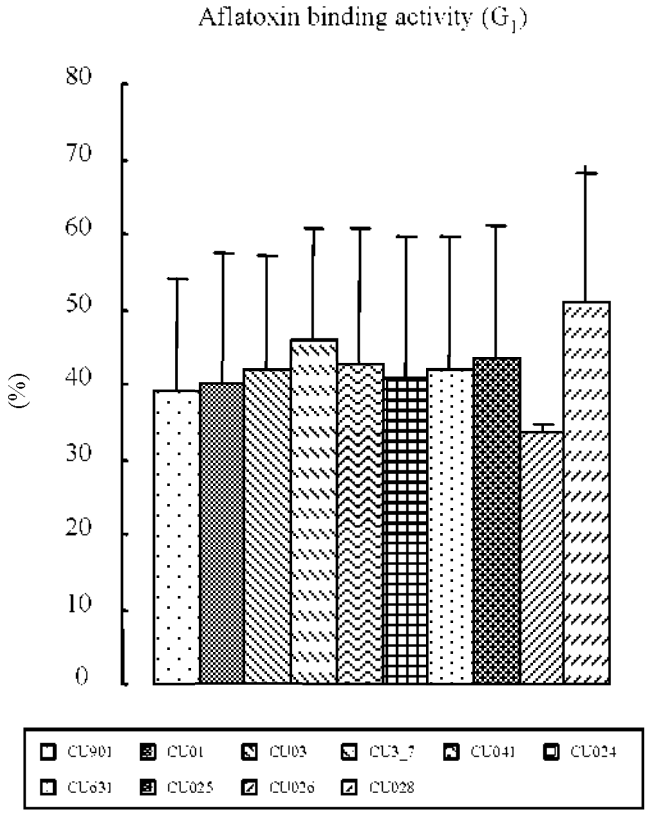

Figure 1. Aflatoxin AFG_1 binding activity of *Lactobacillus* spp.

method for the quantitation of residual AF G_1, G_2 and B_2 in the samples. MQ water/acetronitrile/methanol (6:3:1 v:v:v) was used as a mobile phase and the flow rate was 1 ml min^{-1}. The sample injection volume was set to 50 μl and the retention time of AF G_1, G_2 and B_2 was approximately 9.6 min, 9.5 min. 9.5 min. respectively.

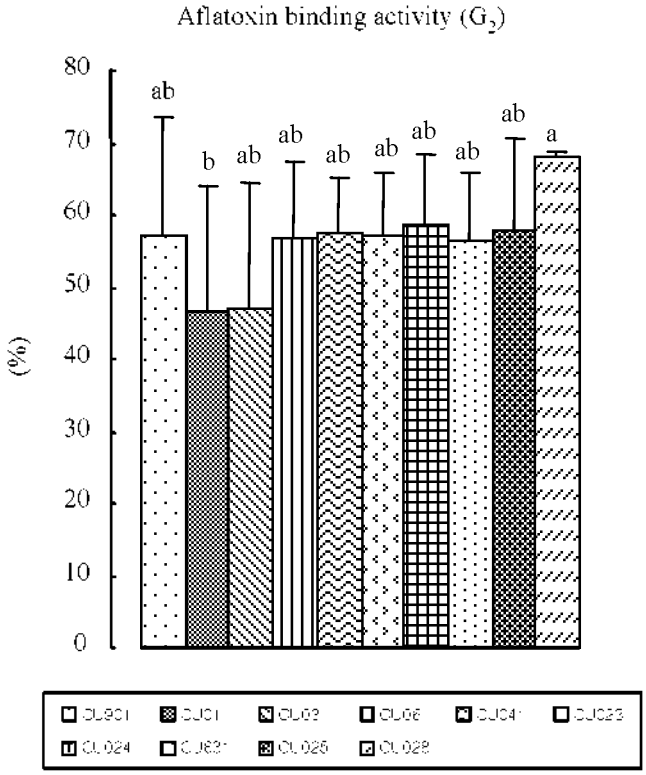

Figure 2. Aflatoxin AFG_2 binding activity of *Lactobacillus* spp.

The percentage of aflatoxin bound to the bacteria was calculated using the formula.

$$1 - \frac{\text{AF } G_1, G_2 \text{ and } B_2 \text{ peak area in sample}}{\text{AF } G_1, G_2 \text{ and } B_2 \text{ peak area in 5 } \mu g\ ml^{-1} \text{ control}} \times 100$$

Statistical analysis

Within the same tratment group. for the comparison of the aflatoxin binding ability % between *Lactobacillus* strains were compared using SAS Duncans' multiple range test.

RESULTS AND DISCUSSION

The removal of aflatoxin G_1 by *Lactibacillus* spp. was measured and was found to be dependent on the strain tested (Figure 1). The range of AFG_1 binding activity was from 33% to 53% and most efficient binding of AFG_1 was observed by *L. acidophilus* CU028 and *L. brevis* CU 06 which bound 50% and 53% of AFG_1 respectively.

The removal of aflatoxin G_2 by *Lactibacillus* spp. was measured and was found to be dependent on the strains (Figure 2). The range of AFG_2 binding activity was from 46% to 68% and most efficient binding of AFG_2 was observed by *L. acidophilus* CU028 and *L.casei* CU 901

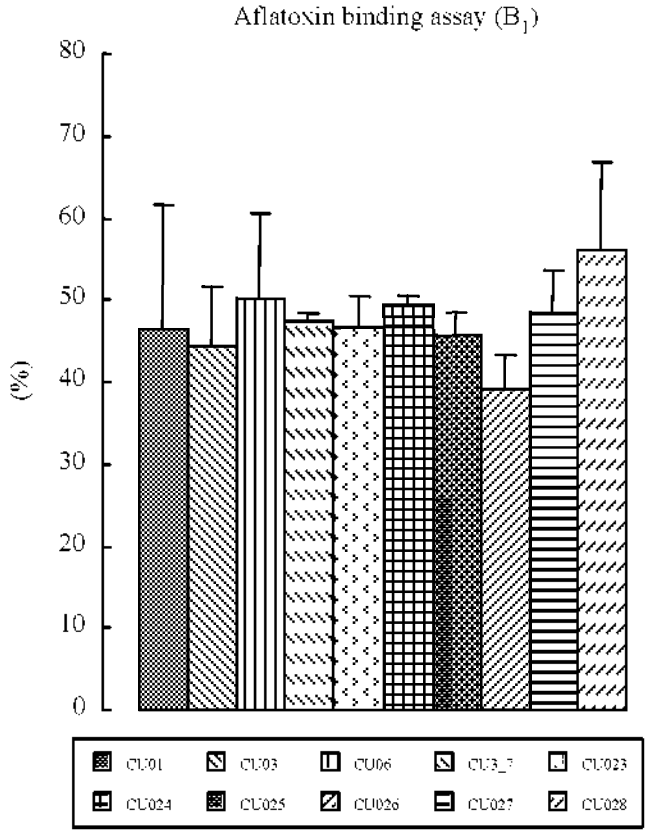

Figure 3. Aflatoxin AFB_2 binding activity of *Lactobacillus* spp. AFB_2.

which bound 68% and 57% of AFG_2 respectively. The differences in the binding activities of AFG_2 between the strains showed stastisical significance ($p>0.05$)

The aflatoxin B_2-binding capacity of the strains is presented in Figure 3. the strians were observed to possess variable AFB_2-binding ability within the ranges from 38% to 56%. *L. acidophilus* CU028 and *L. helveticus* CU 631 were the best binders with approximately 56% and 53% respectively. The most potent removal activity against all the three tested types of aflatoxin was observed by *L. acidophilus* CU 028 which was selected as starter strain for the preparation of probiotic.

Several strategies for the reduction or inactivation of aflatoxins have been reported in the scientific literatures. some methods are more effective and practical than the others. Aflatoxins may be degraded by physical. chemical or biological methods. physical approaches to aflatoxin destruction involve treating with heat, ultraviolet light, adsorption from solution; however none of them are entirely effective. Chemical degradation of aflatoxin is ususally carried out by the addition of chlorinating, oxidizing or hydrolytic agents which may result in losses of nutritional quality of treated commodities. This study was carried out to evaluate the carcinogen-binding abitlty of selected probiotic strains using aflatoxin as a model dietary carcinogen. Probiotic lactobacilli have been found to efficiently bind aflatoxins (El-Nezami et al., 1996. El-Nezami et al., 1998). the alfatoxin binding of the tested strains was found to be variable. Carcinogen and mutagen binding by bacteria is thought to be by the bacterial cell wall (Thyagaraja and Hosono 1994. El-Nezami et al., 1998). The removal was thought to involve sequestration by binding the toxin to the bacterial cell wall instead of metabolic degradation. In this study several bacterial treatments have been shown to alter the bacterial aflatoxin binding capacity. *L. acidophilus* CU028 was noted for the common binders for the three types of aflatoxins. El-Nezami et al. (1998) claimed that *L. rhamnosus* GG was observed to interfere with aflatoxin absorption from the intestinal lumen of broiler chicks. Zhang and Ohta (1993) have shown that lactic acid bacteria able to bind mutagen *in vitro* reduced the amount of mutagen absorbed from the small intestine of rats. Simulataneously a decrease in mutagen level in blood was observed. Aflatoxins are absorbed rapidly from the small intestine and colon and transported to the liver, where they are biotransformed prior to subsequenct biliary and urinary excretion.

The mutagenicity in urine is regarded as an acute response to ingestion of meat and reflects the amount of mutagens metabolised, while the unabsorbed mutagens are mainly excreted via feces. The supplementation of *L. acidophilus* -fermented milk resulted in a reduction in total mutagen excretion in feces and urine compared to the consumption of control milk. However it was noted that mutagen binding could not be solely responsible for the *in vitro* observed reduction in urinary and fecal mutagenicity.

REFERENCES

Eaton, D. L. and E. P. Callanger. 1994. Mechanism of aflatoxin carcinogenesis. Ann. Rev. Pharmacol. Toxicol. 34:135-172.

Ellis, W. O., J. P. Smith and B. K. Simpson. 1991. Aflatoxins in food: occurrence, biosynthesis,effects on organisms, detection, and methods of control.Critc. Rev. Food. Sci. Nutr. 30:403-439.

Marth, E. H. and M. P. Doyle. 1979. Update on molds; degradation of aflatoxin, Food Technol.Jan. 81-87.

El-Nezmi, H. S., E. Apostolou, E. Salminen and J. T. Ahokas. 1996. Probiotic lactic acid bacteria; A new approach to detoxify aflatoxins. International symposium on probiotic dairy microorganisms and Nutrition week. 2-3 May, 1996, Potsdam, Germany.

El-Nezmi, H. S., P. Kankaanpaa, S. Salminen and J. Ahokas. 1998. Physicochemical alterations enhance the ability of dairy strains of lactic acid bacteria to remove aflatoxin from contaminated media. J. Food Protec. 61:466-468.

Thyagaraja, N. and A. Hosono. 1994. Binding properties of lactic acid bacteria from food-borne mutagens. Food Chem. Toxicol. 32:805-809.

Yoon, Y. H. and B. R. Byun. 2002. Antagonism against *Helicobacter pylori* and proteolysis of *Lactobacillus helveticus*

CU 631 and strain identification. Asian-Aust. J. Anim. Sci. 15:1057-1065.

Zhang, X. B. and Y. Ohta. 1993. Microorganisms in the gastrointestinal tract of the rat prevent absorption the mutagen-carcinogen 3-amino-1, 4-dimethyl-5H-pyrido (4, 3-b) indole. Can. J. Microbiol. 39:841-845.