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Short Paper

Aflatoxin detoxification potential of lactic acid bacteria isolated from Iranian poultry

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Summary

This study was carried out to examine aflatoxin B_1 (AFB_1) removal ability of four strains of lactic acid bacteria (LAB). Three indigenous (*Lactobacillus rhamnosus* TMU094, *Lactobacillus fermentum* TMU121 and *Pedioccus pentosaceus* TMU457) and a non-indigenous (*Labacillus rhamnosus* PTCC1637) isolates were studied. The strains were incubated with (AFB_1) at different time. The toxin residual in the supernatant was determined. Reduction of the toxin quantity was observed by all species. Binding of aflatoxin by the studied LAB varied from 19.41 to 75.06%. Aflatoxin-binding activity showed time dependent trend taking into consideration of different incubation periods. *Lactobacillus rhamnosus* TMU094 bound 25.64 to 75.06%, *L. fermentum* bound 38.63 to 72.15%, *P. pentosaceus* bound 24.86 to 63.21% and *L. rhamnosus* PTCC1637 bound 19.41 to 35% of AFB_1 during the studied course of incubation times. These results showed that indigenous strains of LAB are able to bind AFB_1 effectively.

Key words: Aflatoxin B_1, Lactic acid bacteria, Toxin-binding

Introduction

According to Nährer's (2011) report, it is obvious that mycotoxins are an ubiquitous problem. Aflatoxins (AFs) are toxic secondary metabolites produced by filamentous fungi. Aflatoxin B_1 is recognized as the most toxic AF and is a common contaminant of feedstuffs (IARC, 1993).

The ubiquitous nature of fungal spores along with the suitable climatic condition of our country makes it difficult to cope with food and feed commodities from fungal invasion (Yazdanpanah, 2006).

Ingestion of mycotoxin by animals may result in immunosuppression (Dietert *et al*., 1994). Other economic losses by AFs are: feed refusal, reduced daily weight gain and flocks uniformity, impaired food conversion rate, decline in egg production, hatchability and higher mortality rates (CAST, 2003).

Available interventions for AFs risk elimination are physical, chemical and biological treatments (Park, 1993). The shortcomings of physical and chemical AFs detoxification methods have led investigators to seek effective alternatives. Recently biological methods have attracted researcher's attention due to their easy usage and affordable processes. The choice of microorganisms able to diminish AFs is the most critical in screening of probiotic candidates (Teniola *et al*., 2005). Probiotics are defined by Fuller as "live microbial food supplements which beneficially affect the host either directly or indirectly by improving its intestinal microbial balance" (Fuller, 1991). The positive effects of probiotics in terms of performance enhancement are well established (Rahimi and Khaksefidi, 2006). Probiotics function as AFs reducers was recently emphasized (Shahin, 2007).

Toxin decontamination by LAB is highly promising, however, this field is still in its infancy. It is known that toxin binding in LAB is strain dependent and it is very important to test indigenous strains. The present study was carried out to assess the AFs removal potential of local strains of LAB probiotics.

Materials and Methods

Bacterial species and cultural conditions

Four strains of LAB (Table 1) were studied for AFB_1 binding ability. Three strains were isolated from the digestive tracts of Iranian healthy poultry (Karimi Torshizi *et al*., 2007) and the fourth was obtained from Iranian Research Organization for Science and Technology. Species were cultivated in MRS (de Man, Rogosa and Sharpe) broth (Biolifchem, Italy) for 20 h at 37°C in a modified atmosphere (5% CO_2/95% air). Cells were harvested by centrifugation (3000 × g, 10 min, 10°C) and washed twice with phosphate-buffered saline (PBS, pH = 7.2) and once with sterile double-distilled water (El-Nezami *et al.*, 1998).

Table 1: Strains used in the study and their sources

Microorganism	Source/Reference
Lactobacillus rhamnosus TMU094	Karimi Torshizi *et al*., 2007
Lactobacillus fermentum TMU121	//
Pedioccus pentosaceus TMU457	//
Labacillus rhamnosus PTCC1637	IROST*

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AFB_1 binding assay at different times

Solid AFB_1 (Sigma Aldrich, Germany) was suspended in benzene/acetonitrile (97:3 v/v) to obtain an AFB_1 (2 mg/ml). Working solution of AFB_1 (5 µg/ml) was prepared in PBS. The cell count of bacteria was adjusted to 10-15 log CFU/ml. One ml of the suspension was centrifuged (3000 × g, 15 min) and the bacterial pellets were washed with 5 ml of Milli-Q water. Bacterial pellets were re-suspended in 1.5 ml of AFB_1 solution (5 µg/ml) and incubated at 37°C for different times (0, 0.5, 4, 12, 24 and 72 h). The bacteria were pelleted (3000 × g, 15 min) and the supernatant was analysed for AFB_1 (Peltonen *et al*., 2000).

Quantification of unbound AFB_1

Supernatants (1 ml) were transferred to 1.5 ml microtubes, 100 µl of chloroform was added and vortexed for 2 min. The bottom chloroform layer was siphoned off and 20 µl was loaded on thin layer chromatography plates (TLC Silica gel 60 F254, Merck, Germany). The mobile phase was methanol (55% V/V). The AFB_1 quantification was performed using densitometer (TLC scanner CD 60, Desaga, Germany) against AFB_1 standards at 254 nm. All tests were done in triplicate.

Statistical analysis

Data were analysed using a completely randomized design in a factorial arrangement of 4 bacterial strains and 6 levels of incubation times using SAS software (SAS, 1982). Means were compared using least significant differences procedure. All statements of differences were based on a significance level of $P<0.01$.

Results

The amount of AFB_1 bound varied considering LAB strains (Fig. 1). Generally, LAB strains bound 29.33 to 54.95% AFB_1. *Lactobacillus fermentum* and *L. rhamnosus* TMU094 were the most efficient, binding 54.95 and 53.26, respectively.

The amount of AFB_1 bound was time dependent (Fig. 2) and the highest binding occurred at 12 h (60.86%). The binding of

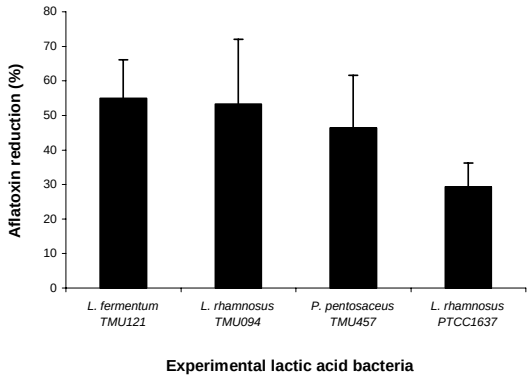

Fig. 1: Effect of experimental lactic acid bacteria on AFB_1 reduction

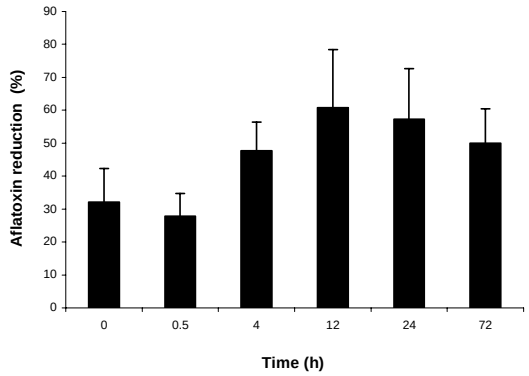

Fig. 2: Aflatoxin reduction in different incubation time intervals

AFB_1 was rapid with 32.13% AFB_1 bound at 0 h.

Considering time and strain interaction, *L. rhamnosus* TMU094 bound 25.64 to 75.06%, *L. fermentum* bound 38.63 to 72.15%, *P. pentosaceus* bound 24.86 to 63.21% and *L. rhamnosus* PTCC1637 bound 19.41 to 35% of AFB_1 during the studied course of incubation times (Table 2). The AFB_1 binding of indigenous LAB was highest in the time of 12 h. On the contrary, at 72 h the AFB_1 binding of these strains was diminished ($P<0.01$). The binding of AFB_1 for *L. rhamnosus* TMU094 increased until 24 h and remained constant. The highest binding throughout the incubation times and strains occurred for *L. rhamnosus* TMU094 at 12 h (75.06%).

Discussion

Lactic acid bacteria have been shown to bind mycotoxins to their cell wall components. It is known that toxin binding in LAB is strain dependent. Strains of LAB with high mycotoxin binding ability have considerable prospects as mycotoxin binding organisms (Shetty and Jespersen, 2006).

Lactic acid bacteria have been previously reported to bind various dietary contaminants such as AFs as well as suppression of mycotoxin-producing fungal growth (Hernandez-Mendoza *et al*., 2009; Dalie *et al*., 2010). It appears that AFB_1 is bound to the surface components of LAB (Haskard *et al*., 2001). The destruction of specific components of the bacterial cell wall, e.g., carbohydrates and proteins, resulted in reduction in AFB_1 binding by *L. rhamnosus* strain GG (Hernandez-Mendoza *et al*., 2009). It is likely, however, that multiple components are involved in AFB_1 binding. In the present study, all strains tested were able to bind AFB_1, but to different extents. The differences in AFB_1 binding by the strains are probably due to different bacterial cell wall and cell envelope structures.

Lactobacillus rhamnosus TMU094 and *L. fermentum* were the most efficient species in AFB_1 binding (75.06 and 72.15%, respectively) and their binding process was rapid (Fig. 2 and Table 2). However, LAB do not bind AFB_1 irreversibly and weak bound AFB_1 may release upon incubation of the lactobacilli/AFB_1 complexes in aqueous solution. This is probably the reason for the decrease in binding at 0.5 h. During incubation, bacterial cells can grow and reproduce, so the population of vegetative cells which can bind AFB_1 score maximum at 12 h, causing the highest binding at this point. Because the binding involved weak non-covalent interactions lactobacilli/AFB_1 bound released gradually, therefore aflatoxin binding capacity diminished at 72 h.

In this experiment, indigenous strains were more effective than the non-indigenous strain. The difference between our local strains and standard strain is probably due to the different mechanism in AFB_1 removal by these microorganisms.

The efficient AFB_1 binding by indigenous strains was a rapid process which

Table 2: Effect of experimental LAB species on AFB_1 reduction in different incubation times

Species	Decreasing of aflatoxin B_1 (%)					
	Incubation time (h)					
	0	0.5	4	12	24	72
L. rhamnosus TMU094	34.25 ± 2.73^{h}	25.64 ± 1.77^{ij}	53.97 ± 1.18^{e}	75.06 ± 1.60^{a}	72.15 ± 1.90^{a}	58.47 ± 0.28^{dc}
L. fermentum	45.90 ± 2.40^{f}	38.63 ± 0.60^{g}	54.94 ± 1.31^{de}	72.15 ± 0.38^{a}	62.32 ± 1.28^{b}	55.77 ± 0.90^{de}
P. pentosaceus	28.97 ± 0.30^{i}	24.86 ± 0.69^{j}	46.72 ± 1.02^{f}	63.21 ± 3.04^{b}	61.67 ± 0.04^{bc}	52.55 ± 0.55^{e}
L. rhamnosus PTCC1637	19.41 ± 0.51^{k}	22.25 ± 3.94^{kj}	35.00 ± 5.73^{h}	33.01 ± 3.08^{h}	33.11 ± 1.04^{h}	33.22 ± 3.05^{h}

^{abc} Different letters show significant differences ($P<0.01$)

took place in a reasonably short time (Fig. 2). This is in agreement with other reports on LAB (El-Nezami *et al*., 1998).

Since the passage rate of feed through the poultry gastrointestinal tract is short the species with rapid binding capability are more appropriate. Aflatoxins reducing ability should be introduced in selection criteria of probiotic candidates. With regards to the instability of bactery -AFB_1 complex the irreversible AFB_1 attachment must be sought. The presented simple and inexpensive method of AFs removal opened a new window for many developing countries that cannot afford to use expensive technologies of AFs elimination. More studies regarding the chemistry of binding and stability of the complex, especially under harsh conditions of GI tract need to be investigated.

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