

## Short Paper

# Aflatoxin detoxification potential of lactic acid bacteria isolated from Iranian poultry

Bagherzadeh Kasmani, F.<sup>1</sup>; Karimi Torshizi, M. A.<sup>1\*</sup>; Allameh, A. A.<sup>2</sup>  
and Shariatmadari, F.<sup>1</sup>

<sup>1</sup>Department of Poultry Sciences, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran;

<sup>2</sup>Department of Clinical Biochemistry, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran

\*Correspondence: M. A. Karimi Torshizi, Department of Poultry Sciences, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. E-mail: karimitm@modares.ac.ir

(Received 13 Feb 2011; revised version 19 Sept 2011; accepted 18 Oct 2011)

## Summary

This study was carried out to examine aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) removal ability of four strains of lactic acid bacteria (LAB). Three indigenous (*Lactobacillus rhamnosus* TMU094, *Lactobacillus fermentum* TMU121 and *Pediococcus pentosaceus* TMU457) and a non-indigenous (*Labacillus rhamnosus* PTCC1637) isolates were studied. The strains were incubated with (AFB<sub>1</sub>) 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<sub>1</sub> during the studied course of incubation times. These results showed that indigenous strains of LAB are able to bind AFB<sub>1</sub> effectively.

**Key words:** Aflatoxin B<sub>1</sub>, 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<sub>1</sub> 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 AFB<sub>1</sub> 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<sub>1</sub> 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<sub>2</sub>/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
<i>Lactobacillus rhamnosus</i> TMU094	Karimi Torshizi <i>et al.</i> , 2007
<i>Lactobacillus fermentum</i> TMU121	//
<i>Pediococcus pentosaceus</i> TMU457	//
<i>Labacillus rhamnosus</i> PTCC1637	IROST*

\*Iranian Research Organization for Science and Technology

### AFB<sub>1</sub> binding assay at different times

Solid AFB<sub>1</sub> (Sigma Aldrich, Germany) was suspended in benzene/acetonitrile (97:3 v/v) to obtain an AFB<sub>1</sub> (2 mg/ml). Working solution of AFB<sub>1</sub> (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<sub>1</sub> 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<sub>1</sub> (Peltonen *et al.*, 2000).

### Quantification of unbound AFB<sub>1</sub>

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<sub>1</sub> quantification was performed using densitometer (TLC scanner CD 60, Desaga, Germany) against AFB<sub>1</sub> standards at 254 nm. All tests were done in triplicate.

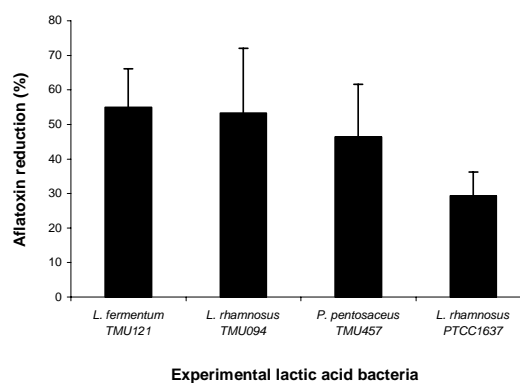
### Statistical analysis

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.

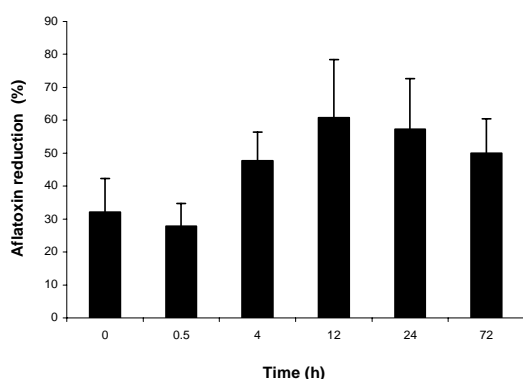
## Results

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

The amount of AFB<sub>1</sub> 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<sub>1</sub> reduction**



**Fig. 2: Aflatoxin reduction in different incubation time intervals**

AFB<sub>1</sub> was rapid with 32.13% AFB<sub>1</sub> 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<sub>1</sub> during the studied course of incubation times (Table 2). The AFB<sub>1</sub> binding of indigenous LAB was highest in the time of 12 h. On the contrary, at 72 h the AFB<sub>1</sub> binding of these strains was diminished ( $P < 0.01$ ). The binding of AFB<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> binding by *L. rhamnosus* strain GG (Hernandez-Mendoza *et al.*, 2009). It is likely, however, that multiple components are involved in AFB<sub>1</sub> binding. In the present study, all strains tested were able to bind AFB<sub>1</sub>, but to different extents. The differences in AFB<sub>1</sub> 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<sub>1</sub> binding (75.06 and 72.15%, respectively) and their binding process was rapid (Fig. 2 and Table 2). However, LAB do not bind AFB<sub>1</sub> irreversibly and weak bound AFB<sub>1</sub> may release upon incubation of the lactobacilli/AFB<sub>1</sub> 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<sub>1</sub> score maximum at 12 h, causing the highest binding at this point. Because the binding involved weak non-covalent interactions lactobacilli/AFB<sub>1</sub> 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<sub>1</sub> removal by these microorganisms.

The efficient AFB<sub>1</sub> binding by indigenous strains was a rapid process which

**Table 2: Effect of experimental LAB species on AFB<sub>1</sub> reduction in different incubation times**

Species	Decreasing of aflatoxin B <sub>1</sub> (%)					
	Incubation time (h)					
	0	0.5	4	12	24	72
<i>L. rhamnosus</i> TMU094	34.25±2.73 <sup>h</sup>	25.64±1.77 <sup>ij</sup>	53.97±1.18 <sup>e</sup>	75.06±1.60 <sup>a</sup>	72.15±1.90 <sup>a</sup>	58.47±0.28 <sup>dc</sup>
<i>L. fermentum</i>	45.90±2.40 <sup>f</sup>	38.63±0.60 <sup>g</sup>	54.94±1.31 <sup>de</sup>	72.15±0.38 <sup>a</sup>	62.32±1.28 <sup>b</sup>	55.77±0.90 <sup>de</sup>
<i>P. pentosaceus</i>	28.97±0.30 <sup>i</sup>	24.86±0.69 <sup>j</sup>	46.72±1.02 <sup>f</sup>	63.21±3.04 <sup>b</sup>	61.67±0.04 <sup>bc</sup>	52.55±0.55 <sup>e</sup>
<i>L. rhamnosus</i> PTCC1637	19.41±0.51 <sup>k</sup>	22.25±3.94 <sup>kl</sup>	35.00±5.73 <sup>h</sup>	33.01±3.08 <sup>b</sup>	33.11±1.04 <sup>h</sup>	33.22±3.05 <sup>h</sup>

<sup>abc</sup> 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 bacteria-AFB<sub>1</sub> complex the irreversible AFB<sub>1</sub> 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.

## References

- CAST (2003). Mycotoxins: risks in plant, animal, and human systems. Ames, Iowa, USA, Task Force Report No. 139.
- Dalie, DK; Deschamps, AM; Atanasova-Penichon, V and Richard-Forget, F (2010). Potential of *Pediococcus pentosaceus* (L006) isolated from maize leaf to suppress fumonisin-producing fungal growth. *J. Food Prot.*, 73: 1129-1137.
- Diertert, RR; Golemboski, KA and Austic, RE (1994). Environment-immune interactions. *Poult. Sci.*, 73: 1062-1076.
- El-Nezami, H; Kankaanpää, P; Salminen, S and Ahokas, J (1998). Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B<sub>1</sub>. *Food Chem. Toxicol.*, 36: 321-326.
- Fuller, R (1991). Probiotics in human medicine. *Gut*. 32: 439-442.
- Haskard, C; El-Nezami, H; Kankaanpää, P; Salminen, S and Ahokas, J (2001). Surface binding of aflatoxin B<sub>1</sub> by lactic acid bacteria. *Appl. Environ. Microbiol.*, 67: 3086-3091.
- Hernandez-Mendoza, A; Garcia, HS and Steele, JL (2009). Screening of *Lactobacillus casei* strains for their ability to bind aflatoxin B<sub>1</sub>. *Food Chem. Toxicol.*, 47: 1064-1068.
- IARC (1993). Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. Vol. 56 of Monographs on the Evaluation of Carcinogenic Risk to Humans. IARC, Lyon.
- Karimi Torshizi, MA; Rahimi, Sh; Mojgani, N; Esmailkhanian, S and Grimes, JL (2007). Screening of indigenous strains of lactic acid bacteria for development of a probiotic for poultry. *Asian-Aust. J. Anim. Sci.*, 21: 1495-1500.
- Nährer, K (2011). Mycotoxin report 3rd Quarter. Biomin Holding GmbH, Industriestrasse 21, A-3130 Herzogenburg, Austria. Available at: <http://www.biomin.net/en/knowledge-center/articles/articles-details/article/biomins-mycotoxim-survey-3rd-quarter-report-2011/>.
- Park, DC (1993). Controlling aflatoxin in food and feed. *Food. Technol.*, 47: 92-96.
- Peltonen, K; El-Nezami, H; Salminen, S and Ahokas, J (2000). Binding of aflatoxin B<sub>1</sub> by probiotic bacteria. *J. Sci. Food Agric.*, 80: 1942-1945.
- Rahimi, Sh and Khaksefidi, A (2006). A comparison between the effects of a probiotic (Bioplus 2B) and an antibiotic (virginiamycin) on the performance of broiler chickens under heat stress. *Iranian J. Vet. Res.*, 7: 23-28.
- SAS (1982). *SAS User's Guide: Statistics*. SAS Institute Inc., Cary, NC.
- Shahin, AAM (2007). Removal of aflatoxin B<sub>1</sub> from contaminated liquid media by dairy lactic acid bacteria. *Int. J. Agric. Biol.*, 9: 71-75.
- Shetty, PH and Jespersen, L (2006). *Saccharomyces cerevisiae* and lactic acid bacteria as potential mycotoxin decontaminating agents. *Trends Food Sci. Tech.*, 17: 48-55.
- Teniola, OD; Addo, PA; Brost, IM; Farber, P; Jany, KD; Alberts, JF; Van Zyl, WH; Steyn, PS and Holzappel, WH (2005). Degradation of aflatoxin B<sub>1</sub> by cell-free extracts of *Rhodococcus erythropolis* and *Mycobacterium fluoranthenvorans* sp. nov. DSM44556(T). *Int. J. Food Microbiol.*, 105: 111-117.
- Yazdanpanah, H (2006). Mycotoxin contamination of foodstuffs and feedstuffs in Iran. *Iran. J. Pharm. Res.*, 1: 9-16.