1st International Conference of
TWAS Young Affiliates Network

ABILITY OF *LACTOBACILLUS PLANTARUM* MON03 TO ADHERE TO CACO-2 CELLS *IN VITRO* AND TO MITIGATE AFB1 INTESTINAL CELLS DAMAGE *IN VIVO*

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INTRODUCTION

Mycotoxins?

- Group of natural toxic produced by fungi

ary metabolites

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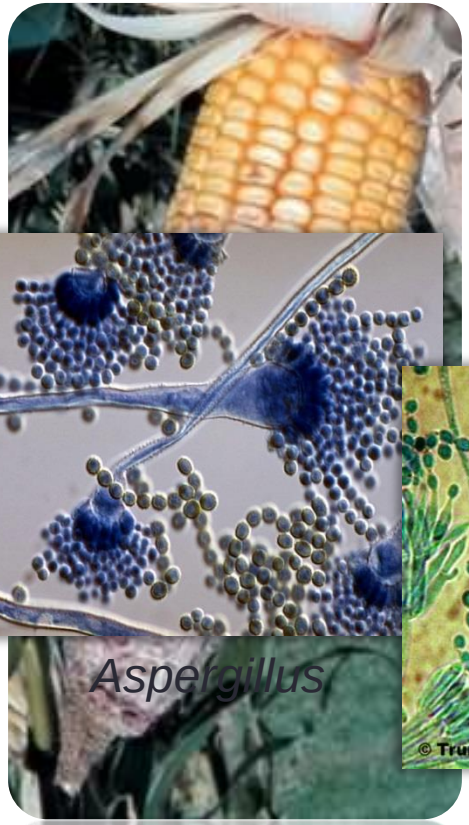
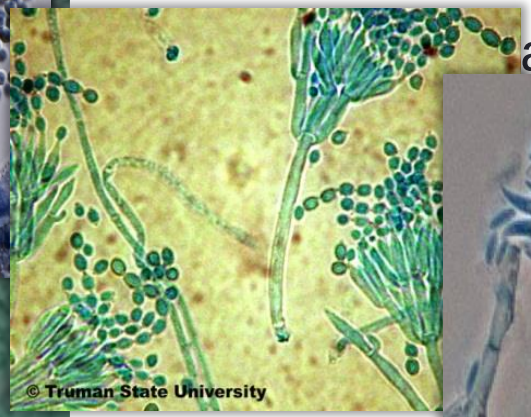


Aspergillus



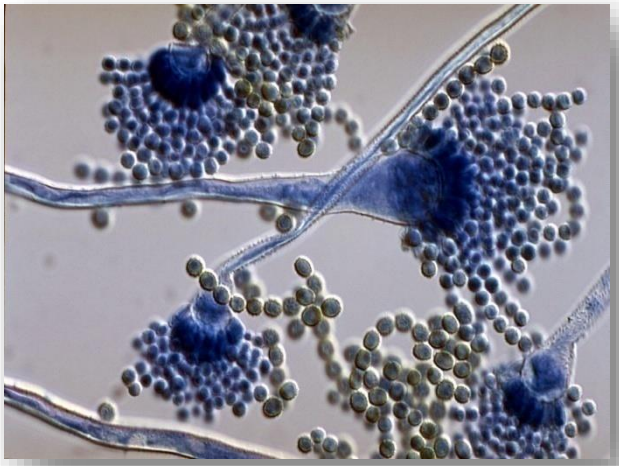
Penicillium

Fusarium

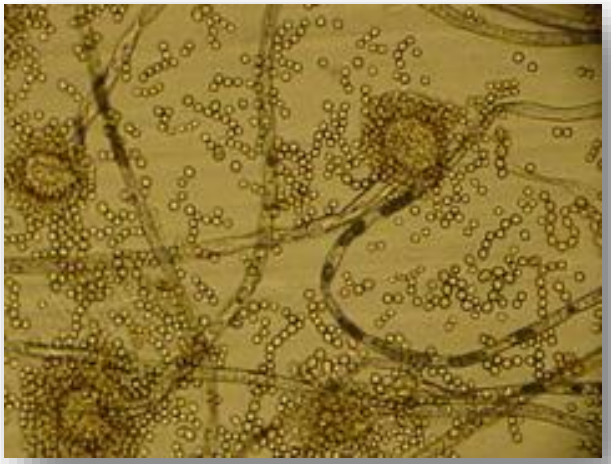
INTRODUCTION

Mycotoxins appear in six major classes:

- **Aflatoxins (AFB1)**
- **Ochratoxins**
- **Trichothecenes**
- **Fumonisins**
- **Zearalenone**
- **Patulin**



A. Flavus



A. parasiticus

INTRODUCTION

INTRODUCTION

Natural occurrence of aflatoxins (B_1 and M_1) in feed, plasma and raw milk of lactating dairy cows in Beja, Tunisia, using ELISA

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Beja is an agricultural area in northwest Tunisia. It contributes to national needs by offering cereals and milk to the market for human and animal consumption. A small number of studies on mycotoxin occurrence in feedstuffs and raw milk from lactating dairy cows in this region are available. Therefore, 226 samples were collected from farms and local markets during November 2008 until April 2010. Samples consisted of 112 raw cow milk, 56 blood from lactating cows and 58 feed destined for dairy cows. Plasma and feed were analysed for aflatoxin B_1 (AFB_1). Milk samples were analysed for aflatoxin M_1 (AFM_1). All samples were treated using a simultaneous methanolic-aqueous extraction, followed by immunoaffinity column clean-ups and were investigated by competitive enzyme-linked immunoabsorbent assay (ELISA). Recoveries were 80%–95% and 81%–92% for AFB_1 and AFM_1, respectively, while the limit of detection (LOD) was 0.01 µg/kg or µg/l for both mycotoxins. Results revealed the presence of AFB_1 in 84.4% of the feed samples (mean 18.7 ± 1.4 µg/kg), and 39.2% of the plasma-examined samples (median 7.1 ± 1.0 µg/l) were found to be contaminated at levels higher than the Tunisian and the European Union (EU) limit for dairy animals, which are 20 and 5 µg/kg in animal feed, respectively. AFM_1 was detected in 60.7% of the cow raw milk samples examined (median 13.6 ± 1.4 µg/l). Contaminated levels were higher than the EU limit of 0.05 µg/l. It was concluded that more precaution should be taken on hygiene controls in order to prevent fungal contamination.

Keywords: raw milk; feedstuff; plasma; aflatoxin B_1; aflatoxin M_1; occurrence; ELISA; risk

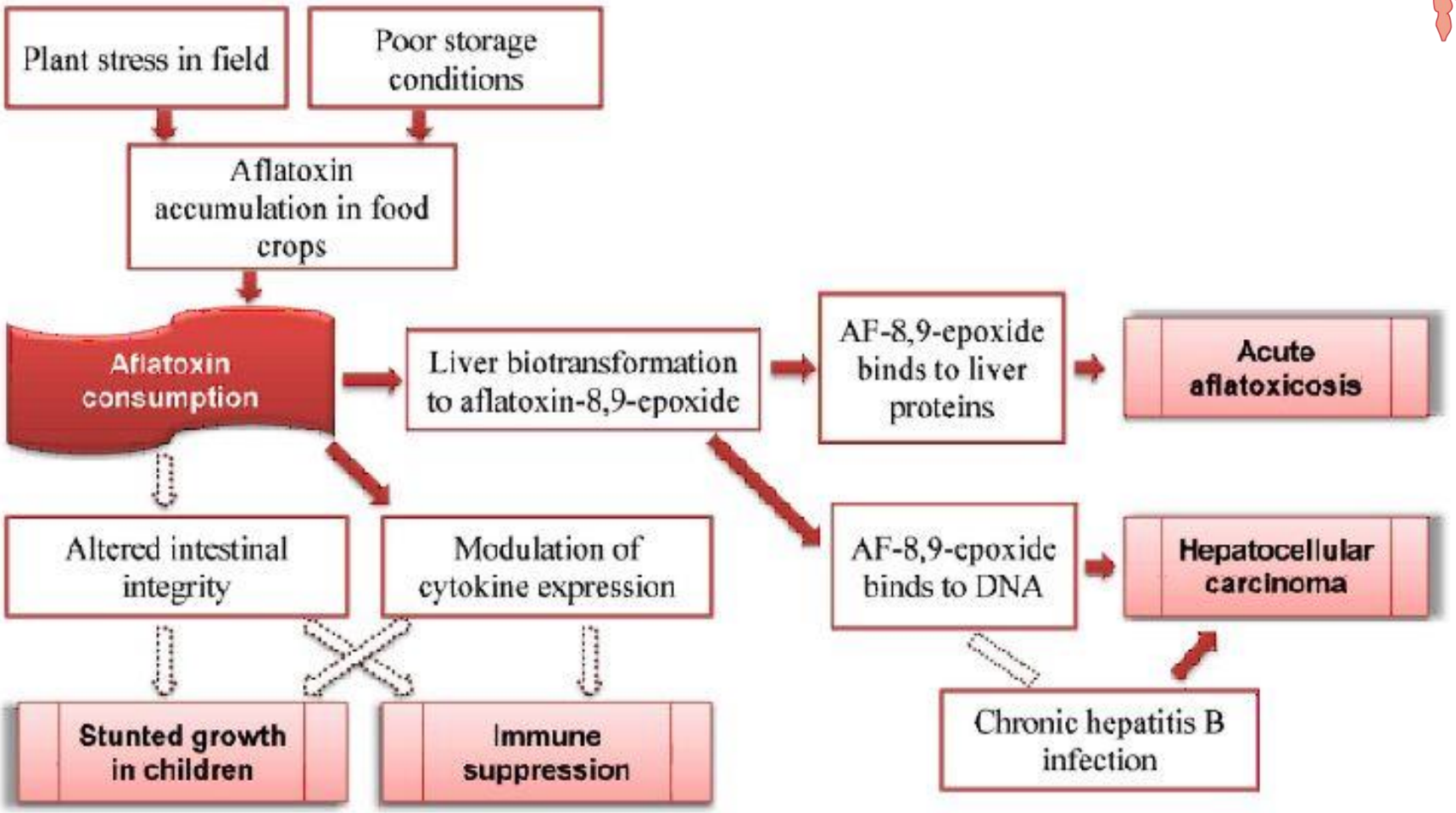
INTRODUCTION

Exposure

Direct

Indirect

INTRODUCTION

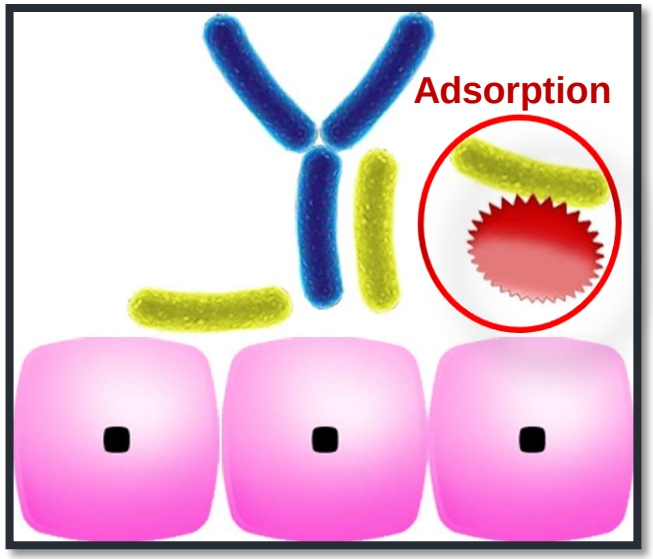


Aflatoxin disease pathways in humans

(Adopted from Wu, 2010; Wu, 2011)

INTRODUCTION

To get ride against this problem numerous strategies of detoxification or/and inactivation were elaborated. Therefore, the use of **lactic acid bacteria** strains has been suggested as a **better technique for removing AFB1** through adsorption, especially using *Lactobacillus rhamnosus* GG. Additionally, many other microorganisms have been reported to convert aflatoxin into less toxic substances.



Adsorption of mycotoxin by *Lactobacilli*

INTRODUCTION

Ability of *Lactobacillus plantarum* MON03 to mitigate aflatoxins (B_1 and M_1) immunotoxicities in mice

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Abstract

Aflatoxin B_1 (AFB_1) and M_1 (AFM_1) are mycotoxins produced by numerous *Aspergillus* species in pre- or post-harvest cereals and milk. AFB_1 and AFM_1 display a potent economic loss in livestock and also cause severe immunological problems. The aims of this study were to: evaluate a new AFB_1 and AFM_1-binding/degrading micro-organism for biological detoxification; examine its ability to degrade AFB_1 and AFM_1 in liquid medium; and evaluate its potential for *in vivo* preventative effects against AFB_1- and AFM_1-induced immunomodulation in mice. *Lactobacillus plantarum* MON03 (LP) isolated from Tunisian artisanal butter was found to display significant binding ability to AFB_1 and AFM_1 in PBS (i.e. 82% and 89%, respectively) within 24 h of incubation and able to tolerate gastric acidity, have strongly hydrophilic cells surface properties, and adhere efficacy to Caco-3 cells *in vitro*. The *in vivo* study was conducted using Balb/c mice that received by oral gavage vehicle (control), LP only (2×10^9 CFU/L, ~2 g/kg BW), AFB_1 or AFM_1 alone (0.25 and 0.27 mg/kg, respectively), or AFB_1 + LP or AFM_1 + LP daily for 15 days. Compared to in control mice, treatments with AFB_1 and AFM_1 led to significantly decreased body weight gains, histopathological changes, and decrements in all hematologic and immune parameters assessed. Co-treatment with LP strongly reduced the adverse effects of each mycotoxin. In fact, the mice receiving AFB_1 + LP or AFM_1 + LP co-treatment displayed no significant differences in the assayed parameters as compared to the control mice. By itself, the bacteria alone had no adverse effects in the mice. From these data, it is concluded that the tested bacteria could be beneficial in biotechnology detoxification of contaminated food and feed for humans and animals.

Keywords

Aflatoxin B_1, aflatoxin M_1, *Lactobacillus*, immunotoxicity, binding, detoxification

History

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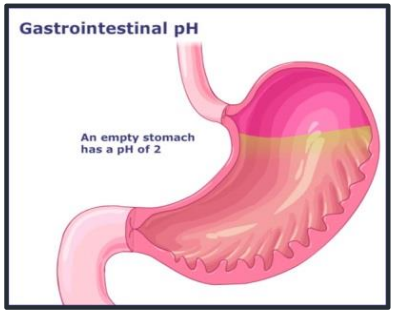
DESIGN / METHODS

In vitro study

❑ *L. plantarum* MON03 tolerance to gastric acidity

The acid tolerance of LP was studied in simulated gastric juices as described by Charteris et al. (1998).

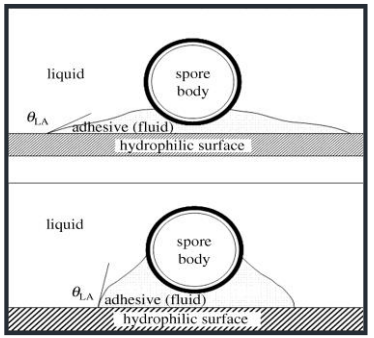


❑ Adhesion ability to caco-2 cells

The ability of LP to adhere to caco-2 intestinal epithelial cells was evaluated using the method of Martin et al. (2006).

❑ Bacterial cells surface propreties

Cell surface propreties were determined by a microbe adhesion to solvent method (Pelletier et al,, 1997) for live and thermally inactivated *Lactobacillus* strains and live *E. coli* (for comparative purposes).



DESIGN / METHODS

In vivo study

- **Preventative effects of *L. plantarum* MON03 against AFB1-induced intestinal cells alterations in balb/c mice**

D1 D15

1 week 15 days

40 Balb/c Mice

-T: 22 ± 2°C
-RH: 40 ± 5%
-Light/Dark Cycle : 12h
-Standard rodent chow and filtered water were available *ad libidum*

Groups

-G1: Control
-G2 : LP ($2x10^9$ CFU/mL)
-G3: AFB_1(100 µg/kg BW)
-G4: AFB_1+LP

-Euthanazy of each mice (cervical dislocation)
-Collect of tissues samples (small intestine)

- DNA Fragmentation
- Gene expression analysis (semi-quantitative PCR)

Gene Expression of p53, Bax and Bcl-2

DATA / RESULTS

In vitro study

Gastric juice tolerance and Caco-2 adherence of LAB strains (log cfu/ml).

Lactobacillus Strain	Initial mean counts	Resistance to gastric juice		Caco-2 adhesion[a]
		pH 2	pH 3	
***L. plantarum* MON03**	**9.77 ± 0.19**	**9.23 ± 0.46**	**9.16 ± 0.56**	**466.3 ± 109.1**
***L. rhamnosus* GG[b]**	**9.92 ± 0.18**	**9.21 ± 0.47**	**9.13 ± 0.58**	**493.2 ± 97.3**

[a]Number of *Lactobacilli* strains adhered to Caco-2 cells in 20 random microscopic fields.
[b]*Lactobacillus rhamnosus* GG: used as control.
Each value shown is the mean ± SD from three trials.

DATA / RESULTS

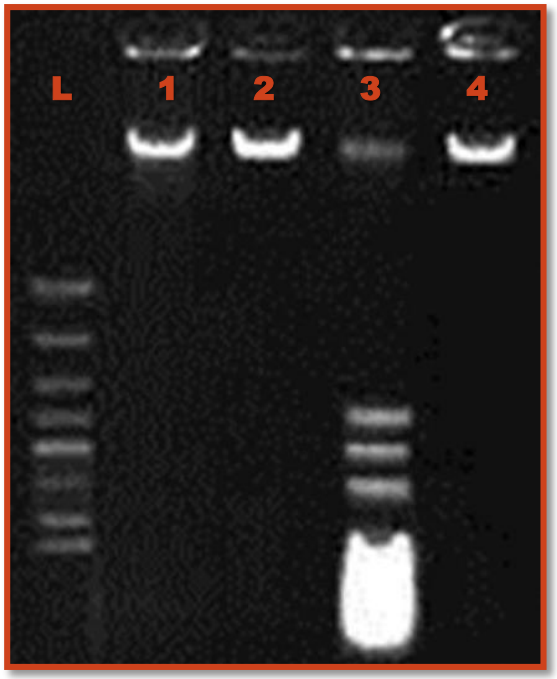
In vitro study

Characterization of bacterial surface - adhesion to solvent (microbial adhesion to solvents method).

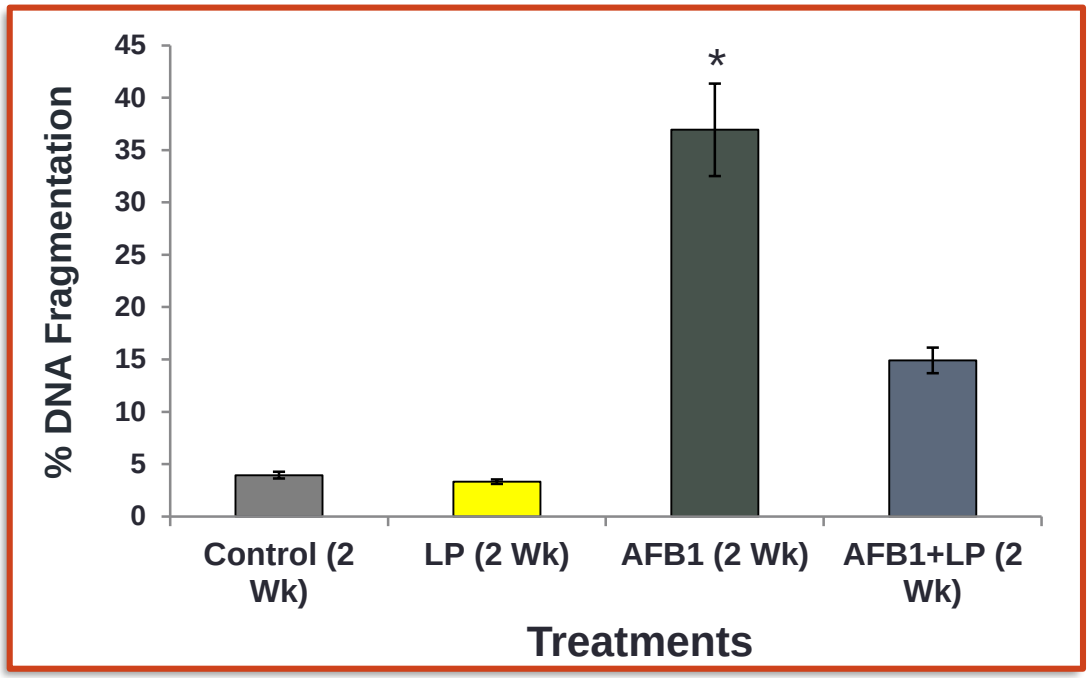
Solvent	% adhesion to solvent		
	L. plantarum MON03		*E. coli* ATCC 10536
	Live biomass	Dead biomass	Live biomass
Hexadecane	7.63 ± 0.35	38.52 ± 0.05	21.13 ± 0.09
Chloroform	59.30 ± 0.24	42.91 ± 0.32	8.78 ± 0.06
Ethyl acetate	12.51 ± 0.05	23.43 ± 0.41	30.85 ± 0.28

DATA / RESULTS

❑ DNA Fragmentation



Ethidium bromide stained agarose gel electrophoresis showing DNA fragmentaion in small intestine of mice.M-100 bp ladder; lane1: Control, lane 2: LP, lane3: AFB1, lane4: LP+AFB1



Percentage of DNA fragmentation in small intestine cells after treatment with LP alone or in combination with AFB1.

DATA / RESULTS

❑ Apoptotic gene expression (P53, Bax and Bcl-2)

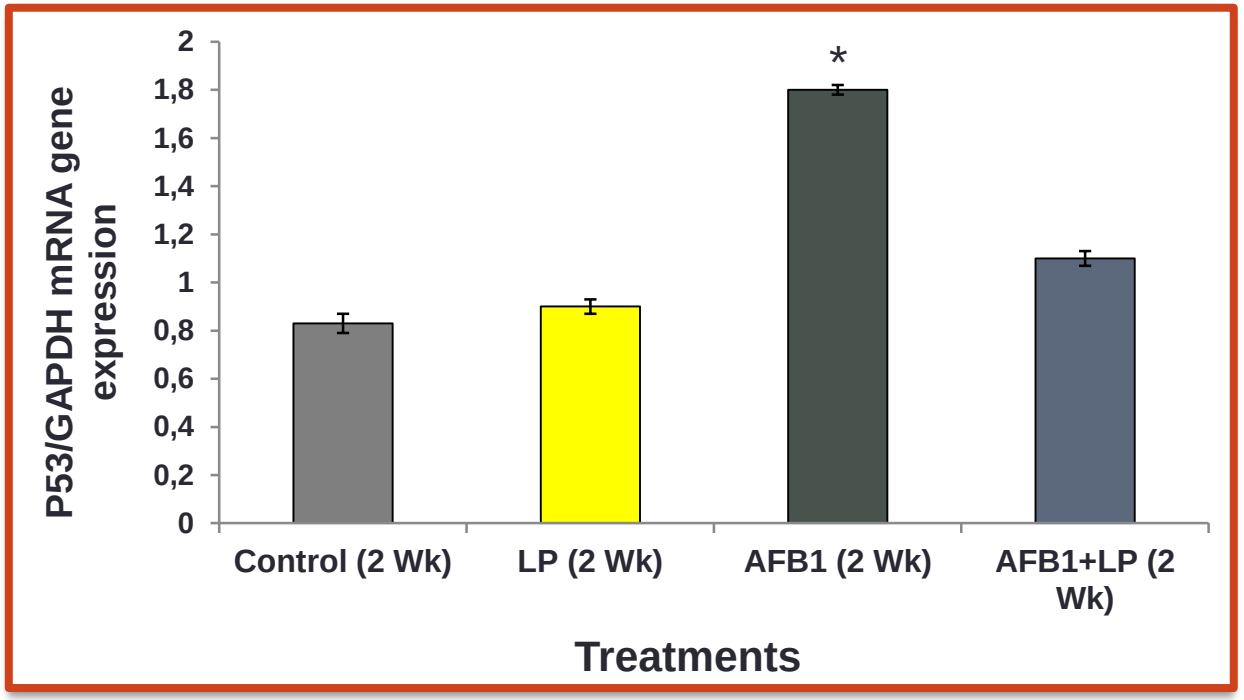
Ethidium bromide stained agarose gel electrophoresis results showing the expression of P53 mRNA in small intestine of mice determined by semi-quantitative-PCR.
M-100 bp ladder; lane1: Control, lane 2: LP, lane3: AFB1, lane4: LP+AFB1.

Ethidium bromide stained agarose gel electrophoresis results showing the expression of Bax mRNA in small intestine of mice determined by semi-quantitative-PCR.

Ethidium bromide stained agarose gel electrophoresis results showing the expression of Bcl-2 mRNA in small intestine of mice determined by semi-quantitative-PCR.

DATA / RESULTS

❑ P53/GAPDH mRNA gene expression



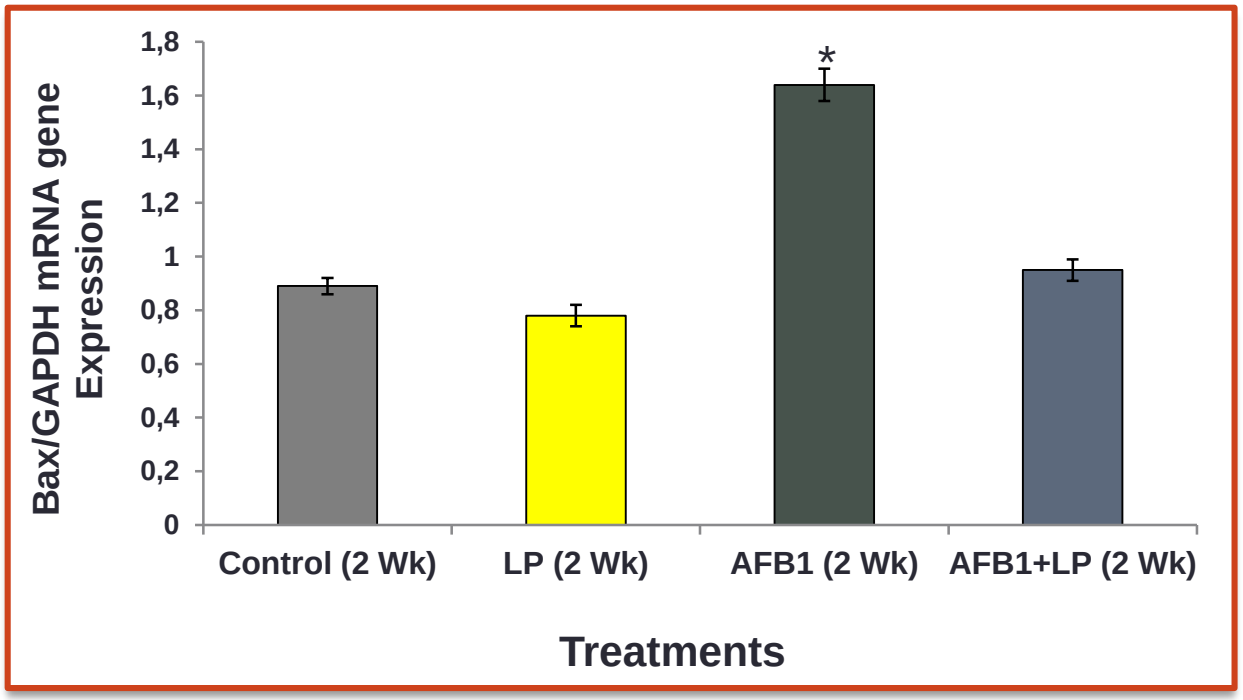
The ratio between p53/GAPDH mRNA in Balb/c mice

Values represent mean ± SE for each group.

* Values significantly differs from control, LP alone or corresponding co-treatment value ($p \leq 0.05$)

DATA / RESULTS

❑ Bax/GAPDH mRNA gene expression



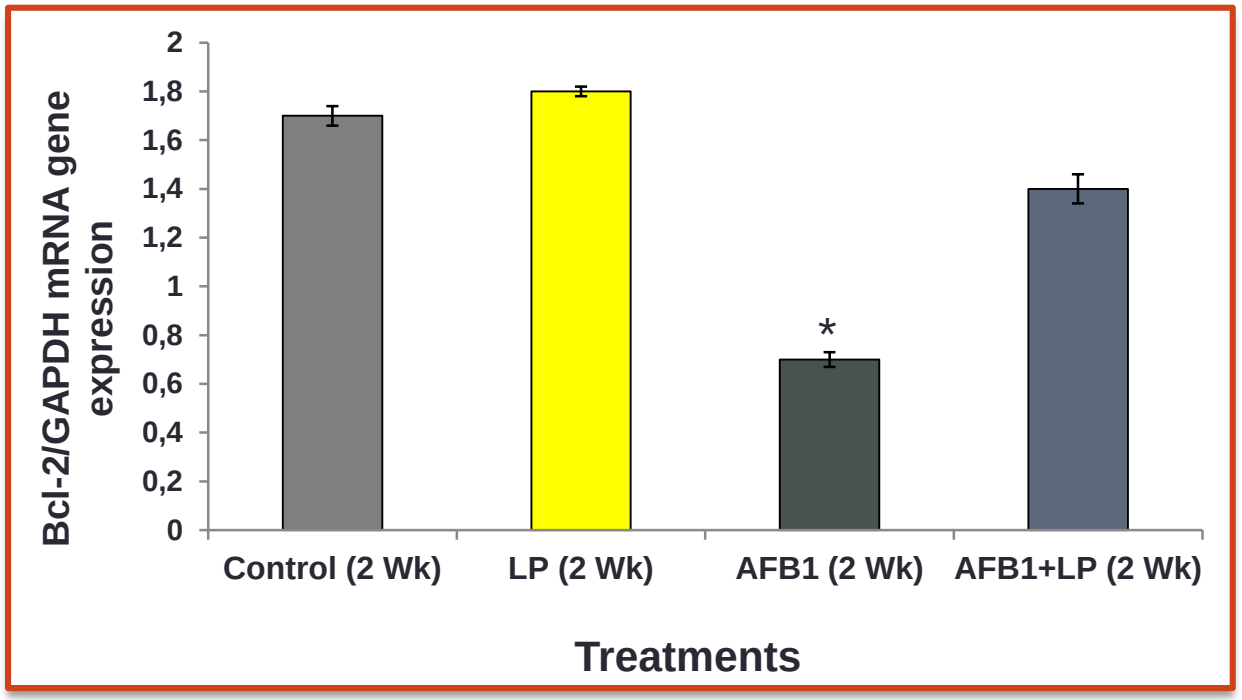
The ratio between Bax/GAPDH mRNA in Balb/c mice

Values represent mean ± SE for each group.

* Values significantly differs from control, LP alone or corresponding co-treatment value ($p \leq 0.05$)

DATA / RESULTS

❑ Bcl-2/GAPDH mRNA gene expression



The ratio between Bcl-2/GAPDH mRNA in Balb/c mice

Values represent mean ± SE for each group.

* Values significantly differs from control, LP alone or corresponding co-treatment value ($p \leq 0.05$)

CONCLUSION

- LP strain was resistant to low (pH 2) and able to adhere to caco-2 cells (adherence ability for intestinal mucosa=important selection creterion for LAB strains intended for probiotic use and/or toxin detoxification).

- AFB1 affect the intestinal integrity by DNA fragmentation and alteration of the apoptotic gene expressions of intestinal cells.

- LP by itself was « safe » and it was able to couteract the alterations caused by AFB1 in the intestinal cells.

- The combined treatment with AFB1+LP succeded to induce a significant protection against AFB1.

➢ **LP can be considered in biotechnological processes that have a major goal of mycotoxin decontamination and Gstro-intestinal tract prevention.**

THANK YOU
FOR YOUR ATTENTION