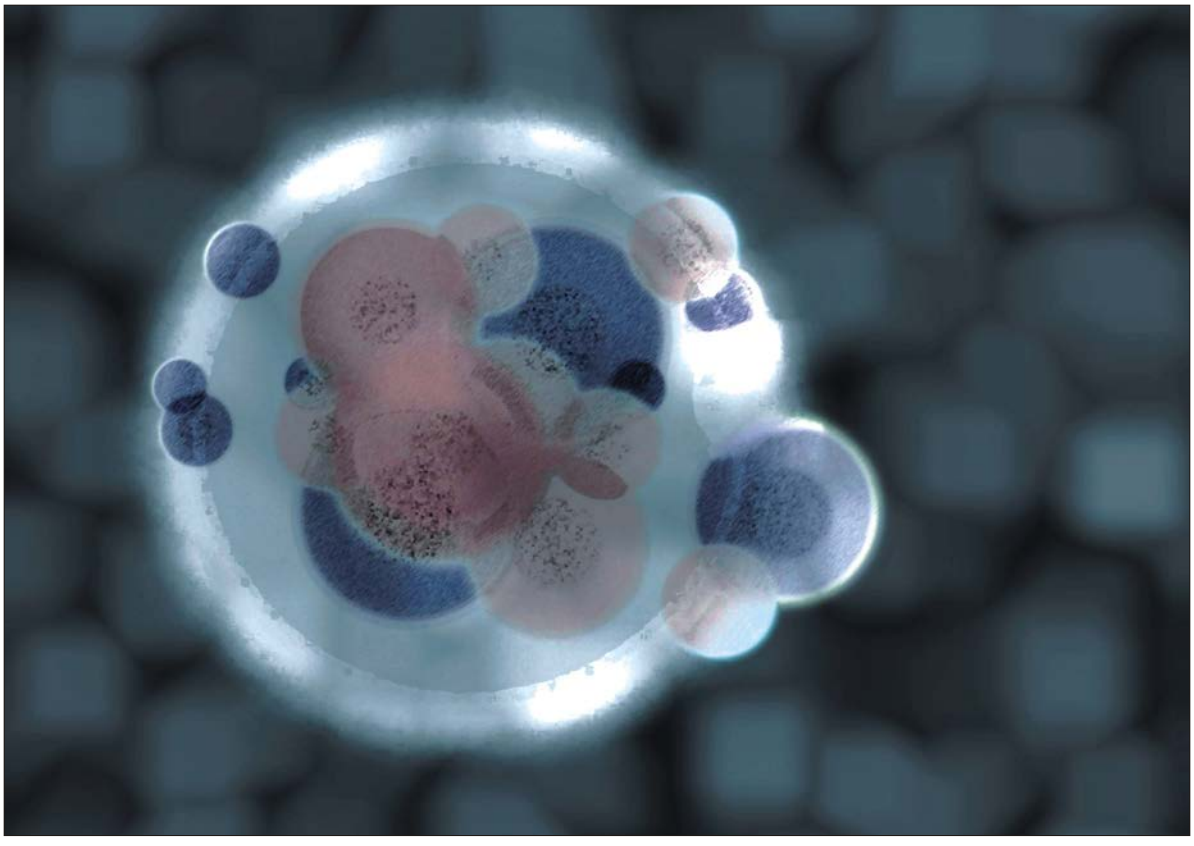

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Research Article
Detoxification of Aflatoxin B1 in Milk Using Lactic Acid Bacteria

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Abstract

Background and Objective: The prevalence of liver cancer in Egypt is particularly worrisome. Aflatoxin B1 (AFB1), a hepatocarcinogenic mycotoxin, is one of the main contributors to the high rate of hepatocellular carcinoma (HCC). This study aimed to determine the ability of four lactic acid bacteria (LAB) species in removing or binding aflatoxin B1 (AFB1) from broth media and whole milk and to evaluate the stability of LAB/AFB1 complex during cold storage at 4°C for 1 week. **Materials and Methods:** Whole milk and MRS broth media were spiked with 50 ng mL^{-1} AFB1 and incubated at 37°C for 24 h with four LAB strains followed by storage at 4°C for 24, 72 and 196 h. AFB1 removal was determined using HPLC. **Results:** The efficiency of LAB strains in removing AFB1 from both media was affected by the type of used strain and media. Whole milk was a favorable media for the tested LAB strain in AFB1 reduction when compared with MRS broth. *Lactobacillus acidophilus* (*L. acidophilus*) achieved 80% reduction in milk within 24 h at 37°C, whereas *Lactobacillus plantarum* (*L. plantarum*) favored cold storage to reduce 85% of the AFB1 content after 1 week. The count of LAB cells in whole milk medium raised in MRS broth by 0.5-2.25 log cycles. None of the tested strains affected by AFB1 (50 ng mL^{-1}) in either MRS or whole milk medium. **Conclusion:** LAB bacteria were an excellent agent used in minimizing AFB1 in milk at both 37 and 4°C.

Key words: Lactic acid bacteria, cold storage, aflatoxin B1, health hazards, bioremoval

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aflatoxins are secondary metabolites produced by *Aspergillus* spp., mainly *A. flavus* and *A. parasiticus*. They are the most common food contaminants notably in sub-tropical and tropical countries[1]. Aflatoxin B1 (AFB1), the most hazardous type affecting liver, is classified as carcinogenic group 1[2]. Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and the third most common cause of cancer-related death[3]. Aflatoxin B1 is found in foodstuffs, such as corn, rice, oil seeds, dried fruits and peanuts that have been stored in abuse conditions[4]. Moreover, Aflatoxin B1 is metabolized to monohydroxy derivative Aflatoxin M1 (AFM1) in milk and subsequently it can be found in human and animal milk, infant formula, powdered milk, cheese and yoghurt[5]. In addition to its metabolites AFM1, it can be also secreted in milk[6].

Aflatoxins have also an industrial importance due to the economic losses resulting from elimination of contaminated crops and impaired animal growth. Consequently there is a great demand for novel strategies to prevent both aflatoxin formation in food and feeds and the impact of existing aflatoxin contamination[6].

Three known strategies have been used to avoid or remove the harmful effects of aflatoxins: Physical, chemical and biological methods[7,8]. Physical approaches include heat treatments, gamma rays or ultraviolet. Chemical degradation of aflatoxin is usually carried out by addition of calcium hydroxide, hydrogen peroxide, chlorine gas or hydrolytic agents[9]. Both physical and chemical methods have limitations, such as losses of nutritional value, high coast and cause undesirable health effects. Therefore, biological control provides attractive and safe methods to remove aflatoxins from foods[10,11].

Several studies have reported the capability of many microorganisms, including bacteria, yeast, fungi, actinomycetes and algae in removing or degradation of aflatoxins from food and feed[12]. Among all types of available microorganisms that may be utilized to remove aflatoxin from contaminated medium, lactic acid bacteria (LAB) would be suitable choice for reducing the bioavailability of aflatoxins because of their unique characteristics, they are Generally Recognized As Safe (GRAS) by USFDA, also some of them have a beneficial effects on health which called probiotics[13]. Both viable and non-viable cells of lactic acid bacteria have the same adsorbent ability to bind AFB1 because adsorption occurs due to the interaction between the toxins and the functional groups of the cell surface[14,15]. The ability of LAB to binding AFB1 is affected by various conditions like temperature, pH, time of incubation and bacterial concentration[16,17].

There are no available researches studied the efficacy of LAB in AFB1 removal from milk and also the ability of LAB cells in the capture of aflatoxin during milk storage in fridge. So, the current study aimed to determine the ability of four LAB strains in minimizing AFB1 in both standard media and whole milk after 24 h incubation at 37°C and also to study the effect of cold storage on either the availability of LAB cells and on its efficacy in binding of AFB1.

MATERIALS AND METHODS

Aflatoxin B1 standard: Aflatoxin B1 standard (Sigma-Aldrich, USA) was diluted in methanol (HPLC grade) in order to obtain 50 μg mL^{-1} stock solution. By methanol evaporation, 5 μg mL^{-1} working solution was prepared in phosphate buffer saline (PBS, pH 7.3).

Lactic acid bacteria strains: Four strains of LAB were used for AFB1 binding ability test. *Lactobacillus acidophilus* CH-2 and *Streptococcus thermophilus* (*S. thermophilus*) CH-1 were obtained by Chr. Hansen's Lab., Denmark, *Lactobacillus rhamnosus* (*L. rhamnosus*) B-445 was provided by Northern Regional Research Laboratory, Illinois, USA and *Lactobacillus plantarum* EMCC-1039 was provided by Cairo MIRCEN, Egypt. All LAB strains were cultured for 24 h in MRS broth (de Man, Rogosa and Sharpe) at 37°C.

Efficacy of LAB cells in binding of AFB1 during storage: In triplicates, each of MRS media and fresh whole milk were divided into 7 groups each LAB strain. Media flasks containing 50 mL each were autoclaved at 121°C for 15 min whereas, milk treatments (50 mL each replicate) were sterilized at 115°C for 10 min. With exception of negative control group (only LAB strains cultures), each replicate of either media or milk was spiked with AFB1 at concentration of 50 ng mL^{-1}. Positive control group represented the spiked media or milk before inoculation. Each replicated of the last 5 groups were inoculated with 1 mL of each strains (2×10^8 CFU mL^{-1}). Third group represented zero time of the inoculated replicates before incubation at 37°C. The ability of LAB cells in AFB1 binding was examined in replicated of 24 h cultures (4th group). The rest cultures of either media or milk were kept in fridge and regularly withdrew after 24 h (5th group), 72 h

(6th group) and 196 h (7th group). Flasks of each treatment were centrifuged at 1800 rpm for 15 min and the supernatants were kept at -20°C for later AFB1 analysis.

Quantification of residual AFB1: Supernatants were transferred to 250 mL separating funnel, 50 mL of chloroform was added and shaken for 10 min. The lower chloroform layer was collected and evaporated in rotary evaporator at 45°C[18]. The residue was quantitatively transferred to a small vial with chloroform and evaporated to dryness under nitrogen.

Two hundred microliters quantities of hexane were added to each sample followed by 50 µL trifluoroacetic acid (TFA). The mixture was well mixed for 30 sec and left for 5 min. The mixture, 950 µL H_2O:acetonitrile (9:1 v/v) was added, mixed for 30 sec and left to stand for 10 min. The lower aqueous layer was used for HPLC analysis.

The HPLC system used for AFB1 determination was Ultimate 3000 Thermo Fisher system (Germany) equipped with auto sampler, pump, fluorescence detector and a C18 column chromatography Phenomenex (250×4.6 mm, 5 µm). The mobile phase, water:methanol:acetonitrile (60:30:10), was isocratically flowed at 1.2 mL min^{-1}, AFB1 was measured at 360 nm excitation and 440 nm emission wave length.

Effect of aflatoxin B1 on the growth and survival of lactic acid bacteria: In glass tubes, 9 mL of either MRS media or milk was inoculated with 1 mL inoculum of each strain (2×10^8 CFU mL^{-1}). AFB1 was added to get 50 ng mL^{-1} final concentration. All tubes were incubated at 37°C. Periodically at zero time, after 24, 48 and 72 h, 3 replicates of each treatment was examined for cell survival. Cell count was done using serial dilution method on same media regarding to MRS culture and on milk plate count media regarding to milk culture. Records of plate counts were compared with those without AFB1[19,20].

Statistical analysis: Statistical significance was determined using Statistica Version 9 (StateSoft, Tulsa, Okla., USA). The means were determined by analysis of variance test (ANOVA, two way analysis) ($p<0.05$). Fisher's LSD (Least Significant Difference) Method ($\alpha = 0.05$) was applied to compare significant differences between treatments.

RESULTS

Efficacy of LAB strains in AFB1 removal: The effects of four LAB strains on minimizing AFB1 in MRS media at both the optimum temperature (37°C) and at 4°C storage are shown in Table 1. The recovery of AFB1 determination in spiked media was higher than 98.2%. All strains significantly decreased AFB1 concentration after 1 day incubation at the optimum condition. The best reduction (29.6%) was observed using *L. plantarum*. Generally, AFB1 reduction continued along with cold storage reaching to 65.7% using *S. thermophilus* after 7 days. Although, *L. acidophilus* and *L. rhamnosus* recorded 59.3 and 44.4% reduction, respectively in AFB1 content after 3 days storage, they again released 10.6 and 29.7% of the absorbed AFB1 in media after 7 days. *L. plantarum* and *S. thermophilus* were able to gradually decrease AFB1 within the whole week of cold storage.

Table 1: Effect of LAB strains on AFB1 binding in MRS media at different storage periods

			AFB1 concentration (ng mL^{-1}) (Mean±S.E)				
			Incubation (37°C)		Storage (4°C)		
LAB strains	Negative control	Positive control	0	24 h	24 h	72 h	196 h
Lactobacillus plantarum							
Value	0	49.6 ± 1.08^{aa}	49.3 ± 1.32^{aa}	34.9 ± 2.68^{bc}	30.15 ± 3.79^{cd}	29.8 ± 1.28^{cb}	21.0 ± 1.58^{dc}
Reduction (%)	0	0	0.6	29.6	39.2	39.9	57.7
Lactobacillus acidophilus							
Value	0	49.4 ± 2.14^{aa}	48.5 ± 1.21^{ab}	41.6 ± 2.26^{ba}	31.6 ± 2.88^{cc}	20.1 ± 0.56^{dc}	23.2 ± 1.98^{ed}
Reduction (%)	0	0	1.8	15.8	36.0	59.3	53.0
Lactobacillus rhamnosus							
Value	0	49.3 ± 1.68^{ab}	48.6 ± 2.11^{ab}	40.8 ± 1.98^{bb}	39.2 ± 1.46^{ba}	27.4 ± 1.88^{cd}	33.5 ± 2.64^{ab}
Reduction (%)	0	0	1.4	17.2	17.2	44.4	32.0
Streptococcus thermophilus							
Value	0	49.1 ± 2.04^{ab}	48.4 ± 1.08^{ab}	41.5 ± 1.48^{ba}	35.9 ± 2.08^{cb}	34.6 ± 2.88^{da}	16.84 ± 1.11^{ed}
Reduction (%)	0	0	1.4	15.5	26.9	29.5	65.7

Means followed by different superscript letters within columns and by different subscript letters within rows are significantly different ($p = 0.05$)

Table 2: Effect of LAB strains on AFB1 binding in whole milk at different storage periods

			AFB1 concentration (ng mL^{-1}) (Mean±S.E)				
			Incubation (37°C)		Storage (4°C)		
LAB strains	Negative control	Positive control	0	24 h	24 h	72 h	196 h
Lactobacillus plantarum							
Value	0	49.2±1.78^{aa}	49.2±1.08^{aa}	36.4±2.53^{ba}	26.7±2.08^{ca}	19.7±2.04^{da}	7.3±0.58^{ed}
Reduction (%)	0	0	0	26.0	45.7	60.0	85.2
Lactobacillus acidophilus							
Value	0	49.6±2.08^{aa}	47.9±1.24^{bb}	11.1±1.36^{ed}	11.8±1.86^{ed}	18.7±1.28^{db}	23.4±1.68^{ca}
Reduction (%)	0	0	3.4	77.6	76.2	62.3	52.8
Lactobacillus rhamnosus							
Value	0	49.1±1.28^{ab}	44.4±1.58^{bc}	15.2±1.50^{cc}	13.2±1.14^{cdc}	12.1±1.22^{dc}	10.1±1.09^{ec}
Reduction (%)	0	0	9.6	69.0	73.1	75.4	79.4
Streptococcus thermophilus							
Value	0	49.4±1.46^{ab}	48.4±1.26^{bab}	21.9±2.28^{cb}	19.5±1.40^{db}	18.6±1.78^{dfb}	17.2±1.12^{fb}
Reduction (%)	0	0	2	55.7	60.5	62.3	65.2

Means followed by different superscript letters within columns and by different subscript letters within rows are significantly different (p = 0.05)

Table 2 illustrates the AFB1 removal by four LAB strains grown in whole milk at optimum condition (37°C) and cold storage (4°C). Similar trend of reduction as media was observed in whole milk. Interestingly, around 10% of AFB1 content in milk was adsorbed at 0 time by *L. rhamnosus* inoculation. Comparing with MRS media, the used LAB strains achieved higher reduction in milk at optimum condition within 24 h ranging from 26.0-77.6%. AFB1 reduction continued along with the cold storage recording 85.2, 79.4 and 65.2% using *L. plantarum*, *L. rhamnosus* and *S. thermophilus*, respectively. Regarding to *L. acidophilus*, cold storage was unsuitable condition for AFB1 binding. Instead of reduction, 18.2 and 30.7% of the adsorbent AFB1 were released in milk after 3 and 7 days, respectively of cold storage.

Impact of AFB1 on the survival of LAB strains: The effect of AFB1 (50 ng mL^{-1}) on the growth and survival of the tested LAB strains on MRS media within 3 days is illustrated in Fig. 1. In general, treating media with AFB1 had no effect on the growth of all LAB strains when compared with control. The highest growth was recorded after 1 day incubation followed by gradual decaying after 48 and 72 h. The *S. thermophilus* achieved the fastest growth among all strains in both control and treated media recording 13.36 and 13.21 log CFU mL^{-1}, respectively after 1 day. While, *L. plantarum* recorded the lowest growth (10 and 9.84 log CFU mL^{-1} in both control and treated media, respectively).

Figure 2 shows the impact of AFB1 addition in whole milk on the growth of the tested LAB strains. None of the examined LAB strains were affected by AFB1 addition in whole milk. In either control or treated milk, log phase of all strains was observed after 24 h followed by cell decaying after 48 and 72 h representing in lag phase. Interestingly, milk was a favorable media for the growth of *L. plantarum* (12 log CFU mL^{-1}) when compared with MRS media (10 CFU mL^{-1}). Similar to MRS media, *S. thermophilus* recorded the highest growth after 24 h in both control and spiked medium with 13.80 and 13.38 log CFU mL^{-1}, respectively. While, the lowest growth was recorded using *L. acidophilus* in both control and treated medium (11.36 and 11.25 log CFU mL^{-1}, respectively). Generally, the growth of LAB strains in whole milk was favorable when compared with MRS broth rising in range between 0.50-2.25 log cycles.

DISCUSSION

Normal healthy intestinal microflora contains many strains of lactic acid bacteria (LAB), some of which have been isolated, ascribed health benefits and termed probiotic strains. Lactic acid bacteria have been widely used in binding of aflatoxins in contaminated media. Both viable and non-viable cells have the same adsorbent ability to bind aflatoxins[6]. The ability of LAB bacteria in AFB1 removal from whole liquid milk and broth media greatly affected by several factors, AFB1 concentration, bacterial counts, time of incubation, temperature and pH[16,21]. However, there are no available works studied the efficacy of LAB bacteria in minimizing AFB1 during cold storage. So, the present study focused on the impact of culture media type (MRS and

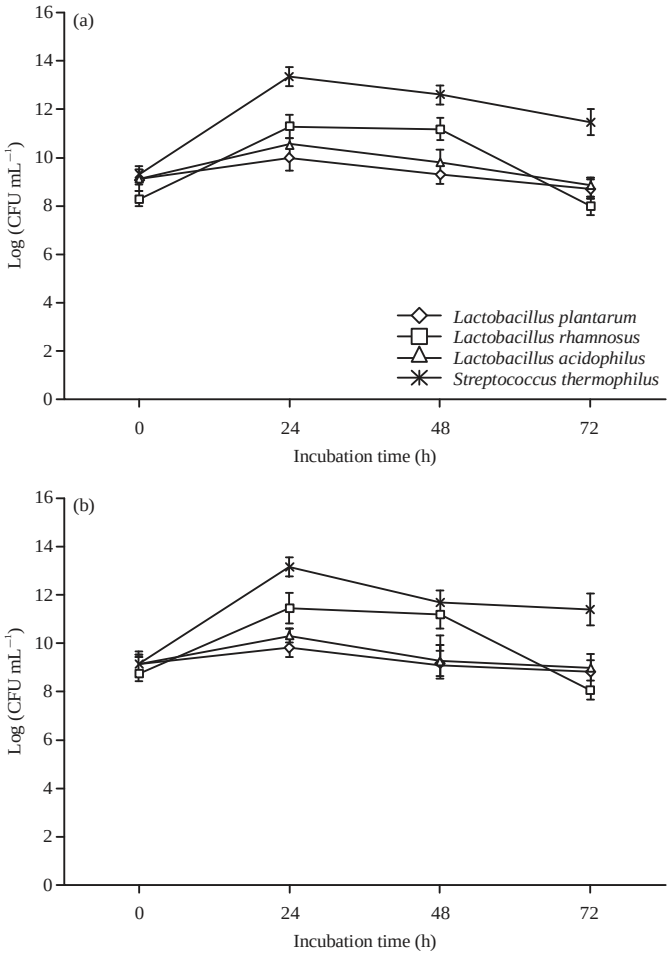

Fig. 1(a-b): Effect of Aflatoxin B1 on the survival of some LAB strains on MRS broth (a) MRS broth and (b) MRS broth contains 50 ng mL^{-1}

milk) on AFB1 binding by LAB strains and on the stability of LAB/AFB1 complex during cold storage.

In the current study, *L. plantarum* had the highest reduction effect on AFB1 (30%) in MRS for 24 h among the tested LAB species followed by *L. rhamnosus* (18%), *S. thermophilus* and *L. acidophilus* (17%). In contrast, Zinedine *et al.*[22], found the examined *L. plantarum* strains had a negligible reduction effect (2-5%) and the highest removal was using *L. rhamnosus* strains (25-45%) in MRS broth at 30°C after 48 h. Likewise, Peltonen *et al.*[23] studied the binding of AFB1 in phosphate buffer solution (PBS) by 12 *Lactobacillus*, 5 *Bifidobacterium* and 3 *Lactococcus* strains. They found that *L. rhamnosus* strains were able to minimize >50% of AFB1 content at 37°C within 24 h. El-Nezami *et al.*[21] reported that *L. rhamnosus* strains followed by *L. acidophilius* removed around 80 and 60% of AFB1, respectively in PBS. Kankaanpaa *et al.*[24] and Gratz *et al.*[25] found that the ability in AFB1 binding *in vitro* and *in vivo* depended on LAB strain.

Although, the examined LAB species reduced AFB1 effectively at 4°C within 196 h notably *S. thermophilus* (66.4% reduction). None of the previous studies evaluated the effect of cold storage on AFB1 binding in MRS or other media.

With exception of *L. plantarum*, the binding ability increased in whole milk media at 37°C reaching to 78% using *L. acidophilus*. In contrary, incubation at 4°C discouraged AFB1 binding by these species. This finding was in accordance with Rayes[16], who found that the optimum temperature for AFB1 removal from milk was 37°C and the lowest removal at 5°C. Otherwise, Haskard *et al.*[6] reported that the incubation temperature did not significantly affect the stability of AFB1/LAB complexes formed between *L. rhamnosus* strains and AFB1 in the range from 4-37°C.

The present study revealed that the noticeable reduction in AFB1 were observed after 24 h incubation at 37°C in MRS. The only *L. rhamnosus* showed 10% reduction at 0 time in whole milk media at 37°C. In this regard, El-Nezami *et al.*[21] and

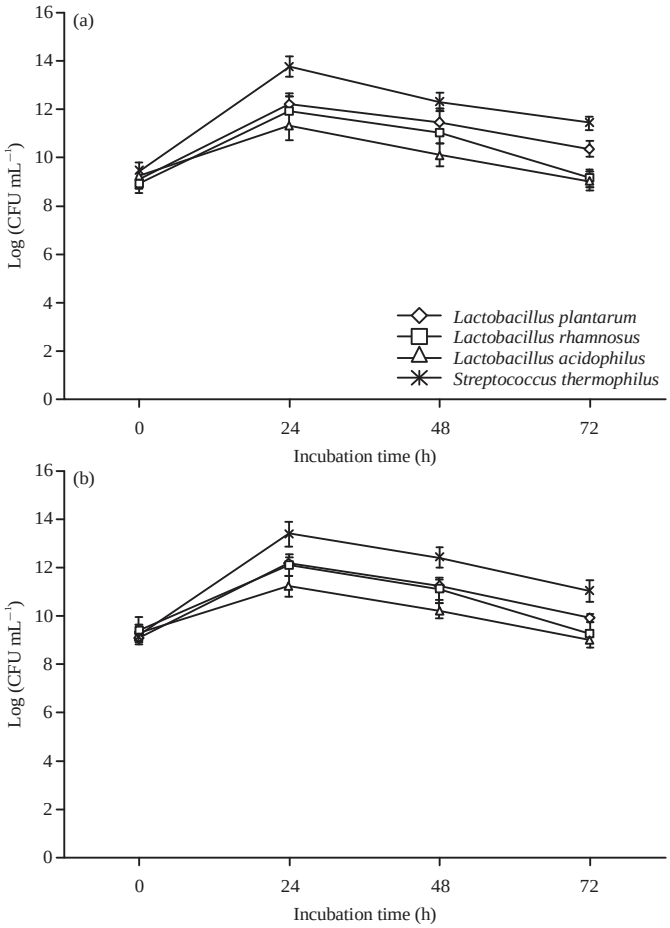

Fig. 2(a-b): Effect of Aflatoxin B1 on the survival of some LAB strains on whole milk, (a) Whole milk media and (b) Whole milk media contains 50 ng mL^{-1}

Peltonen *et al.* [23] found that by varying the incubation time, no significant difference in the amount of the removed AFB1 by LAB strain were observed and the process was fast, since the 1st min. While, Kasmani *et al.*[26] reported that the amount of the adsorbed AFB1 in PBS buffer by LAB strains was time dependent recording the best adsorbing after 12 h. Khanafari *et al.*[27] showed that *L. plantarum* bound AFB1 at the rate of 45% in 1 h and total binding after 90 h was observed. Likewise, Sezer *et al.*[17] found that AFB1 binding by *L. plantarum* and *L. lactis* was almost complete in the first 6 h. Hussein[28] observed an increase in AFB1 binding by *L. casei* and *L. acidophilus* within the first 60 h of incubation followed by a constant binding rate for the next 20 h.

Bovo *et al.*[29] found that there were no significant differences between MRS broth and milk whey based medium in the adsorption of AFB1 by *L. rhamnosus*. These findings disagree with those of the present study. Whole milk media was a favorable media for AFB1 reduction when compared with MRS at both 37°C and during the cold storage for 1 week.

Ringot *et al.*[30] attributed the toxin binding on the surface of microbial cells to the fast physicochemical interaction with the functional group of the cells surface. El-Nezami *et al.* [21] noticed a rapid toxin binding rate (80%) in 1 h by lactic acid bacteria. Bovo *et al.*[29] refer to the production of greater quantities of *L. rhamnosus* cells using milk whey medium (MWM) compared with that of MRS broth. This result may explain why AFB1/LAB binding in milk was greater than MRS in the present study.

The concentration of 50 ng mL^{-1} AFB1 in either MRS or whole milk media had no effect on the viability of the tested LAB species at 37°C for 72 h. In this regard, very few studies were found. Khanafari *et al.*[27] observed that *L. casei* and *L. acidophilus* concentration gradually decreased by AFB1 increasing.

CONCLUSION

Whole milk media was the favorable media for both the growth of the tested LAB bacteria and for minimizing the AFB1 content when compared with MRS broth. *L. plantarum* reduced effectively AFB1 (85%) in whole milk at 4°C, whereas *L. acidophilus* preferred the optimum temperature (37°C) to achieved 80% of AFB1 reduction. Finally, it is recommended to use LAB bacteria in food and dairy industries as a bioremoval agent of aflatoxins contamination.

SIGNIFICANCE STATEMENT

This study considers the first one used lactic acid bacteria to eliminate AFB1 in milk during cold storage for different times. The current study will encourage scientists to use LAB in detoxification of other potential toxins in dairy products rather than aflatoxin B1. Also, it considers an applicable study that can be used in food and dairy industries to bio-remove aflatoxins contamination using useful bacteria.

REFERENCES

1. Guan, S., M. Gong, Y. Yin, R. Huang, Z. Ruan, T. Zhou and M. Xie, 2011. Occurrence of mycotoxins in feeds and feed ingredients in China. J. Food Agric. Environ., 9: 163-167.
2. Bhatnagar, D., K.C. Ehrlich and T.E. Cleveland, 2003. Molecular genetic analysis and regulation of aflatoxin biosynthesis. Applied Microbiol. Biotechnol., 63: 83-93.
3. Bosch, F.X., J. Ribes, R. Cleries and M. Diaz, 2005. Epidemiology of hepatocellular carcinoma. Clin. Liver Dis., 9: 191-211.
4. Kensler, T.W., B.D. Roebuck, G.N. Wogan and J.D. Groopman, 2011. Aflatoxin: A 50-year odyssey of mechanistic and translational toxicology. Toxicol. Sci., 120: S28-S48.
5. Ketney, O., A. Santini and S. Oancea, 2017. Recent aflatoxin survey data in milk and milk products: A review. Int. J. Dairy Technol., 70: 320-331.
6. Haskard, C.A., H.S. El-Nezami, P.E. Kankaanpaa, S. Salminen and J.T. Ahokas, 2001. Surface binding of aflatoxin B_1 by lactic acid bacteria. Applied Environ. Microbiol., 67: 3086-3091.
7. Bueno, D., M.A. Salvano, J.O. Silva, S.N. Gonzalez and G. Oliver, 2001. Mycotoxins: Diagnosis and prevention in poultry. Boletin Micologico, 16: 23-36.
8. Kabak, B., A.D.W. Dobson and I. Var, 2006. Strategies to prevent mycotoxin contamination of food and animal feed: A review. Crit. Rev. Food Sci. Nutr., 46: 593-619.
9. Karlovsky, P., M. Suman, F. Berthiller, J. De Meester and G. Eisenbrand *et al*., 2016. Impact of food processing and detoxification treatments on mycotoxin contamination. Mycotoxin Res., 32: 179-205.
10. Alberts, J.F., Y. Engelbrecht, P.S. Steyn, W.H. Holzapfel and W.H. van Zyl, 2006. Biological degradation of aflatoxin B_1 by *Rhodococcus erythropolis* cultures. Int. J. Food Micobiol., 109: 121-126.
11. Fan, Y., L. Zhao, Q. Ma, X. Li and H. Shi *et al*., 2013. Effects of *Bacillus subtilis* ANSB060 on growth performance, meat quality and aflatoxin residues in broilers fed moldy peanut meal naturally contaminated with aflatoxins. Food Chem. Toxicol., 59: 748-753.
12. Hathout, A.S. and S.E. Aly, 2014. Biological detoxification of mycotoxins: A review. Ann. Microbiol., 64: 905-919.
13. Fuchs, S., G. Sontag, R. Stidl, V. Ehrlich, M. Kundi and S. Knasmuller, 2008. Detoxification of patulin and ochratoxin A, two abundant mycotoxins, by lactic acid bacteria. Food Chem. Toxicol., 46: 1398-1407.
14. Lee, Y.K., H. El-Nazami, C.A. Haskard, S. Gratz, K.Y. Puong, S. Salminen and H. Mykkanen, 2003. Kinetics of adsorption and desorption of aflatoxin B_1 by viable and nonviable bacteria. J. Food Prot., 66: 426-430.
15. Bueno, D.J., C.H. Casale, R.P. Pizzolitto, M.A. Salano and G. Olivier, 2007. Physical adsorption of aflatoxin B_1 by lactic acid bacteria and *Saccharomyces cerevisiae* : A theoretical model. J. Food Prot., 70: 2148-2154.
16. Rayes, A.A.H., 2013. Removal of aflatoxin B_1 from experimentally contaminated whole milk using a pool of probiotic strains of lactic acid bacteria and baker's yeast *Saccharomyces cerevisiae*. N. Y. Sci. J., 6: 84-90.
17. Sezer, C., A. Guven, N.B. Oral and L. Vatansever, 2013. Detoxification of aflatoxin B_1 by bacteriocins and bacteriocinogenic lactic acid bacteria. Turk. J. Vet. Anim. Sci., 37: 594-601.
18. Peltonen, K.D., H.S. El Nezami, S.J. Salminen and J.T. Ahokas, 2000. Binding of aflatoxin B_1 by probiotic bacteria. J. Sci. Food Agric., 80: 1942-1945.
19. De Caire, G.Z., J.L. Parada, M.C. Zaccaro and M.M.S. de Cano, 2000. Effect of *Spirulina platensis* biomass on the growth of lactic acid bacteria in milk. World J. Microbiol. Biotechnol., 16: 563-565.
20. Balouiri, M., M. Sadiki and S.K. Ibnsouda, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. J. Pharma. Anal., 6: 71-79.
21. El-Nezami, H., P. Kankaanpaa, S. Salminen and J. Ahokas, 1998. Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B_1. Food Chem. Toxicol., 36: 321-326.
22. Zinedine, A., M. Faidand and M. Benlemlih, 2005. *In vitro* reduction of aflatoxin B_1 by strains of lactic acid bacteria isolated from Moroccan sourdough bread. Int. J. Agric. Biol., 7: 67-70.

23. Peltonen, K., H. El-Nezami, C. Haskard, J. Ahokas and S. Salminen, 2001. Aflatoxin B_1 binding by dairy strains of Lactic acid bacteria and bifidobacteria. J. Dairy Sci., 84: 2152-2156.
24. Kankaanpaa, P., E. Tuomola, H. El-Nezami, J. Ahokas and S.J. Salminen, 2000. Binding of aflatoxin B_1 alters the adhesion properties of *Lactobacillus rhamnosus* strain GG in a Caco-2 model. J. Food Prot., 63: 412-414.
25. Gratz, S., H. Mykkanen, A.C. Ouwehand, R. Juvonen, S. Salminen and H. El Nezami, 2004. Intestinal mucus alters the ability of probiotic bacteria to bind aflatoxin B_1 *in vitro*. Applied Environ. Microbiol., 70: 6306-6308.
26. Kasmani, F.B., M.A.K. Torshizi, A.A. Allameh and F. Shariatmadari, 2012. Aflatoxin detoxification potential of lactic acid bacteria isolated from Iranian poultry. Iran. J. Vet. Res., 13: 152-155.
27. Khanafari, A., H. Soudi, M. Miraboulfathi and R.K. Osboo, 2007. An *in vitro* investigation of aflatoxin B_1 biological control by *Lactobacillus plantarum*. Pak. J. Biol. Sci., 10: 2553-2556.
28. Hussein, H.A., 2008. Effect of certain factors on surface binding of aflatoxin B_1 by some probiotic strains. J. Radiat. Res. Applied Sci., 1: 349-361.
29. Bovo, F., L.T. Franco, R.E. Rosim and C.A.F. de Oliveira, 2014. Ability of a *Lactobacillus rhamnosus* strain cultured in milk whey based medium to bind aflatoxin B_1. Food Sci. Technol., 34: 566-570.
30. Ringot, D., B. Lerzy, K. Chaplain, J.P. Bonhoure, E. Auclair and Y. Larondelle, 2007. *In vitro* biosorption of ochratoxin A on the yeast industry by-products: Comparison of isotherm models. Bioresour. Technol., 98: 1812-1821.