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Original Article

Probiotic Strategies for Detoxification of AFM1 in Skim Milk Using *Bifidobacterium Lactis* and *Streptococcus Thermophiles*

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ABSTRACT

This study was conducted to evaluate the efficacy of *Bifidobacterium lactis and Streptococcus thermophilus*, both independently and in combination, in detoxifying skim milk contaminated with aflatoxin M1 (AFM1). To achieve this, two concentrations of the bacteria (8 and 10 log CFU/mL) were inoculated into skimmed milk contaminated with three levels of AFM1 (0.1, 0.25, and 0.5 µg/mL) and incubated at two different temperatures (4 and 42°C). High-performance liquid chromatography (HPLC) was employed to measure the removal percentage of AFM1 at various intervals (30, 60, 120 minutes, and 24 hours). Results indicated a significant time-dependent increase in AFM1 removal from the skim milk. The removal efficiency of AFM1 by these bacterial strains ranged from 12% to 87%, influenced by bacterial concentration, incubation time, toxin concentration, and whether the bacteria were used alone or in combination. *B. lactis* exhibited a superior capacity for AFM1 removal compared to *S. thermophilus*. The optimal strategy for maximum AFM1 removal (87%) involved treating contaminated milk spiked with 0.5 µg/mL of AFM1 with a mixture of *B. lactis* and *S. thermophilus* at concentrations of 10 and 8 log CFU/mL, respectively, and incubating at 42ºC for 24 hours. This study suggests a potentially effective method for reducing AFM1 concentrations in the dairy industry, thereby mitigating public health risks associated with aflatoxin contamination. The implications of these findings could significantly contribute to improving food safety standards and reducing exposure to harmful toxins in dairy products. Further research is recommended to explore the underlying mechanisms of AFM1 removal by these probiotic strains and to validate these findings under commercial dairy processing conditions.

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1. Introduction

Aflatoxins (AFs), as some of the most important mycotoxins, are natural by-products that cause serious food quality and safety problems worldwide. AFs are produced by the fungal species *Aspergillus*, particularly *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (1, 2). They are commonly found in foods and feeds such as cereals, oilseeds, spices, and nuts, especially in tropical regions. Among the 20 known AFs, AFB1, AFB2, AFG1, and AFG2 are the primary ones. The highest toxicity is associated with AFB1, produced by *A. flavus,*which is often found in the feed of dairy ruminants. Aflatoxin M1 (AFM1) is a hydroxylated metabolite of AFB1 formed in the liver and excreted in the milk of animals or humans who have consumed an aflatoxin-contaminated diet. Various factors, including the species type, diet, and individual factors such as lactation period and milk production yield, influence the conversion of AFB1 to AFM1 (3, 4). Milk and dairy products with high consumption rate across all ages, especially children, serve as a vehicle for contaminants that pose serious risk to human health. Aflatoxins are known for their heat-resistant properties, which means that even the pasteurization process of milk doesnot effectively inactivate AFM1 contamination. (5).

Despite affecting numerous important agricultural products and increasing economic costs, AFs are carcinogens and hepatotoxic agents. Due to the high incidence of AFM1 in milk, several countries have implemented strict control policies to reduce the risk of aflatoxin exposure (6). Efforts to detoxify contaminated products have been ongoing for decades (7). Several strategies have been developed to prevent the growth of mycotoxigenic fungi and to eliminate or inactivate AFM1 in milk. However, these strategies have limitations, such as reduced nutritional value, low organoleptic qualities, low efficiency and safety concerns, and high costs. Recently, biological methods have gained attention as alternative strategies to chemical and physical treatments (8, 9). Certain lactic acid-producing microorganisms have recently attracted interest due to their ability to detoxify AFM1 in contaminated milk (10, 11).

Aflatoxin detection and quantification of aflatoxins are critical for safety concerns. Various methods are available, while enzyme-linked immune-sorbent assay (ELISA) and High-performance liquid chromatography (HPLC) are the most widely used. Given food safety, public health risks, and economic factors related to the presence of aflatoxin in food and animal diets such as silage, combining beneficial microorganisms is probably the most effective strategy for achieving the optimal effect. *Bifidobacteria* are abundant in the normal gut flora, used in dairy products as probiotics, and have shown potential in aflatoxin detoxification. Some species of *Bifidobacteria*, such as *Bifidobacterium bifidum* and *Bifidobacterium lactis*, have been reported to possess aflatoxin detoxification properties. Additionally, *S. thermophilus*, as a major dairy starter, exhibits antimicrobial, antioxidant, and antitoxin effects (12).

Even though there are some reports on AFB1 detoxification by different microbes, the effects of *Streptococcus thermophilus* and *Bifidobacterium lactis* have not been compared. The objective of this study was to identify the most effective method for detecting aflatoxin M1 contamination in skim milk using HPLC. Given thatphysicochemical parameters, such as temperature and the concentrations of AFM1 and probiotics, could affect the detoxification of AFM1 (13, 14), we initially investigated the detoxification effects of bacteria at two levels of bacteria concentration (8 and 10 logs CFU/mL) and incubation temperature (4 and 42ºC)), along with three levels of AFM1 concentrations (0.1, 0.25 and 0.5 µg /mL) during storage at the different time point (30, 60, 120 min and 24h), using HPLC. In the second step, we chose the best strategies for each bacterium and compared the individual probiotics to determine the most effective strategy for detoxifying AFM1.

2. Materials and Methods

2.1.Preparation of Bacteria

The bacterial strains used in this study, *Bifidobacterium animalis* subsp. *lactis Bb12* and *Streptococcus thermophilus* PTCC7788, were purchased from Chr. Hansen (Denmark). To prepare the cell suspension, 1g of lyophilized bacteria was cultivated in 100 mL of De Man, Rogosa, and Sharpe (MRS) broth and M17 broth and incubated at 37°C for 24 hours. The culture media was then centrifuged at 3500 ×g at 4°C for 15 minutes to harvest the cells. The turbidity of suspension was standardized to match that of a 10 McFarland standard, which corresponds to approximately 3×10^{10} CFU/ mL. The cell suspension was counted using a hemocytometer (Neubauer counting chamber) to obtain two final concentrations of 10 and 8 log CFU/ mL(15).

2.2.Preparation of AFM1

AFM1 powder (6795-23-9, Aokin, Germany) was diluted in acetonitrile to achieve a concentration of 10 µg/mL. The AFM1 standard solution was further diluted in acetonitrile to obtain a concentration of 1 µg/mL and stored at 4°C until use(16).

2.3.Contamination and Inoculation of Skim Milk

Skimmed milk was prepared by mixing skim milk powder (115363, Merck, Germany) with distilled water in a ratio of 1:10 (w/v). The skim milk samples were agitated for 5 minutes and then centrifuged at 3500 ×g at 4ºC for 10 minutes to separate the cream. After centrifugation, the upper cream layer was completely removed from the skim milk. Subsequently, samples were spiked with three different concentrations of AFM1 working solutions (0.1, 0.25, and 0.5 µg /mL) at 42ºC. After milk contamination, 9 mL samples,both separately and in combination, were inoculated with bacteria at two concentrations (10 and 8 log CFU /mL) and incubated at two temperatures (4 and 42ºC) for different time points (30, 60,120 min, and 24 h). The skim milk samples with AFM1 and bacteria were the positive control, while samples without bacteria acted as the negative control. After due time, the samples were centrifuged at 2750 ×g for 5 minutes to harvest the supernatant,which was then to evaluate the residual aflatoxin (17). Each treatment sample was named according to Table 1.

2.4.AFM1 Determination By HPLC Method

The detection of AFM1 residues in skim milk was evaluated using the HPLC method, as described by Sarlak et al.(18), with minor modifications. An HPLC system (Waters Alliance 2695 Separations Module) equipped with a column (Grom Sil C18 ODS-5ST, 5µm×250×4.6mm) and a fluorescence detector 2475 were used in this study. The excitation and emission wavelengths were set at 365 and 465 nm, respectively. The mobile phase consisted of water, methanol, and acetonitrile in a ratio of 60:20:20. The flow rate was set at 1 mL/min and the injection volume was 150µl.

The percentage of AFM removed by bacteria was calculated as follows.

%AFM1 removal=100 * [1 - (peak area of sample)/ peak area of positive control)].

2.5.Statistical Analysis

The data in the study were described using frequency (percent) for qualitative variables and Mean±SD for quantitative variables. The normality of the distribution of quantitative variables was assessed using the Shapiro–Wilk test. To compare the means of normal and non-normal quantitative variables between the two treatment groups (*B. lactis* and *S. thermophilus*), the independent T-test and Mann-Whitney U test were applied, respectively. The Kruskal-Wallis test was used to compare the mean of quantitative variables between the three toxin concentrations (0.1, 0.25, and 0.5 µg /mL). If there was a significant difference between the three toxin concentrations, the Dunn-Bonferroni test was utilized to identify which toxin concentration pairs caused these differences. In addition, the effects of the treatment type, temperature, toxin concentration, and bacterial concentration on AFM1 removal percent were evaluated using the generalized estimating equations (GEE) model. All statistical analyses were performed using SPSS version 20 , with a significant level of 0.05.

3. Results

We examined the percentage of AFM1 removal by two bacteria (*B. lactis* and *S. thermophilus*) at two levels of bacteria concentration (8 and 10 logs CFU/mL) , two incubation temperatures (4 and 42ºC) ,and three levels of AFM1 concentrations (0.1, 0.25 and 0.5 µg /mL) during storage at the different time points (30, 60, 120 and 1440 min) using HPLC.

3.1. Effect of Bacterial Concentration

Our findings showed a significant difference between the two treatment types at both bacteria concentrations after 30 minutes (P <0.05). At 60, 120, and 1440 minutes, the mean of AFM1 removal was significantly higher in the *B. lactis* treatment compared to *S. thermophiles* at the 10 log CFU/mL concentration (P <0.05). No significant difference was observed in the mean of AFM1 removal percentage between the two levels of bacteria concentrations within treatment types (P>0.05)(Table 2). Figures 1F-A and 1-B show the trends of AFM1 removal percentage over time in both treatment types by bacterial concentration (BC). Results from the Friedman test revealed that the mean AFM1 removal rate at each treatment type increased significantly over time in both bacterial concentrations(P <0.05).(Table-5)

3.2. Effect of incubation temperature

Table 3 shows the results of evaluating the percentage of AFM1 removal between treatment types at different time points and temperatures. We found that the mean percentage of AFM1 removal showed a significant difference between the two treatment types at 30, 60, and 120 minutes (P <0.05).).(Table-5)

Table 1. Culture condition for tested strains.

Treatment type	Bacterial Concentration (BC) 8 and 10 log CFU /mL	Temperature (Tem) 4 and 42ºC	Toxin concentration (TC) 0.1, 0.25 and 0.5 µg /mL
B. lactis	B.L-8, B.L-10	B.L-4, B.L-42	B.L-0.1, B.L-0.25, B.L-0.5
S. thermophilus	S.T-8 S.T-10	S.T-4 S.T-42	S.T-0.1, S.T-0.25, S.T-0.5

B.L: *B. lactis*, S.T: *S. thermophilus*

Table 2. The Comparison of AFM1 removal percent between treatment types and bacterial Concentration within each treatment type by time.

Time (min)	Bacterial Concentration (BC)	Treatment type		P-value
		B. lactis	***S. thermophilus***	
30	8 log CFU /mL	30.50±8.68	17.83±6.21	0.02*
	10 log CFU /mL	34.83±9.74	18.50±3.93	0.005*
	P-value	0.52	0.62	
60	8 log CFU /mL	34.17±10.26	22.50±6.80	0.054
	10 log CFU /mL	40.00±12.06	21.00±4.85	0.01*
	P-value	0.33	0.68	
120	8 log CFU /mL	36.50±9.87	26.33±5.82	0.055
	10 log CFU /mL	43.00±12.99	23.17±4.44	0.008*
	P-value	0.37	0.33	
1440	8 log CFU /mL	51.50±19.21	40.17±8.88	0.14
	10 log CFU /mL	52.00±14.01	32.00±7.15	0.01*
	P-value	0.68	0.12	

*Significant at the level of 0.05; # Values are reported as Mean ± SD.

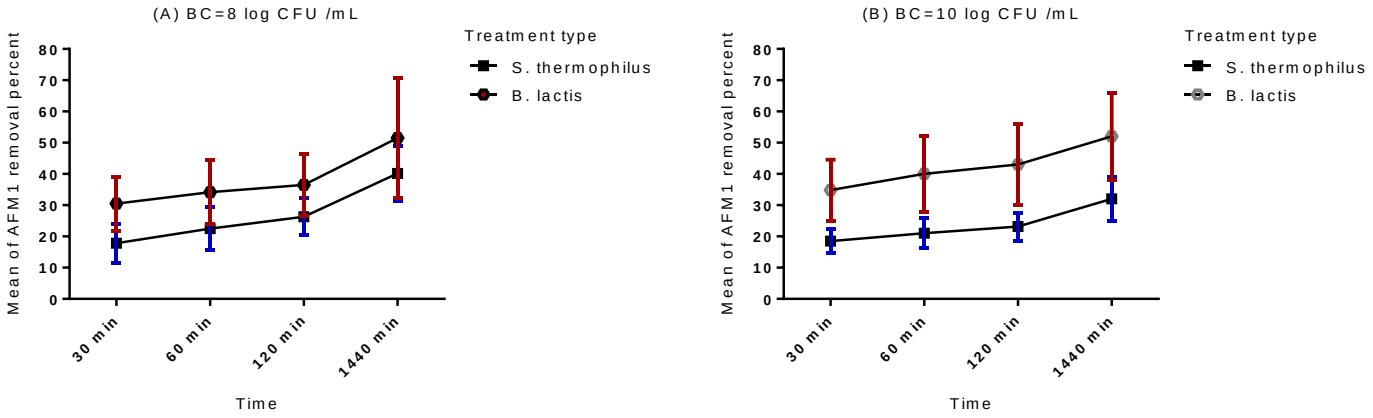

Figure 1. Change trends of AFM1 removal percent in both treatment types in terms of bacterial concentration (BC).

Table 3. The Comparison of AFM1 removal percent between treatment types and temperature by time.

Time (min)	Temperature (T)	Treatment type		P-value
		B. lactis	*S. thermophilus*	
30	4 ºC	28.33±8.16	13.67±1.63	0.004*
	42 ºC	37.00±8.36	22.67±1.75	0.004*
	P-value	0.07	0.004*	
60	4 ºC	32.00±10.11	16.67±1.03	0.003*
	42 ºC	42.17±10.34	26.83±2.85	0.006*
	P-value	0.09	0.003*	
120	4 ºC	34.67±10.25	21.33±1.96	0.004*
	42 ºC	44.83±11.16	28.17±5.26	0.006*
	P-value	0.09	0.04*	
1440	4 ºC	51.33±20.13	33.67±4.17	0.03*
	42 ºC	52.17±12.64	38.50±11.77	0.055
	P-value	0.68	0.22	

*Significant at the level of 0.05; # Values are reported as Mean ± SD.

At 1440 minutes, the mean percentage of AFM1 removal in the *S. thermophiles* group was significantly lower compared to the B. lactis group,but only at 4ºC (P <0.05). In the *S. thermophilus* group, a significant difference was observed in the mean percentage of AFM1 removal between the two temperatures at 30, 60, and 120 minutes (P<0.05). However, in the *B. lactis* group, no significant difference was observed in the mean percentage of AFM1 removal between the two temperatures (P>0.05). As shown in Figures 2-A and 2-B, the mean percentage of AFM1 removal in both treatment types increased over time at both temperatures. Results from the Friedman test indicated that the mean AFM1 removal rate at each treatment type increased significantly over time at both temperatures (P <0.05).

3.3. Effect of treatments on AFM1 concentration

Table 4 presents a comparison of the AFM1 removal percentage between two treatment types at different time points across three toxin concentrations (0.1, 0.25, and 0.5 µg/mL). Our findings revealed a significant difference between the two treatment types at 0.25 and 0.50 µg/mL toxin concentrations at 30 and 60 minutes (P <0.05). At 120 and 1440 minutes, we observed that the mean AFM1 removal rate in the *B. lactis* group was significantly higher than that in the *S. thermophiles* group at 0.50 µg/mL toxin concentration (P<0.05). In the *B. lactis* group, a significant difference was observed in the mean AFM1 removal percentage among the three toxin concentrations (0.1, 0.25, and 0.5 µg/mL) at 30, 60, and 120 minutes, based on the results of the Kruskal–Wallis test (P <0.05). To understand which mean differences between the two toxin concentrations had caused significant differences among the three toxin concentrations, we used the Dunn-Bonferroni post-hoc test. According to Dunn-Bonferroni post-hoc test results in the *B. lactis* group, there was a statistically significant difference in mean AFM1 removal percentage between 0.1 and 0.5 µg/mL toxin concentrations at times 30, 60, and 120 minutes (P <0.05). In contrast, no significant difference was observed in the mean AFM1 removal percentage between the three toxin concentrations (0.1, 0.25, and 0.5 µg/mL) in the *S. thermophiles* group (P>0.05). Figures 1-A, 1-B, and 1-C show trends of AFM1 removal percentage over time in both treatment types by toxin concentrations (TC). The Friedman test results showed that the mean AFM1 removal rate at each treatment type increased significantly over time across all three toxin concentrations (P <0.05).

The generalized estimating equations (GEE) model was used to investigate the effects of treatment type, temperature, toxin concentration, and bacterial concentration on AFM1 removal percentage. Results from the GEE model showed that there was a statistically significant difference in the mean AFM1 removal percentage between two treatment types, two temperatures, two bacterial concentrations, and three toxin concentrations at baseline or first measurement (30 minutes) (P<0.05) (Figure 3).(Table-5)

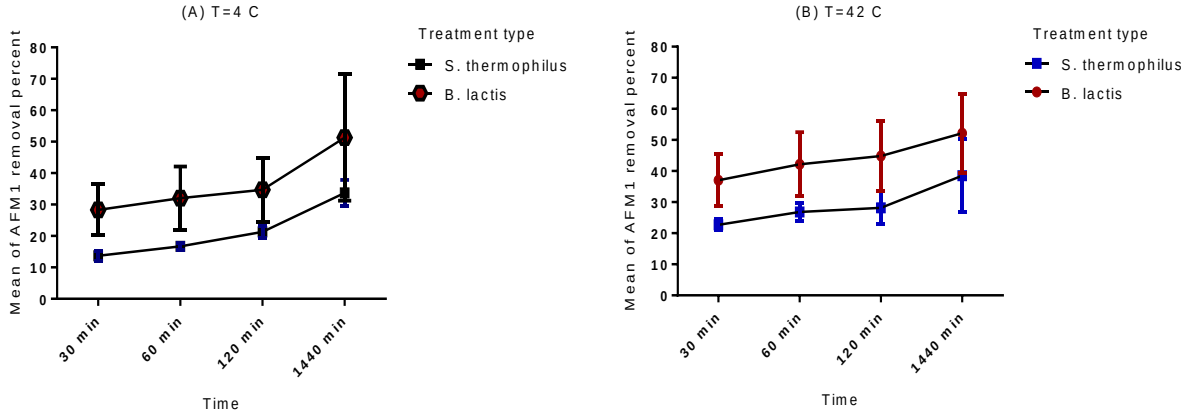

Figure 2. Change trends of AFM1 removal percent in both treatment types in terms of temperature (T).

Table 4. The Comparison of AFM1 removal percent between treatment types and toxin concentration by time.

Time (min)	Toxin concentration (TC)	Treatment type		P-value
		B. lactis	*S. thermophilus*	
30	0.1 µg /mL	25.25±4.78	19.25±6.23	0.19
	0.25 µg /mL	30.50±6.45	17.75±4.64	0.02*
	0.5 µg /mL	42.25±6.02	17.50±5.26	0.02*
	P-value	0.02*	0.69	
60	0.1 µg /mL	28.50±7.14	22.50±7.89	0.38
	0.25 µg /mL	33.75±5.85	22.00±4.89	0.04*
	0.5 µg /mL	49.00±8.04	20.75±5.50	0.02*
	P-value	0.03*	0.80	
120	0.1 µg /mL	31.00±6.05	24.50±7.32	0.14
	0.25 µg /mL	36.00±6.27	26.25±4.34	0.08
	0.5 µg /mL	52.25±8.99	23.50±4.65	0.02*
	P-value	0.02*	0.58	
1440	0.1 µg /mL	51.00±23.62	36.75±4.57	0.19
	0.25 µg /mL	43.50±8.18	37.00±2.94	0.18
	0.5 µg /mL	60.75±10.87	34.50±15.78	0.04*
	P-value	0.15	0.48	

*Significant at the level of 0.05; # Values are reported as Mean ± SD.

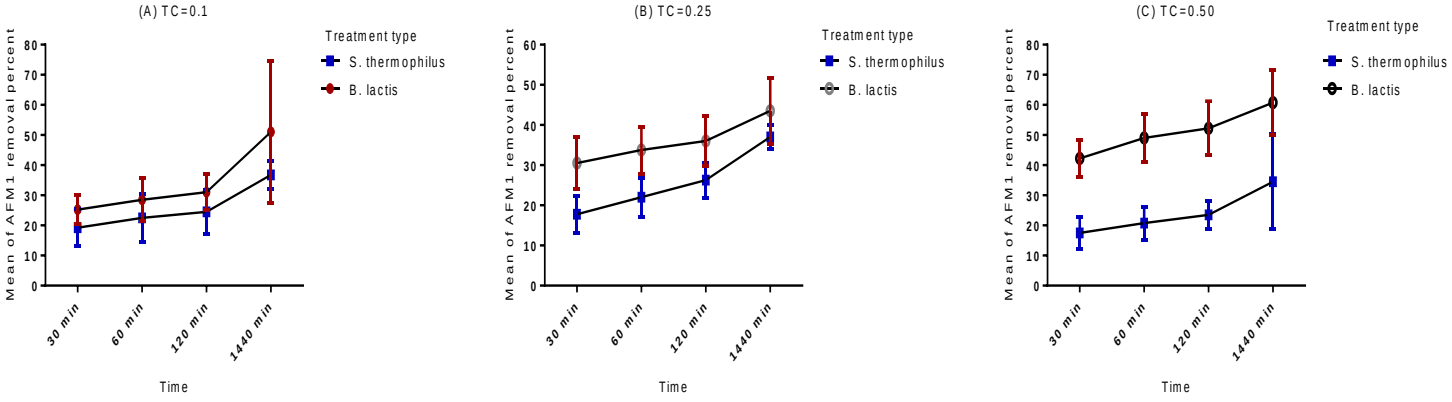

Figure 3. Change trends of AFM1 removal percent in both treatment types in terms of Toxin concentration (TC).

After adjusting for the effects of other variables in the model, we found that the mean AFM1 removal percentage in the *B. lactis* group was 14.90 units higher than in the *S. thermophiles* group at baseline or the first measurement (30 minutes). Additionally, the mean AFM1 removal percentage at 4ºC was 9.84 units lower than at 42 ºC at the first measurement (30 minutes). When comparing bacterial concentrations, the mean AFM1 removal percentage at 8 log CFU/mL was 3.04 units lower than at 10 log CFU/mL at the first measurement. Regarding toxin concentration, the mean AFM1 removal percentage at baseline (30 minutes) was 13.31 and 9.32 units lower in 0.10 µg/mL and 0.25 µg/mL, respectively compared to 0.5 µg/mL. However, other variables, including time and interaction of time with treatment type, temperature, toxin concentration, and bacterial concentration, had no significant effect on the rate of AFM1 removal (P>0.05) (Table 4).

4. Discussion

Milk and dairy products contaminated with AFM1 have become major food safety concerns. Thus, it is important to implement strategies for reduction and to monitor the presence of AFB1 in feedstuffs. The present study investigated the ability of *S. thermophilus* and *Bifidobacterium animalis* (subspecies lactis) as probiotic bacteria, to detoxify AFM1 in contaminated milk, considering factors such as bacterial population, incubation temperature, and toxin concentration. We found that AFM1 detoxification from milk was time-dependent, with significant AFM1 removal occurring at an earlier time of exposure. Other studies confirm that the removal of AFM1 is a rapid process that depends on the bacterial strain (19). We found that *B. lactis* and *S. thermophilus* had significant ability to remove AFM1 at 120 minutes and 60 minutes, respectively.

Bacterial concentration was one of the factors influencing AFM1 reduction in skim milk for both *B. lactis* and *S. thermophiles*. Similar results were reported by Sarlak et al. (18), who investigated the removal of AFM1 from fermented milk drinks (doogh) by probiotic strains. They showed the percentage of AFM1 removal was higher at 10 log CFU/mL of *Lactobacillus. acidophilus* compared to 7 log CFU/ mL (99 vs 95%) over 28 days. Additionally, 7 log CFU/mL of *L. acidophilus* exhibited more AFM1 binding capacity than 7 log CFU /mL of *B. lactis* (75%).

Table 5. Determining the effect of the treatment type, temperature, toxin concentration, and bacterial concentration on AFM1 removal percent using the GEE model.

Variables (Reference)	Coefficients	95% CI	P-value
Treatment type (*S. thermophiles*)	-	-	-
B. lactis	14.90	(10.78, 19.02)	<0.001
Temperature (42 ºC)	-	-	-
4 ºC	-9.84	(-15.13, -4.55)	<0.001
Toxin concentration (0.5 µg /mL)	-	-	-
0.10 µg /mL	-13.31	(-19.43, -7.19)	<0.001
0.25 µg /mL	-9.32	(-13.56, -5.07)	<0.001
Bacterial Concentration (10 log CFU /mL)	-	-	-
8 log CFU /mL	-3.04	(-8.00, 1.91)	0.22
Time	0.005	(-0.005, 0.01)	0.34
Time* [Treatment type= *S. thermophiles*]	-	-	-
Time* [Treatment type= *B. lactis*]	0.001	(-0.006, 0.007)	0.86
Time* [Temperature=42 ºC]	-	-	-
Time* [Temperature=4 ºC]	0.005	(-0.002, 0.01)	0.13
Time* [Bacterial Concentration=10 log CFU /mL]	-	-	-
Time* [Bacterial Concentration=8 log CFU /mL]	0.005	(-0.002, 0.01)	0.15
Time* [Bacterial Concentration=0.5 µg /mL]	-	-	-
Time* [Bacterial Concentration=0.10 µg /mL)	0.005	(-0.004, 0.01)	0.30
Time* [Bacterial Concentration=0.25 µg /mL)	-0.0003	(-0.006, 0.006)	0.92

We found that the high concentration of *B.lactis* (10 logs CFU/ mL) led to 67.65 % AFM1 removal after 24 hours in milk. We also found that the lower concentration level of *S. thermophiles* (8 logs CFU/ mL) could remove more AFMI, suggesting that the cell wall structure is more related to the type of microorganisms involved in removing toxins.

Two enzymatic and absorption mechanisms have been proposed to reduce aflatoxin by microorganism strains. Since it has been reported that viable and non-viable bacteria can bind AF, the surface of the cell wall is the dominant mechanism of toxin elimination. The removal of AFM1 in contaminated skim milk with 0.5 ng/mL of AFM1 inoculated with 10^{10} cells /mL of heat-killed strains, including *Bifidobacterium lactis* FLORA-FITBI07 and a pool of LAB, was approximately 12% at 60 minutes at 42ºC (10). The stability of bacterial-AFM1 binding was evaluated using repeated washing by Panwar et al. (20). They highlighted of the bacterial cell walls due to the release of AFM1 after washing and suggested mechanisms of action in aflatoxin detoxification likely involving noncovalent binding rather than metabolic inactivation.

Our result indicated that the highest percentage of toxin removal for both bacterial types related to an incubation temperature of 42ºC compared to 4ºC. It may be attributed to to the heat treatment affecting components of the cell wall, such as polysaccharides and peptidoglycans, which can disrupt the the cell membrane and enhance aflatoxin binding to cell wall components and plasmatic membrane.

We also found that the highest affinity for *B. lactis* binding to AFM1 was observed when the toxin concentration was high (0.5 µg /mL). Our findings agree with previous studies showing that toxin binding increases with higher toxin concentration (13, 21, 22). For example, Karazhiyan et al. showed a similar upward trend in toxin removal by yeasts with increasing toxin concentrations from 100 to 750 pg /mL (21).

The level of AFM1 binding by *S. thermophilus* in PBS and yogurt spiked with 50 µg /L and incubated at 42 ºC increased over time and was approximately 35% and 38% after 6 hours, respectively. The higher removal rate in yogurt may be associated with the better binding ability of AFM1 to casein molecules (23). Such data were in good correlation with our finding, which indicated that the highest removal AFM1 for *S. thermophiles* in milk was related to 0.1 and 0.25 µg /mL (24 and 22.8% respectively) after 60 minutes at 42°C and reaching 45% at 24 hours with 0.5 µg/mL.

The beneficial effect of lactic acid fermentation on the reduction of AFM1 level using starter cultures of *L. bulgaricus* and *S. thermophiles* in milk fermentation showed a significant reduction in AFM1 concentration from 0.075 and 0.207 to 0.068 and 0.198 ppb, respectively. Barukcic et al. (24) investigated the potential of the probiotics (*Lactobacillus acidophilus La-2*, *Bifidobacterium animalis subsp. lactis BB-12* and *Streptococcus thermophiles*) to reduce AFM1 in milk contaminated with 54 ng /L AFM1 over a period of 21 days. Their results demonstrated approximately a 50% reduction in AFM1 concentration. These findings align with our results, showing the ability of probiotics in detoxification of AFM1.The present study confirmed the detoxification capability of probiotic bacteria. They indicated that the amount of AFM1 removal by tested bacteria depends on the strain, bacterial population, incubation temperature, and toxin concentration, while storage time had a significant effect. Our findings showed that the significant removal of AFM1 in skim milk contaminated with 0.5 µg/mL and treated with 10 log CFU/mL *B. lactis* was 57.7% at 120 minutes at 42ºC. Similarly , the significant removal of AFM1 in skim milk spiked with 0.1 and 0.5 µg/mL of AFM1 and inoculated with 8 log CFU/mL *S. thermophiles* was 24% and 45% at 60 minutes and 24 hours both at 42 ºC. Additionally, the best strains showed the highest AFM1 removal (87%) at 0.5 µg/mL at 24 hours. These findings suggest potential future application of these future applications of these bacteria to control AFM1 in the dairy industry. However, more studies are needed to investigate the mechanisms involved in toxin removal by *B. lactis* and *S. thermophiles*, especially considering changes in physicochemical factors.

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Not applicable

Authors' Contribution

Study concept and design: M. A. SAA. A
Acquisition of data: AE. K.
Analysis and interpretation of data: M. T.
Drafting of the manuscript: N. SH.

Critical revision of the manuscript for important intellectual content: SAA. A, M. A.
Statistical analysis: M. T, N. SH.
Administrative, technical, and material support: SAA. A, AE. K.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare no relevant financial or non-financial interests to disclose

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Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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