Binding ability of aflatoxin M₁ to yoghurt bacteria

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Summary: Aflatoxin M₁ (AFM₁) is a highly toxic compound found in milk. Several microorganisms have been previously reported to bind or degrade AFM₁ from liquid media. This study was performed to assess the binding of AFM₁ in contaminated phosphate buffered saline (PBS). Lactobacillus delbrueckii subsp. bulgaricus CH-2 and Streptococcus thermophilus ST-36 were used for this purpose. Removal activities of two strains were also assessed using contaminated reconstituted milk and contaminated yoghurt made from reconstituted milk. ELISA procedure was used in this study Lactobacillus delbrueckii subsp. bulgaricus CH-2 bound in PBS at 18.70% and in milk at 27.56% while Streptococcus thermophilus ST-36 bound in PBS at 29.42% and in milk at 39.16%. AFM₁ was bound at the level of merely 14.82% in yoghurt. The results indicated that binding ability of Streptococcus thermophilus ST-36 was higher than that of Lactobacillus delbrueckii subsp. bulgaricus CH-2 in both PBS and reconstituted milk. Both of microorganisms bound higher in milk than in PBS. Also, AFM₁ binding levels were at least level in yoghurt (%14.82). These findings supported that specific yoghurt bacteria used in this study can offer decontaminating AFM₁ from milk.

Key words: Aflatoxin M₁, binding, yoghurt bacteria

Aflatoxins M₁'in yoğurt bakterilerine bağlanma yeteneği

Özet: Bu çalışmada, kontamine PBS'de AFM₁'in bağlama yeteneği araştırıldı. Bu amaçla, Lactobacillus delbrueckii subsp. bulgaricus CH-2 ve Streptococcus thermophilus ST-36 bakterileri kullanıldı. AFM₁'in bu iki bakteriye bağlama yeteneği, aynı zamanda kontamine süte ve kontamine sütten yapılan yoğunlukta da araştırıldı. Çalışmada, ELISA yöntemi kullanıldı. Lactobacillus delbrueckii subsp. bulgaricus CH-2'nin AFM₁'i PBS'de % 18.70, süte % 27.56; Streptococcus thermophilus ST-36'nın ise PBS'de % 29.42 ve süte % 39.16 düzeylerinde bağlama yeteneğinde olduğu belirlendi. Yoğunlukta ise bağlanma en düşük düzeyde sırtlandı()% 14.82. Sonuç olarak, gerek PBS'de gerekse süte Streptococcus thermophilus'un bağlama yeteneği, Lactobacillus delbrueckii subsp. bulgaricus CH-2'den daha yüksek bulundu. Aynı zamanda her iki bakterinin de PBS'e göre süte daha fazla bağlama yeteneği olduğu sırtandi. Elde edilen bulgulara göre, bu çalışmada kullanılan spesifik yoğun bakterilerinin AFM₁'i sütten uzaklaştırma yeteneğinde olduğu belirlendi.

Anahtar sözcükler: Aflatoxin M₁, bağlama, yoğun bakterileri

Introduction

Aflatoxins are the most potent toxic, mutagenic, teratogenic and carcinogenic metabolites produced by the species of Aspergillus flavus, Aspergillus flavus subsp. parasiticus and Aspergillus nomius on food and feed materials. There are four main toxins which have been divided into B and G groups (B₁, B₂, G₁ and G₂). Of these, aflatoxin B₁ is most toxic and most carcinogenic. Aflatoxin M₁, a hydroxylated metabolite of aflatoxin B₁ is an important toxin present in the milk of lactating animals fed with aflatoxin B₁ contaminated feeds. Presence of aflatoxin M₁ in milk is a public health hazard. There is a general consensus that approximately 1-3% of the aflatoxin B₁ initially present in the animal feedstuff appears as aflatoxin M₁ in milk (5,8,18,23). Evidence of potential hazardous human exposure to AFM₁ from dairy products arises from many studies on the occurrence of AFM₁ in dairy products (15,27). Since milk has the greatest demonstrated potential for introducing aflatoxins residues from foods of animal origin into the human diet and is also the main nutrient for infants and children, the occurrence of aflatoxin M₁ in milk and dairy products is a concern (9,10,12,21,22). The best way to control the presence of AFB₁ in foods and feeds is to prevent their formation. Various physical, chemical and biological agents have been used to detoxify aflatoxins from food and feed materials (2,11,20). But there are currently no acceptable chemical, physical or biological methods to counteract the AFM₁ problem in milk (24). Thus, a practical and effective method is needed to be developed for the detoxification of AFM₁ contaminated milk.

Some strains of lactic acid bacteria have been reported to be effective in removing AFB₁ and AFM₁ from contaminated liquid media and milk(1,14,16,17). For this purpose, this study was carried out in order to investigate the ability of Lactobacillus delbrueckii subsp.
bacterial pellets were collected as described earlier, but bacterial pellets were suspended in contaminated nonfat reconstituted milks. Stock solution (1 µg AFM₁/1 ml benzene/acetonitrile) was evaporated to dryness under a smooth N₂ stream. The AFM₁ residue was redissolved in 1 ml methanol. A volume of 0.01 ml was transferred from the contaminated methanol to 1.5 ml of reconstituted skim milk, resulting in milk containing 10 ng/ml AFM₁. Bacterial pellets was suspended in reconstituted milk contaminated with AFM₁ and incubated at 37°C for 4 h. After incubation period, suspensions were centrifuged. Unbound AFM₁ content in the supernatant was determined by ELISA (14). Each sample for the ELISA analysis was diluted 1:125 in PBS.

Cell-free reconstituted milk contaminated with AFM₁ was used for positive control. Bacteria suspended in reconstituted milk were used for negative control. All assays were performed at control groups, too. Procedure of contamination with AFM₁ in reconstituted milk was that of Pierides et al (17).

Contamination of reconstituted skim milk and yoghurt production

Yoghurt was made from the reconstituted skim milk presented 12% nonfat dry matter. Prepared skim milk was heated at 90°C for 5 min and then cooled to 42°C. Stock solution (5 µg/ml AFM₁ in benzene/acetonitrile) was collected as described earlier. But AFM₁ residue was redissolved in 2 ml methanol. A volume of 0.08 ml was transferred from the contaminated methanol to 20 ml of skim milk, resulting in milk containing 10 ng/ml AFM₁. After that, 20 ml milk was inoculated with 2% starter cultures. The ratio of Lactobacillus delbrueckii subsp. bulgaricus CH-2 : Streptococcus thermophilus ST-36 was 1:1.

Cell-free reconstituted milk contaminated with AFM₁ was used for positive control, yoghurt made from reconstituted milk and uncontaminated as negative control. All groups were incubated at 42°C for 4 h. Yoghurt was centrifuged at the end of incubation and unbound AFM₁ content in the supernatant was determined by ELISA. Each sample for the ELISA analysis was diluted 1:125 in PBS. ELISA procedure was performed according to R-biopharm GmbH recommendations. Binding of AFM₁ by Lactobacillus delbrueckii subsp. bulgaricus CH-2 and Streptococcus thermophilus ST-36 cells was analysed according to Pierides et al (17). Cell-free PBS contaminated with AFM₁ was used for positive control. Bacteria suspended in PBS were used for negative control. All assays were performed at control groups, too.

Statistical analysis

The variance analysis (with two factors) was done for determining the difference as binding amount of aflatoxin M₁ in two medium of two bacteria. In addition, one-way ANOVA variance analysis was also done for comparison of binding in yoghurt. DUNCAN test was used for determining the different groups after the one-way variance analysis.
Results

In this study, in vitro binding ability of AFM$_1$ to *Lactobacillus delbrueckii* subsp. *bulgaricus* CH-2 and *Streptococcus thermophilus* ST-36 was investigated in the liquid medium (PBS), reconstituted milk and yoghurt comparatively.

Comparing two strains for statistical analysis, *Streptococcus thermophilus* ST-36 showed significantly high (p< 0.01) percentage of AFM$_1$ binding ability according to *Lactobacillus delbrueckii* subsp. *bulgaricus* CH-2 in PBS and milk (Table 1). On the other hand the percentage of removal activity of both *Lactobacillus delbrueckii* subsp. *bulgaricus* CH-2 and *Streptococcus thermophilus* ST-36 in PBS showed significant differences (p< 0.01) according to milk. At the same time the differences between milk and yoghurt were found statistically important (P< 0.01) (Table 1).

Table 1. Comparison of strains-media and yoghurt

<table>
<thead>
<tr>
<th>Strain-Media</th>
<th>X± SX</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus delbrueckii</em> subsp. <em>bulgaricus</em> CH-2</td>
<td>£18.7± 0.582</td>
</tr>
<tr>
<td><em>Lactobacillus delbrueckii</em> subsp. <em>bulgaricus</em> CH-2 PBS</td>
<td>£27.56±0.699</td>
</tr>
<tr>
<td><em>Streptococcus thermophilus</em> ST-36</td>
<td>£29.42±0.601</td>
</tr>
<tr>
<td><em>Streptococcus thermophilus</em> ST-36 PBS</td>
<td>£39.16±0.459</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>£14.82±0.558</td>
</tr>
</tbody>
</table>

Discussion

It was determined that *Streptococcus thermophilus* ST-36 has a more binding ability in comparison *Lactobacillus delbrueckii* subsp. *bulgaricus* CH-2 both in PBS and reconstituted milk (Table 1).

Lactic acid bacteria are known to bind aflatoxins. Recently, heat-killed bifidobacteria have been reported to bind aflatoxin B$_1$ in PBS (14). *Lactobacillus rhamnosus* GG, *Bifidobacterium bifidum* BGN4, *Bifidobacterium sp.* JO3, *Bifidobacterium longum* JR 20 and *Bifidobacterium* sp. CH4 bound to AFB$_1$ by 37±1%, 46±4%, 41±3%, 37±3% and 37±1%, respectively. Pierides et al (17) reported that viable *L. rhamnosus* GG bound to AFM$_1$ by 77±0.4%, *L. rhamnosus* LC-705 bound by 75.2±1.2% and *L. gasseri* (ATCC 33323) bound by 51.4±1.9% after 4 h incubation in PBS. However the binding ability of heat-killed same bacteria was determined as 57.8±3.3%, 51.4±3.0% and 61.5±0.7%, respectively after the 15-16 h incubation in PBS. In the same study, binding of AFM$_1$ to both viable and heat-killed *L. rhamnosus* GG was reported as 18±1.9% and 26.6±3.2%; *L. rhamnosus* LC-705 was reported as 69.6±0.9% and 27.4±4.8% in skim milk. Peltonen et al (16) investigated the AFB$_1$ binding ability of 12 *Lactobacillus*, 5 *Bifidobacterium* and 3 *Lactococcus* in PBS. In their study, *Lactobacillus* strains bound by 17.3-59.7% AFB$_1$, *Bifidobacterium* strains by 18.0-48.7% and *Lactococcus* strains by 5.6-41.1% AFB$_1$.

These studies suggested that significantly different binding abilities of lactic acid bacteria were due to different cell-wall structure. Thus, in this study binding ability of yogurt cultures examined were found different. Also, Pierides et al (17) reported that *L. rhamnosus* 1/3 presented a less binding ability than *L. rhamnosus* GG in spite of the same genetic structure, and they presumed that this was caused by different biological activities of the strain.

When the binding ability of yoghurt cultures in PBS and reconstituted milk were compared, the binding was much greater in milk (Table 1). The principal reason of that may be due to the binding properties of aflatoxin to casein. So, Brackett and Marth (3) reported that an average of 30.7% more AFM$_1$ was found in milk treated with proteolytic enzyme than in untreated milk and they suggested that AFM$_1$ is bound by milk protein. Also, the same authors (4) reported that AFM$_1$ did not display a homogeneous distribution in milk and a part of AFM$_1$ could not be extracted from milk. Tabata et al (25) reported that milk concentrations had an effect on AFM$_1$.

It was seen that the binding after yoghurt manufacturing was less than that in milk, examined separately (Table 1). This may be caused by fermentation, which is greater in yogurt than in milk and by the fact that AFM$_1$, which is bound to casein is extracted better than milk(19). Van Egmond et al (26) found AFM$_1$ was recovered in slightly greater amounts from yoghurt than from the original milk. They believe the increased AFM$_1$ content in yoghurt possibly results from a more complete recovery of AFM$_1$ from yoghurt than milk. Munksgaard et al (13) found the level of AFM$_1$ during production of yoghurt to be increased on average by 9%. They explained that AFM$_1$ is extracted better from cultured products. At the same time, the binding abilities may be decreased because of synergetic reproduction in yoghurt, although it is reported by El-Nezami (6) that the binding abilities increased in acid treatment in PBS experimentally. In fact the binding determined in yoghurt, was found to be even less than the bindings determined separately in PBS.

In this study it was determined that both *Lactobacillus delbrueckii* subsp. *bulgaricus* CH-2 and *Streptococcus thermophilus* ST-36 have binding abilities during yoghurt manufacturing. Thus, it could be suggested that yoghurt cultures could be used in the removal of AFM$_1$.
References


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