Antimycotic and Antiaflatoxicogenic Effect of Lactic Acid Bacteria: A Review

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ABSTRACT

Lactic acid bacteria are extensively used in the fermentation of a wide variety of food products and are known for their preservative and therapeutic effects. Many lactic acid bacteria species have been reported to inactivate bacterial pathogens, and numerous antibacterial substances have been isolated. However, the antimycotic and antiamyocytotoxic potential of lactic acid bacteria has still not been fully investigated. Fermented foods such as cheese can be contaminated by molds and mycotoxins. Mold causes spoilage and renders the product unsuitable for consumption, and the presence of mycotoxins presents a potential health hazard. A limited number of reports have shown that lactic acid bacteria affect mold growth and aflatoxin production. Although numerous lactic acid bacteria such as Lactobacillus spp. were found to inhibit aflatoxin biosynthesis, other lactic bacteria such as Lactococcus lactis were found to stimulate aflatoxin production. The morphology of lactic acid bacteria cells has also been found to be affected by the presence of fungal mycelia and aflatoxin. Lactococcus lactis cells became larger and formed long chains in the presence of Aspergillus flavus and aflatoxins. Numerous investigations reported that low pH, depletion of nutrients, and microbial competition do not explain the reason for aflatoxin inhibition. Some investigators suggested that the inhibition of aflatoxin is due to lactic acid and/or lactic acid bacteria metabolites. These metabolites have been reported to be heat-stable low-molecular-weight compounds.

Key words: Lactic acid bacteria, molds, antimycotic substances, aflatoxins, Lactobacillus, Lactococcus

Aflatoxins are secondary metabolites produced by Aspergillus flavus, Aspergillus flavus subsp. parasiticus, and Aspergillus nomius in various foods and agricultural commodities. Aflatoxins have been demonstrated to be carcinogenic, teratogenic, and mutagenic (4, 21, 31, 38). Aflatoxin B₁ is the most potent hepatocarcinogen in many animal species (20). Aflatoxins have been reported to be produced in cereal grains, peanuts, tree nuts, figs, seeds, and fermented products including cheese (10, 14, 58) and fermented meats such as salami, sausage, and country cured hams (1, 17, 18, 60, 66, 73, 74).

Lactic acid bacteria (i.e., Lactobacillus, Lactococcus, and Pediococcus species) are used extensively for the manufacture and preservation of many fermented products. Many investigations have reported the control of microbial growth by lactic acid bacteria through production of acid and/or antimicrobial substances. These fermented products have been reported to have therapeutic and preservative properties. The preservative effect is generally attributed to acid production and low pH. The presence of lactic acid bacteria in fermented foods mainly affects the growth of spoilage and pathogenic bacteria. However, molds are also capable of growing in these fermented products. Mold growth can cause spoilage or may render the fermented foods hazardous to human health through the production of mycotoxins. Since lactic acid bacteria are present in fermented products, it is important to understand their interaction with mycotoxigenic fungi. Some lactic acid bacteria have been shown to affect mold growth and mycotoxin production. The inhibition of spoilage and mycotoxigenic fungi by fermenting lactic acid bacteria could improve the shelf life of fermented products and reduce the health hazard of mycotoxins.

EFFECT OF LACTIC ACID BACTERIA ON MOLD GROWTH AND AFLATOXIN PRODUCTION

Antifungal properties of lactic acid bacteria

Antifungal properties of lactic acid bacteria have been reported by many investigators. Wiseman and Marth (78) studied growth and aflatoxin production by A. parasiticus in the presence of Lactococcus lactis subsp. lactis (formerly called Streptococcus lactis). Each organism was found to influence the growth of the other, although inhibition of L. lactis could have been due to the accumulation of lactic acid (68). Due to a rise in pH caused by fungal metabolism, L. lactis remained viable after being added to a mold culture. The inhibition of L. lactis started with the production of aflatoxins and other fungal secondary metabolites. The growth of A. parasiticus was also affected by L. lactis. Mold growth was inhibited when mold spores were added to a 3-day L. lactis culture and when both organisms were added simultaneously (78). Fungal inhibition was probably due to the production of...
lactic acid. However, when *L. lactis* was added to a 3-day mold culture, there was stimulation of mold growth. This effect could be due to the removal of acids and/or to the production of stimulatory metabolites by the bacteria (5, 52, 53, 54).

El Gendy and Marth (30) investigated the interaction between *A. parasiticus* and *Lactobacillus casei*. They used two treatments: the first treatment consisted of simultaneous addition of bacteria and fungus to the medium, while in the second treatment the fungus was added to a 3-day culture of *L. casei*. Mold growth was reduced in the second treatment. The authors suggested that such reduction was due to some nutritional change in the medium after the growth of *L. casei*. In the first treatment it was observed that there was stimulation of fungal growth by the bacteria. *A. flavus* also affected the metabolism and shape of lactic acid bacteria cells, which became elongated (75, 76).

Batish et al. (6) screened 19 lactic bacteria strains for their antifungal activity against *A. parasiticus*, *A. fumigatus*, *Rhizopus stolonifer*, and *Rhizopus* sp. *Lactococcus lactis* subsp. *diacetylactis* DRC1 and *Streptococcus thermophilus* 489 were found to be the most inhibitory to all fungal cultures and *A. fumigatus* was found to be the most sensitive. In another study, Batish et al. (8) found that incubation of *S. lactis* subsp. lactis DRC1 (formerly called *Streptococcus lactis* subsp. *diacetylactis*) at 30°C for 48 to 72 h led to the maximum production of the inhibitory substance against *A. fumigatus*. These results were in agreement with the findings of other investigators (11). The inhibitory compound was found to be polypeptide in nature, since it was inactivated by pronase E and trypsin. Batish et al. (8) studied the interaction of *S. lactis* subsp. *lactis* DRC-1 with *A. parasiticus* and *A. fumigatus* in milk. The authors found that *S. lactis* subsp. *lactis* inhibited the growth of *A. fumigatus* when they were grown together in milk. Amemiya et al. (3) reported that *A. fumigatus* growth was affected by *Lactobacillus acidophilus*. Collins and Hardt (25) found that *L. acidophilus* inhibited *Candida albicans* and other spoilage and pathogenic yeasts. However, other investigators (46) could not demonstrate the inhibition of yeast by lactic acid bacteria.

Another study by Batish et al. (7) showed that the optimal incubation temperature for *L. acidophilus* to produce an antifungal substance was 30°C for 48 h. Extended incubation decreased the antifungal activity, which could be caused by enzymatic degradation of the antifungal compound. The optimal pH for the production of the antifungal substance was between 6.8 and 7.6, which agreed with the findings of other investigators (11, 63, 64, 65, 67). Batish et al. (7) reported that the optimal pH for *L. lactis* subsp. *lactis* DRC-1 to produce antifungal substances was 6.8. Incorporation of 1% yeast extract into the basal medium was found to stimulate the production of the antifungal compounds by *L. acidophilus*. Higher concentrations of yeast extract resulted in a decrease of the antifungal substance. Similar results were found with beef extract. Supplementation of the basal medium (Elliker's medium) with 2% tryptone and 1 to 2.5% Casamino Acids increased the production of the antifungal substance. Addition of glucose up to 1% was stimulatory, but higher levels were inhibitory. The use of 3.5% salt gave the maximum level of the antifungal substance (7).

Karunaratne et al. (36) studied the inhibition of mold growth and aflatoxin production by *Lactobacillus* spp. The authors used three lactobacilli species (*L. acidophilus*, *L. bulgaricus*, and *L. plantarum*) and a commercial silage inoculant mixture of the same three *Lactobacillus* species. They worked with three substrates, a liquid semisynthetic broth, corn, and rice. *Lactobacillus* cells inhibited mold growth, which was suggested to be due to competitive growth and low pH. However, on solid substrates mold growth was not affected.

Gourama (32) found that the same commercial mixture of lactobacilli totally inhibited the germination of mold spores, while in cell-free supernatants mold spore growth was only slightly affected. *Lactobacillus* spp. cell-free supernatant also prevented germinated spores from growing further. Mold growth was not affected by the presence of *Lactobacillus* species.

**Antiaflatoxigenic properties of lactic acid bacteria**

When Wiseman and Marth (78) added mold spores to a 13-day culture of *S. lactis*, total aflatoxin decreased. However, when both microorganisms were added simultaneously, the amount of aflatoxins increased during the incubation period. The interaction between *L. lactis* and *A. parasiticus* was described as an amensalism coaction, where the strong benefits and the weak is unaffected (78). Mohran et al. (51) found that the proteolytic activity of some lactic bacteria strains was affected in the presence of aflatoxin B1. A good correlation was found between the inhibition of the bacterial proteinases and aflatoxin concentration. However, the activities of proteinase produced by *Streptococcus thermophilus* and *L. lactis* subsp. *diacetylactis* increased in the presence of the toxin. These findings are of practical importance to the dairy industry, since they may explain some of the defects in proteolysis by lactic acid bacteria starters in cheese manufacturing.

Coallier-Ascah and Idziak (24) studied the effect of the interaction between *Lactococcus lactis* and *A. flavus* on the production of aflatoxin. They found that when the two organisms were in mixed culture in lablemco tryptone broth, (LTB), few or no aflatoxins were produced in the medium. Supplementation of glucose consumed by lactic acid bacteria at the time of mold sporulation did not restore aflatoxin production. In addition a decrease in pH was not responsible for aflatoxin inhibition. The authors suggested that an inhibitory compound(s) produced by *L. lactis* during early stages of the exponential growth phase was responsible. Partial purification of the inhibitory substance suggested that it was a heat-stable, low-molecular-weight compound. *L. lactis* was also found to degrade the existing aflatoxin. A similar finding was reported by Wiseman and Marth (78), when they found that *S. lactis* decreased aflatoxin levels in *A. parasiticus* cultures. Maing et al. (45) reported that aflatoxin B1 was degraded in the presence of *A. oryzae* and *Lactobacillus delbrueckii* during soy sauce fermentation.

Ciegler et al. (23) found that *Flavobacterium aurantiacum* removed aflatoxins from a nutrient solution. Various investigators have proposed adsorption of aflatoxins into the bacterial cell wall as a mechanism of this degradation (22, 28, 43, 47). Coallier-Ascah and Idziak (24) tested the mutagenicity of the mixed culture using the Ames test and
found that *L. lactis* totally removed the mutagenic activity caused by *A. flavus* mutagenic compounds. Luchesse and Harrigan (44) studied growth and aflatoxin production by *A. parasiticus* in the presence of *Lactococcus lactis*. When *A. parasiticus* was grown in mixed culture with *L. lactis* higher total aflatoxins (B1 + G1) were produced. Megalla and Mohran (50) reported the transformation of aflatoxin B1 to aflatoxin B2 and aflatoxicol (R0) in milk fermented with *Streptococcus lactis*.

Aflatoxin B2 is nontoxic while aflatoxicol is less toxic than aflatoxin B1. Megalla and Hafez (49) found that aflatoxin B2 was transformed to aflatoxin B2a in acidogenic yoghurt. Basie et al. (61) reported that the fermentation and acidified milk containing aflatoxin B1 greatly reduced the amount of the toxin.

Karunaratne et al. (36) found that *Lactobacillus* cell-free supernatant inhibited the production of aflatoxins. It was shown that the aflatoxin inhibition was probably due to an inhibitory metabolite other than hydrogen peroxide and low pH. However, on rice there was stimulation of aflatoxin B1 production in the presence of the silage inoculant. Gourama (32) found that *Lactobacillus* cell-free supernatants inhibited aflatoxin production without greatly affecting mold growth. The inhibition was probably due to a bacterial metabolite which may interfere with the biosynthesis of aflatoxin. Preliminary study with a tip culture technique showed that the inhibition of aflatoxins in the biosynthetic pathway may occur between versicolorin A and sterigmatocystin intermediates (32).

**Effect of lactic acid bacteria antimicrobial metabolites**

Many investigators have suggested that production of certain metabolites by lactic acid bacteria affects mold growth and aflatoxin production. The release of the intracellular pool of the lactic acid bacteria during bacterial cell lysis may influence mold growth and mycotoxin production. Lactic acid bacteria could also produce certain metabolites during the growth phase which could interfere with aflatoxin biosynthesis. This was shown by Coallier-Ascah and Idziak (24) and Gourama (32) who grew lactic acid bacteria inside dialysis sacks that were immersed in a mold broth culture. A significant reduction in aflatoxin biosynthesis was obtained. The aflatoxin reduction was due to low-molecular-weight inhibitory compounds that diffused through the membrane. Coallier-Ascah and Idziak (24) found that continuing the incubation of the lactic acid bacteria culture to 16 h decreased the inhibitory effect of the compound(s). Partial purification and characterization of the inhibitor showed that it was a heat-stable compound (24).

Gourama (32) tested the effect of *Lactobacillus* cell-free supernatant on mold growth and aflatoxin production. The cell-free supernatant was placed inside a dialysis sack, and the whole sack was immersed in LTB inoculated with mold spores. With all the molecular weight cutoffs used, there was a reduction in aflatoxin biosynthesis without any effect on mold growth. Microgard, which is a fermented milk product containing antimicrobial metabolites, was found to be inhibitory to *Penicillium expansum* (2).

Many investigators have isolated and identified different lactic acid bacteria antibacterial substances (48, 57, 60, 64, 71). However, other than nisin, the identity of the antifungal substances produced by lactic acid bacteria have not been fully investigated.

**Effect of lactic acid**

Among the many factors that influence the growth and metabolism of microorganisms is the level of acid. During the fermentation of many foods, lactic acid is the primary acid produced. Many investigations indicated the possibility of mold growth and aflatoxin production in fermented, lactic acid-containing products such as cheese, sausages, bacon, and salami (17, 18, 20, 40, 73, 74). Ciegler et al. (23) failed to detoxify shelled corn containing aflatoxin using *Lactobacillus plantarum* and *Streptococcus faecalis*, because the lactic acid bacteria did not produce enough acid. When lactic acid was added to the grain at a level of 10% (wt/vol), a considerable reduction of aflatoxin was obtained. Coallier-Ascah and Idziak (24) reported that lactic acid was not inhibitory to aflatoxin biosynthesis. When *A. flavus* was grown in LTB that was acidified using lactic acid to pH 4.3 (amount of lactic acid equivalent to the amount of acid in a 16-h *L. lactis* culture with pH 4.3), it produced the same amount of aflatoxin as in the control, which used LTB with an initial pH of 6.8.

El Gazzar et al. (29) studied the effect of lactic acid on growth and aflatoxin production by *A. parasiticus* NRRL 2999. The levels of lactic acid used varied between 0 and 2% with an initial pH of 3.5 or 4.5. Aflatoxin production was influenced by the concentrations of lactic acid, initial pH, and extent of mycelial growth. Lactic acid at pH 4.5 had no effect on mold growth. There was a slight stimulation of mold growth at 0.75, 1, and 1.5% of lactic acid after 3 days of incubation. Pohlmeir and Bullerman (59) reported that lactic acid has an inhibitory effect on aflatoxin biosynthesis. When *A. parasiticus* was grown in LTB that was acidified using lactic acid to pH 4.3, aflatoxin production was suppressed. Many investigations indicated the possibility of mold growth and aflatoxin production in fermented, lactic acid-containing products such as cheese, sausages, bacon, and salami (17, 18, 20, 40, 73, 74). Ciegler et al. (23) failed to detoxify shelled corn containing aflatoxin using *Lactobacillus plantarum* and *Streptococcus faecalis*, because the lactic acid bacteria did not produce enough acid. When lactic acid was added to the grain at a level of 10% (wt/vol), a considerable reduction of aflatoxin was obtained. Coallier-Ascah and Idziak (24) reported that lactic acid was not inhibitory to aflatoxin biosynthesis. When *A. flavus* was grown in LTB that was acidified using lactic acid to pH 4.3 (amount of lactic acid equivalent to the amount of acid in a 16-h *L. lactis* culture with pH 4.3), it produced the same amount of aflatoxin as in the control, which used LTB with an initial pH of 6.8.

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found no difference in the production of aflatoxins B₁ and G₁ at pH 4.5 and 5.5. Coallier-Ascah and Idziak (24) found that after 16 h of growth of L. lactis in LTB the pH decreased from 6.8 to 4.3; this pH decrease was not responsible for aflatoxin inhibition. When the initial pH of the medium was adjusted to 4.3 using 5 N HCl or 85% lactic acid, aflatoxin production was not affected.

Wiseman and Marth (78) reported that when A. parasiticus was grown first, the pH dropped from 6 to 4.5 and was raised to 7 after 10 days of incubation. The pH drop was suggested to be due to the fermentation of sugars by the fungi. However, when L. lactis was grown first it produced lactic acid early during growth. After A. parasiticus was added it raised the pH by metabolizing the acid. El Gazzar et al. (29) showed that when A. parasiticus NRRL 2999 was grown in a medium with an initial pH of 3.5, there was more production of aflatoxin B₁ in the presence of lactic acid. Karunaratne and Bullerman (36) reported that the prevention of mold growth by Lactobacillus spp. was mainly due to a pH effect and microbial competition; however, the cause of aflatoxin inhibition could not be linked to the pH or microbial competition. Luchesse and Harrigan (44) reported that the initial pH affected aflatoxin production and was linked to the culture medium used. Adjusting the initial pH of LTB to 4.2 with HCl or lactic acid rather than using an initial pH close to neutrality increased aflatoxin production. The same results were found by Gourama (32).

Lie and Marth (41) reported that when casein was used as the substrate the maximum aflatoxin content occurred at both acidic and alkaline pHs. Kiermeier and Behringer (39) found that the maximum aflatoxin production occurred using pasty milk powders with an initial pH of 4.6. When the pH was raised to neutrality, lower aflatoxin levels were obtained. Low pH was reported to favor aflatoxin production in Czapek liquid medium (34), in a synthetic low-salt medium (65), and in a nitrate medium (26). However, Davis et al. (27) found that with yeast extract-sucrose broth the initial pH had no effect. Horn and Wicklow (33) reported that acidic conditions caused reduction of aflatoxin production in a corn meal. The effect of pH on mold growth seems to be a matter of controversy between the different investigations. Such an effect depends on many factors such as substrate, temperature, and mold strain. However, it is commonly reported that low pH favors the production of aflatoxin B₁, while mold growth is hindered.

MOLD GROWTH AND AFLATOXIN PRODUCTION IN PRODUCTS FERMENTED BY LACTIC ACID BACTERIA

Mold growth

Mold growth on fermented products such as cheese and meats is a common problem. Penicillium species are the most common mold species to grow on cheese. Mycotoxigenic fungal contaminants of dairy products and stability and penetration of mycotoxins in cheese have been recently reviewed by Scott (70). Bullerman (14) investigated the incidence of mycotoxigenic molds in domestic and imported cheese and found that the majority of the potential mycotoxigenic molds were Penicillium spp., such as P. cyclopium and P. viridicatum, and a number of isolates belonged to the A. flavus and A. ochraceus groups. Various fungal genera that were isolated from cheeses around the world were found to be toxic to biological systems (70). These fungal genera include various Penicillium spp., A. versicolor, A. flavus, A. glaucus, Cladosporium spp., Alternaria spp., Geotrichum candidum, Mucor spp., Rhizopus spp. and Fusarium spp. Toxigenic strains of A. flavus have also been isolated from yogurt (35) and country cured ham (73, 74). Sutic et al. (74) found that the majority of the mycoflora of country cured ham was made up of Penicillium spp., Aspergillus spp., Cladosporium spp. and Alternaria spp.

Production of mycotoxins

Contamination of fermented products by toxigenic molds can lead to the production of various mycotoxins. However, mold growth does not necessarily mean mycotoxin production. We should be aware that most of the experimental work on mycotoxin production has been done under aerobic conditions. As mentioned earlier, Penicillium spp. are the predominant contaminants of cheese and other dairy products. Different mycotoxins can be produced by Penicillium species, including penicillic acid, patulin, ochratoxin A, and citrinin (16, 70, 77).

Most of the work in the literature on the production of mycotoxins in dairy and meat products has focused on the production of aflatoxins. Many reports have been published on the production of sterigmatocystin and aflatoxins B₁, B₂, G₁, and G₂ in different kinds of cheeses. Lie and Marth (40) inoculated the surface of 3-month-old cheddar cheese with toxigenic strains of A. flavus and measured the amounts of aflatoxins B₁ and G₂ after 10 and 52 days of incubation. The top 0.64-cm layer of cheese contained 2,900 µg of aflatoxin B₁ per kg of cheese and 14,400 µg of aflatoxin G₂ per kg of cheese. There was no detection of aflatoxin beyond 1.3 cm of the cheese surface. Oldham et al. (56) reported that there was no aflatoxin production in cheddar cheese and luncheon meat incubated at normal refrigeration temperatures.

Shih and Marth (72) tested the growth and the production of aflatoxin on brick cheese by A. flavus and A. parasiticus at 7°C. No aflatoxins were detected. However, at 12.8°C A. parasiticus produced aflatoxins B₁ and G₂. At 23.9°C both mold species produced aflatoxins. Bullerman and Olivigni (19) screened different cheddar cheese mold isolates for their toxicity to chicken embryos and to Bacillus megaterium, and found that 29.2% of the isolates were toxic to chicken embryos and 20.1% of the isolates were toxic to B. megaterium. Bullerman (13) reported that 35% of the Penicillium spp. isolates from Swiss cheese were found to be toxic to chicken embryos. Lieu and Bullerman (42) found that after storing Swiss cheese for 16 h at 5°C, 8% of the original amount of penicillic acid remained. Penicillium spp. isolated from domestic and imported cheeses by Bullerman (15) produced patulin, penicillic acid, ochratoxin A, and citrinin, and A. flavus isolates produced aflatoxins in laboratory media. Zerfiridis (79) found that keeping Teleme cheese immersed in brine at 5°C hindered mold growth and aflatoxin production. Blanco et al. (10) studied the production of aflatoxin in a Manchego type of cheese, a typical Spanish cheese. Aflatoxin was not detected in cheese that was covered with paraffin, while in non-paraffin-covered cheese aflatoxin was found in the top layer.
Production of aflatoxins has also been reported to occur in other dairy products besides cheese. These products include sterilized homogenized milk, cream, milk powder, unsweetened condensed milk, butter, and yogurt (70). Blanco et al. (9) inoculated commercial yogurt with A. parasiticus NRRL 2999 and followed the production of aflatoxin in different incubation conditions. Aflatoxin content was higher at 28°C and no fungal growth was observed at 10°C. Mohran et al. (51) found that the proteolytic activity of different lactic acid bacteria was affected in the presence of aflatoxin B1. Bullerman et al. (18) found that Italian-type salamis that were inoculated with A. flavus developed aflatoxins during aging. Low temperatures (10°C), low humidities (65 to 70%), and high salt concentrations prevented mold growth and aflatoxin production on country cured ham.

CONCLUSIONS

The antibacterial effects of lactic acid bacteria and their metabolites are well documented and have been extensively investigated, but more research on their antifungal effects is needed. From the available literature on the effect of lactic acid bacteria on mold growth and mycotoxin production, it would appear that lactic acid bacteria have the potential to be used as biological control agents in foods to prevent mold growth. The antimicrobial biopreservatives, reviewed by Ray and Daeschel (62), have the potential to constitute suitable food preservatives that are safe, effective, and acceptable to consumers, regulatory agencies, and the food industries. Many reports showed that the inhibition of mycotoxins by lactic acid bacteria was due to factors other than acidity and hydrogen peroxide, and there is a strong indication that some inhibitory compounds are protein in nature. More research is needed to purify and identify these compounds. One of the difficulties in this regard is the lack of suitable assay procedures.

Another aspect that needs further investigation is to determine why some lactic acid bacteria inhibit aflatoxin biosynthesis, while others have a stimulatory effect. Finding the answers to these questions will enlarge the scope of the preservative capacity of lactic acid bacteria and their use as biological control agents in food systems. Molds can grow in fermented products and cause spoilage and they can be extremely hazardous to human health through the production of mycotoxins. The antifungal and antimycotoxigenic potential of lactic acid bacteria cultures might have commercial applications and could be of great significance to both industry and consumers if properly investigated and developed.

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