APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1985, p. 163–167
0099-2240/85/010163-05$02.00/0

Vol. 49, No. 1

Interaction between *Streptococcus lactis* and *Aspergillus flavus* on Production of Aflatoxin

J. COALLIER-ASCAH AND E. S. IDZIAK*

Department of Microbiology, Faculty of Agriculture, Macdonald Campus of McGill University, Ste. Anne de Bellevue, Quebec, Canada H9X 1C0

Received 2 July 1984/Accepted 23 October 1984

The inoculation of *Aspergillus flavus* spores into a culture of *Streptococcus lactis* in Lablemco tryptone broth medium resulted in little or no aflatoxin accumulation even though the growth of the fungus was not hindered. The drop in pH and reduced nutrient levels in the medium as a result of the *S. lactis* growth were not the cause of the observed inhibition. The inhibition was not eliminated by the addition of carbohydrate equal to the amount used by the bacterium before the inoculation with the fungus. Aflatoxin levels were also markedly reduced when *S. lactis* was inoculated into a growing *A. flavus* culture. In addition to inhibiting the synthesis of aflatoxin, *S. lactis* also degraded preformed toxin. *A. flavus*, on the other hand, not only reduced the growth of *S. lactis* but also affected the morphology of the bacterial cell; the cells became elongated and formed long chains. *S. lactis* produced and excreted the inhibitor into the medium late in its growth phase. The inhibitor was a heat-stable low-molecular-weight compound. Chloroform extracts of *A. flavus* grown in the presence of *S. lactis* were toxic to *Bacillus megaterium* but did not exhibit mutagenic or carcinogenic activity in the *Salmonella*/mammalian microsome mutagenicity test.

Aflatoxin B_1 is considered to be the most potent hepatocarcinogen causing hepatocellular carcinoma and cholangiocarcinoma in the liver of many animal species, including humans (7). *Penicillium* spp. and *Aspergillus* spp. are common contaminants of cheese during ripening and refrigerated storage. Bullerman and Olivigni (6) showed that *Aspergillus* strains isolated from contaminated cheese produced aflatoxins in culture media, but the toxicity of the cheese itself was not demonstrated. Lie and Marth (15), however, have shown that aflatoxin can be produced in cheddar cheese by *A. flavus* and *A. parasiticus*.

It is known that different acid-producing bacteria possess antimicrobial activity against pathogenic organisms. Branen et al. (5) have isolated antimicrobial substances from *Streptococcus diacetilacticus* and *Leuconostoc citrovorum*, and Pulusani et al. (20) purified and characterized antimicrobial compound(s) from *Streptococcus thermophilus*-fermented milk. Recently, Rutzinksi and Marth (23) noticed inhibitory effects of *Streptococcus lactis* and *Streptococcus cremoris* against *Enterobacter* and *Hafnia* species.

In our laboratory, F. Ward (MSc thesis, McGill University, Montreal, Canada, 1975) found that *Streptococcus lactis* (cheese starter) inhibited aflatoxin produced by *A. flavus* (cheese contaminant). Similar findings were reported by Wiseman and Marth (26) and El-Gendy and Marth (11). In the present study, the inhibitory system was further characterized. *S. lactis* was found to degrade the preformed aflatoxin. The viability and morphology of *S. lactis* is greatly affected by aflatoxin and compounds extracted with chloroform from *S. lactis* cultures. Finally, we show that, according to the *Salmonella*/mammalian-microsome mutagenicity test (3), mixed culture supernatants are devoid of any residual mutagenic or potential carcinogenic activity.

MATERIALS AND METHODS

Stock cultures. *S. lactis* (ATCC 11454) and *A. flavus* (V3734/10) were used for these experiments. Stock frozen cultures of *S. lactis* were prepared by freezing cultures grown for 48 h at 28°C in brain heart infusion agar slants (Difco Laboratories, Detroit, Mich., 1.0 ml of medium in 2-ml glass vials) in liquid air. These were subsequently stored at −80°C. Stock spore suspensions of *A. flavus* were prepared by the method of Grivell and Jackson (12).

Working cultures. An *S. lactis* frozen stock culture was defrosted, and transfers were made to 15 ml of Lablemco tryptone broth (LTB; 14). After incubation for 4 h at 28°C, 1 ml of suspension was transferred to 15 ml of LTB which was incubated for a further 3 h at 28°C. This latter suspension constituted the *S. lactis* working culture.

A few granules of silica gel from the *A. flavus* stock cultures were transferred to potato glucose agar slants (Difco Laboratories). The slants were incubated at 28°C for 7 days. The spores were washed off with Tween 80 (0.05% [vol/vol]) by the method of van Walbeek et al. (24). The spores were counted with a hemacytometer, and the suspension was adjusted to 10^7 spores · ml^{-1}.

Media. To meet the fastidious requirements for the growth of *S. lactis*, LTB (glucose, 1%; yeast extract [Difco], 1%; Lablemco beef extract [Oxoid, London, United Kingdom], 1%; tryptone [Difco], 1%; NaCl, 0.5%; Na_2HPO_4, 0.2%) (14) was used. The pH was adjusted to 6.8 with 5 N HCl before sterilization at 121°C for 15 min. When LTB at pH 4.3 was needed, the pH was adjusted aseptically with HCl (5 N) or lactic acid (85%) after sterilization.

Microbial interaction. Inhibition of aflatoxin synthesis. Cultures containing 0.2 ml of *S. lactis* working culture and 15 ml of LTB in 25-by-150-mm culture tubes were incubated at 28°C. At various times, the pH and the residual glucose levels (10) were measured, and 0.1 ml of an *A. flavus* working culture was added. The incubation was continued (mixed culture) with the tubes in a slanted position, and the amount of aflatoxin produced was measured at regular intervals. In some experiments, additional glucose was added to the *S. lactis* culture before the inoculation with *A. flavus* spores.

* Corresponding author.

***S. lactis* destruction of aflatoxin.** *A. flavus* working cultures were inoculated into LTB, and the suspension was incubated at 28°C for 3 to 4 days to allow for aflatoxin production. *S. lactis* working cultures were injected into the medium (beneath the mycelium mat) with a syringe, the incubation was continued, and at different times aflatoxin levels were measured.

Localization of inhibitor. Cultures containing 0.2 ml of *S. lactis* working culture and 15 ml of LTB were incubated for 8 h at 28°C. Subsequently, the cultures were centrifuged at 13,200 × *g* (Sorvall RC2B), and the supernatant fraction was collected and sterilized by filtration (Millipore Corp., 45-μm-pore-size filter). The residue (cells) was washed twice with a sterile physiological saline solution, suspended in LTB to the original volume, and heated for 10 min at 95°C. Toxin production by *A. flavus* in both the supernatant solution and cell fraction was subsequently determined.

S. lactis working culture (0.2 ml) was added to 15 ml of LTB in a dialysis sack (cellulose, 4.8 nm pore diameter). The sack was aseptically placed inside a 250-ml Erlenmeyer flask containing 50 ml of sterile LTB. The flask was incubated for 16 h at 28°C. *A. flavus* spores were then inoculated into the LTB in the flask, and the incubation continued for a further 3 days. Toxin levels were subsequently measured in LTB both inside and outside the dialysis sack.

Aflatoxin determination. (i) Chemical. Extraction. The mycelium from a culture was removed, macerated in a mortar with a pestle, and added back to the culture fluid. The resultant suspension was extracted twice with chloroform (1:1 [vol/vol]) at 60°C. The chloroform extracts were concentrated under a nitrogen stream or on a rotary evaporator (19).

Separation. The concentrated chloroform extract (10 to 25 μl) was spotted and separated on a 20-by-20-cm silica gel thin-layer chromatographic plate. The gel was 0.25 mm thick and was prepared by mixing 25 g of silica gel 7G (Baker) with 50 ml of glass distilled water. The developing solvent was 90% toluene-ethyl acetate-formic acid (6:3:1 [vol/vol/vol]). Known quantities of aflatoxins B_1 and G_1 (Calbiochem) standards in benzene-acetonitrile (spectro grade, 98:2 [vol/vol]) were always spotted for reference (19).

Quantification. The developed thin-layer chromatographic plates were scanned with a Turner fluorometer model 111 (excitation filter no. 7-60 and transmission filter no. 48) equipped with a thin-layer chromatography automatic scanner and a recorder (Heathkit model EU-20B). The areas under the peaks were measured, and the aflatoxin concentration of the unknown was determined by the method developed by Pons et al. (19), using the formula developed by Rodericks and Stoloff (20).

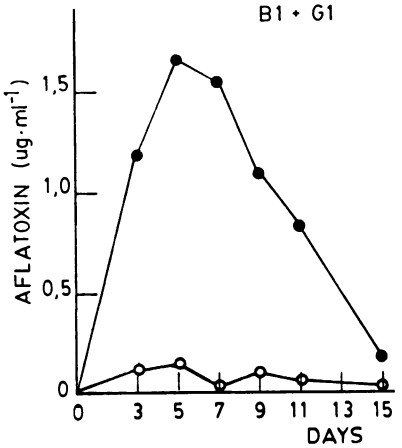

FIG. 1. Aflatoxin B_1 and G_1 production by *A. flavus* in monoculture and in mixed culture with *S. lactis*. Symbols: ●, *A. flavus*; ○, *A. flavus* in *S. lactis* culture.

(ii) Biological. Spore suspensions of *Bacillus megaterium* (ATCC 25848; $10^{10} \cdot ml^{-1}$) were prepared and stored at 4°C. Tryptone-glucose-yeast extract agar (pH 6.2) was inoculated with the spore suspension (final concentration, 1%), and 6 ml was poured into a petri plate and allowed to harden. Sterile filter disks (6 mm diameter) were loaded with 200 μl of the test solutions, dried in air, and placed on top of the agar surface. The plates were incubated at 37°C for 24 h. Inhibition zones were measured, and cell morphology at the periphery of the zone was observed (9).

Dry weight. The mycelium of the *A. flavus* culture was removed and washed three times with water. The mats were then placed in preweighed aluminum cups, dried in an oven at 100°C for 24 h, and cooled in a desiccator to constant weight (9).

Detoxification. Detoxification of *A. flavus* cultures by *S. lactis* growth was determined by using the *Salmonella*/mammalian-microsome mutagenicity test developed by Ames et al. (1–3). The necessary *Salmonella typhimurium* strains were obtained from B. Ames (University of California, Berkeley).

RESULTS AND DISCUSSION

***S. lactis* inhibition of aflatoxin production.** The levels of aflatoxins B_1 and G_1 produced by *A. flavus* in monoculture increased during the first 5 days and decreased thereafter to very low levels after 15 days (Fig. 1). In the mixed-culture system in which *A. flavus* was inoculated and incubated in a 16-h *S. lactis* culture, extremely low levels of the toxins (determined chemically and biologically [9]) were present during the entire 15 days of incubation. The amount of *A. flavus* growth was the same in mono- and in mixed-culture systems.

During the 16-h growth of *S. lactis* in LTB, the pH and the glucose concentration decreased from 6.8 to 4.3 and from 1.0 to 0.6%, respectively. Subsequent findings indicated that the decrease in pH and glucose levels in the medium were not responsible for the inhibition of toxin production. The amount of toxin produced by *A. flavus* in LTB initially adjusted to pH 4.3 with HCl was equal to that produced in the control (LTB at pH 6.8). Further, growth of *A. flavus* and increase in pH from 4.3 to 8.0 were identical in acid-adjusted LTB and in the 16-h *S. lactis* culture.

Supplementation of the 16-h *S. lactis* culture (residual glucose, 0.6% [wt/vol]) with 1.0% (wt/vol) glucose resulted in a substantial increase in toxin production by *A. flavus*. When, however, compared with the control (LTB containing 1.6% glucose) aflatoxin production in the mixed-culture system was considerably lower.

The inhibitory compound produced during the growth of *S. lactis* was excreted into the medium; activity resided in the supernatant fraction of the culture. Heating of the supernatants did not affect their inhibitory characteristics. No inhibition was observed with a disrupted cell fraction. On the contrary, the latter fraction stimulated toxin production. Further, growth of *S. lactis* inside a dialysis bag inhibited toxin production by *A. flavus* in the external medium; whereas, in the control (medium inside dialysis bag uninoculated) toxin was detected in the medium inside and outside the dialysis bag).

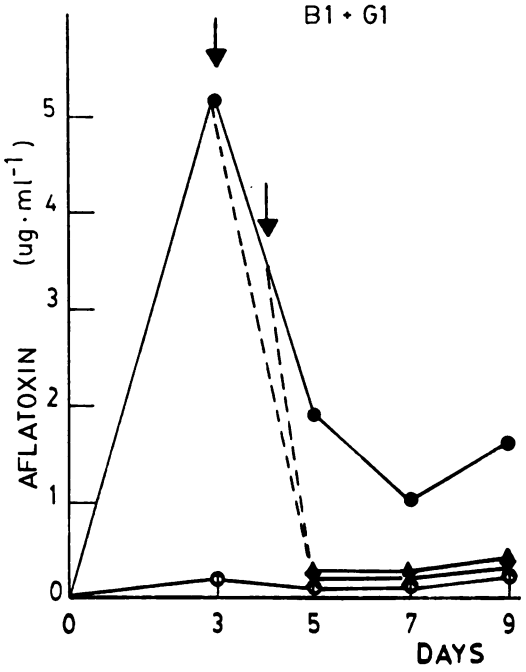

FIG. 2. Effect of *S. lactis* (10^7 CFU · ml^{-1}, final concentration) on preformed aflatoxins B_1 and G_1 in 3- and 4-day-old *A. flavus* cultures. Symbols: ●, *A. flavus*; ○, *A. flavus* into 16-h *S. lactis* culture; ↓ ×, *S. lactis* into 3-day-old *A. flavus* culture; ↓ ▲, *S. lactis* into 4-day-old *A. flavus* culture.

A potential inhibitor was lactic acid (21), as was also suggested by Wiseman and Marth (26). This was discounted when *A. flavus* grown in LTB and in LTB supplemented with lactic acid, pH 4.3, (concentration equal to that found in a 16-h *S. lactis* culture) produced equal amounts of toxin. Another potential inhibitor was nisin, a product of our *S. lactis* strain. Ward (MSc thesis), however, has shown that up to 20 μg of nisin · ml^{-1} of LTB (activity 30,000 U · mg^{-1}; Koch-Light Laboratories, Colnbrook, United Kingdom) had no effect on aflatoxin production by *A. flavus*. Further, Yousef et al. (27) reported that 200 to 5,000 reading units of nisin · ml^{-1} partially delayed the growth of *A. parasiticus* but had no effect on toxin production.

S. lactis commences the production of the inhibitor early in the logarithmic phase of growth. Cultures in their early stationary phase of growth (6 h) were the most inhibitory to subsequent toxin production by *A. flavus*. Continued incubation of the culture to 16 h resulted in an apparent decrease (or inactivation) of the inhibitor. This decrease in inhibitor concentration may have been the result of secondary metabolism of the inhibitor (a primary metabolite) or due to the instability of the inhibitor. Indeed, in later experiments, it was observed that the partially purified inhibitor compound lost activity upon storage, suggesting a labile compound.

***S. lactis* degradation of aflatoxin.** *S. lactis* (16-h culture, 10^7 CFU · ml^{-1} final concentration) added to either a 3-day or a 4-day *A. flavus* culture reduced, within a 1 or 2 days, the toxin levels to those observed in the mixed-culture systems (Fig 2). Wiseman and Marth (26) also found that *S. lactis* decreased toxin levels in *A. parasiticus* cultures. These authors also reported that the toxin levels in mixed cultures were at times higher than those in *A. flavus* monocultures. We have never observed this phenomenon. Degradation of toxin was suggested when *S. lactis* grown in LTB supplemented with either a chloroform extract of a 5-day-old culture of *A. flavus* (final concentration of toxins in LTB, 18 μg · ml^{-1}) or aflatoxins B_1 and G_1 standard solutions (each at a final concentration of 10 μg · ml^{-1}) reduced the toxin levels in both systems (Table 1).

Ciegler et al. (8) surveyed 1,000 microorganisms for their ability to degrade aflatoxin and found that only *Flavobacterium aurantiacum* removed aflatoxin B_1 from a nutrient solution. *Tetrahymena pyriformis* also reduced toxin levels but was not as efficient. The mechanism proposed was that aflatoxin was adsorbed to the cell wall, a mechanism similar to the adsorption of toxins in inert compounds such as clay. The latter has been suggested as a method of detoxification by Masimango et al. (17), who showed that aflatoxin was degraded by intact and fragmented mycelium. In addition, the greater the disintegration of the mycelium, the higher was the adsorption and disappearance of aflatoxin in the environment. This was attributed to a higher mycelium surface contact area being present. Lillehoj and Ciegler (16) also showed that aflatoxin B_1 binds tightly to *B. megaterium* cells, and the toxin can be removed by ultrasonic treatment of the organism and a chloroform extraction of the macerate. Adsorption of the aflatoxin on *S. lactis* cells cannot explain the decreased amount of aflatoxin in mixed-culture systems because the *S. lactis* cells were not removed from the system at the time of extraction with chloroform. From the results presented in Table 1, it appears that although *S. lactis* is capable of degrading aflatoxin, the amount of reduction is insufficient to account fully for the low levels of aflatoxins observed in the mixed-culture systems. Therefore, *S. lactis* must also, in some way, interfere with the synthesis of aflatoxin.

***A. flavus* effects on *S. lactis*.** Although *S. lactis* affected a reduction in aflatoxin production by *A. flavus*, the organism did not remain untouched. *S. lactis* in monoculture with inoculum levels of 10^2 and 10^7 CFU · ml^{-1} (final concentration) grew to ca. 10^9 CFU · ml^{-1} within 8 h. With the same inocula in 3-day-old *A. flavus* cultures, the numbers of *S.*

TABLE 1. Disappearance of aflatoxin in LTB-containing chloroform extracts of a 5-day-old *A. flavus* culture or aflatoxins B_1 and G_1 standard solutions inoculated with *S. lactis*

Time after *S. lactis* inoculation (h)	*S. lactis* (CFU · ml^{-1})[a] in:			Residual toxin (% remaining in LTB) with added:			
	LTB	LTB and added toxin from:		Extract		Standard solution	
		Extract[b]	Standard solution[c]	B_1	G_1	B_1	G_1
24	6×10^8	4×10^2	1×10^8	67 ± 5	50 ± 3	44 ± 5	35 ± 3
48	5×10^8	1×10^2	9×10^7	12 ± 4	13 ± 4	ND[d]	ND

[a] *S. lactis* inoculum; 10^7 CFU · ml^{-1} (final concentration). Data are means of triplicate samples.
[b] Extract, chloroform extract of 5-day-old *A. flavus* culture (18 μg of B_1 and G_1 · ml^{-1}, final concentration).
[c] Standard solutions, aflatoxins B_1 and G_1 added to LTB (each 10 μg · ml^{-1}, final concentration).
[d] ND, Not done.

TABLE 2. Mutagenic and toxic compounds in extracts of *A. flavus* cultures grown in LTB and in *S. lactis* culture filtrates

Compound added	No. of revertants per plate by Ames test	
	TA98	TA100
None	36	120
Chloroform	36	123
Benzene-acetonitrile (98:2)	31	130
Standard B_1[a]	461	25,000
Standard G_1[b]	311	19,440
Extracts from *A. flavus* grown in LTB	830	1,266
Extract from *A. flavus* grown in *S. lactis* culture filtrate	10	8
Extract of *S. lactis* culture filtrate	24	100

[a] B_1, 3.5 μg · (100 μl)$^{-1}$.
[b] G_1, 5.0 μg · (100 μl)$^{-1}$.

lactis decreased exponentially such that after 8 and 24 h the viable counts were 10 and 4×10^2 CFU · ml^{-1}, respectively. Wiseman and Marth (26) also observed that *S. lactis* numbers decreased during incubation in an *A. parasiticus* culture. Their suggestion was that as the mould grows and produces toxin and other metabolites, the aflatoxin destroys the *S. lactis* cells. Our results do not support this assumption. Aflatoxin did not affect the growth of *S. lactis* in LTB, but other compounds produced by *A. flavus* did (Table 1, viable counts). The fact that *S. lactis* in the presence of aflatoxin grew as enlarged cocci in long chains suggests that some metabolic functions have been altered (4, 7, 13, 26).

***S. lactis* detoxification of *A. flavus* cultures.** *S. lactis* produces a compound(s) which inhibits aflatoxin production by *A. flavus*. The question, however, remained whether in the process of inhibiting aflatoxin production, the *S. lactis* had actually detoxified the system. It is possible, for instance, that as a consequence of the inhibition, precursors or degradative products which are more toxic or mutagenic or both may have accumulated. The *Salmonella*/mammalian-microsome mutagenic test developed by Ames et al. (1–3) is simple, and although "it is one or two orders of magnitude less sensitive than the most sensitive bioassay and is therefore of little relevance to mycotoxin detection," it does serve as an indicator of mutagenicity or potential carcinogenicity (25) and can identify chemical carcinogens and mutagens with an accuracy of 90% (18).

The test to determine the presence of mutagenic agents was done with strains TA98 and TA100, which were found to be active against aflatoxin in preliminary tests. Spontaneous revertants were quantified in systems containing no mutagen or solvent. With TA98, the number of spontaneous revertants was 36, and with TA100 the number was 120 (Table 2). These levels were not affected by the addition of either chloroform or benzene-acetonitrile (98:2 [vol/vol]). A slight decrease in the number of revertants was observed with extracts of *S. lactis* culture filtrates. When an aflatoxin B_1 standard solution (3.5 μg · [100 μl]$^{-1}$) was added to the plate, the number of revertants increased to 461 with TA98 and 2,500 with strain TA100. Standard aflatoxin G_1 (5 μg · [100 μl]$^{-1}$) also increased the number of revertants to 311 with strain TA98 and to 19,440 with strain TA100. The chloroform extract of the *A. flavus* culture increased the number of revertants to 830 and 1,266 for strains TA98 and TA100, respectively. On the other hand, the chloroform extract of the *A. flavus* culture grown in *S. lactis* culture filtrate did not have any effect on either strain of the *Salmonella* test organisms. On the contrary, the number of revertants fell below the spontaneous background levels, e.g., 10 and 8 revertants per plate for strains TA98 and TA100, respectively. Thus it would appear from the results of the Ames test that *S. lactis* suppresses the production of mutagenic or potentially carcinogenic compounds by *A. flavus*.

In conclusion, each organism in the mixed culture system comprising *S. lactis* and *A. flavus* affects one or another activity of the other organism. *S. lactis* produces an inhibitor(s) during the logarithmic phase of growth which reduces the amount of aflatoxin produced by *A. flavus*, resulting in a system lacking mutagenic or carcinogenic properties. *S. lactis*, in its turn, is destroyed by compounds produced by *A. flavus*. The nature of the inhibitor(s) produced by *S. lactis* is the subject of another paper.

LITERATURE CITED

1. **Ames, B. N., W. E. Durston, E. Yamasaki, and F. D. Lee.** 1973. Carcinogens and mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc. Natl. Acad. Sci. U.S.A. **70**:2281–2285.
2. **Ames, B. N., F. D. Lee, and W. E. Durston.** 1973. An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Natl. Acad. Sci. U.S.A. **70**:782–786.
3. **Ames, B. N., J. McCann, and E. Yamasaki.** 1975. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res. **31**:347–364.
4. **Beuchat, L. R., and R. V. Lechowich.** 1971. Biochemical alterations in *Bacillus megaterium* as produced by aflatoxin B1. Appl. Microbiol. **21**:119–123.
5. **Branen, A. L., H. C. Go, and R. P. Genske.** 1975. Purification and properties of antimicrobial substances produced by *Streptococcus diacetilactis* and *Leuconostoc citrovorum*. J. Food Sci. **40**:446–450.
6. **Bullerman, L. B., and F. J. Olivigni.** 1974. Mycotoxin producing potential of moulds isolated from cheddar cheese. J. Food Sci. **39**:1166–1168.
7. **Chu, F. S.** 1977. Mode of action of mycotoxins and related compounds. Adv. Appl. Microbiol. **22**:83–143.
8. **Ciegler, A., R. E. Peterson, A. A. Lagoda, and H. H. Hall.** 1966. Aflatoxin production and degradation in 20 L fermentors. Appl. Microbiol. **14**:826–833.
9. **Clements, N. L.** 1968. Note on a microbiological assay for aflatoxin B1: a rapid confirmatory test by effects on growth of *Bacillus megaterium*. J. Assoc. Off. Anal. Chem. **51**:611–612.
10. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for determination of sugar and related substances. Anal. Chem. **28**:350–356.
11. **El-Gendy, S. M., and E. H. Marth.** 1980. Growth of toxigenic and nontoxigenic aspergilli and penicillia at different temperatures and in the presence of lactic acid bacteria. Arch. Lebensmittelhyg. **31**:192–195.
12. **Grivell, A. R., and J. F. Jackson.** 1969. Microbial culture preservation with silica gel. J. Gen. Microbiol. **58**:423–425.
13. **Harley, E. H., K. R. Rees, and A. Cohen.** 1969. A comparative study of the effect of aflatoxin B1 and actinomycin D on HeLa cells. Biochem. J. **114**:289–298.
14. **Hurst, A.** 1966. Biosynthesis of the antibiotic nisin by whole *Streptococcus lactis* organisms. J. Gen. Microbiol. **44**:209–220.
15. **Lie, J. L., and E. H. Marth.** 1967. Formation of aflatoxin in cheddar cheese by *Aspergillus flavus* and *Aspergillus parasiticus*. J. Dairy Sci. **50**:1708–1710.
16. **Lillehoj, E. B., and A. Ciegler.** 1968. Aflatoxin B1 binding and toxic effect on *Bacillus megaterium*. J. Gen. Microbiol. **54**:185–194.
17. **Masimango, N., J. Remacle, and J. L. Ramaut.** 1978. The role of adsorption in the elimination of aflatoxin B1 from contaminated

media. Eur. J. Appl. Microbiol. Biotechnol. **6**:101–105.
18. **McCann, J., E. Choi, E. Yamasaki, and B. N. Ames.** 1975. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. Proc. Natl. Acad. Sci. U.S.A. **72**:5135–5139.
19. **Pons, W. A., J. A. Robertson, Jr., and L. A. Goldblatt.** 1966. Objective fluorometric measurement of aflatoxins on TLC plates. J. Am. Oil Chem. Soc. **43**:665–669.
20. **Pulusani, S. R., D. R. Rao, and G. R. Sunki.** 1979. Antimicrobial activity of lactic cultures: partial purification and characterization of antimicrobial compound(s) produced by *Streptococcus thermophilus*. J. Food. Sci. **44**:575–578.
21. **Reiss, J.** 1976. Prevention of the formation of mycotoxins in whole bread by citric acid and lactic acid (Mycotoxins in foodstuffs. IX). Experientia **32**:168–169.
22. **Rodericks, J. V., and F. L. Stoloff.** 1970. Determination of concentration and purity of aflatoxin standards. J. Assoc. Off. Anal. Chem. **53**:92–93.
23. **Rutzinski, J. L., and E. H. Marth.** 1980. Behaviour of *Enterobacter* species and *Hafnia* species in skim milk during fermentation by lactic acid bacteria. J. Food Prot. **43**:720–728.
24. **van Walbeek, W., P. M. Scott, and F. S. Thatcher.** 1968. Mycotoxins from food-borne fungi. Can. J. Microbiol. **14**: 131–137.
25. **Watson, D. H., and D. G. Lindsay.** 1982. A critical review of biological methods for the detection of fungal toxins in foods and foodstuffs. J. Sci. Food Agric. **33**:59–77.
26. **Wiseman, D. W., and E. H. Marth.** 1981. Growth and aflatoxin production by *Aspergillus parasiticus* when in the presence of *Streptococcus lactis*. Mycopathologia **73**:49–56.
27. **Yousef, A. E., S. M. El-Gendy, and E. H. Marth.** 1980. Growth and biosynthesis of aflatoxin by *Aspergillus parasiticus* in cultures containing nisin. Z. Lebensm. Unters. Forsch. **171**:341–343.