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Research Note

Lactobacillus casei CRL 431 and *Lactobacillus rhamnosus* CRL 1224 as Biological Controls for *Aspergillus flavus* Strains

DANTE J. BUENO,[1,2]* JULIO O. SILVA,[3] GUILLERMO OLIVER,[1] AND SILVIA N. GONZÁLEZ[1,4]

[1]*Centro de Referencia para Lactobacilos (CERELA)-CONICET, Chacabuco 145, 4000, Tucumán, Argentina;* [2]*Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria, Concepción del Uruguay, Casilla de Correo No. 6, 3260, Entre Ríos, Argentina; and* [3]*Cátedra de Micología and* [4]*Cátedra de Salud Pública, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 471, Tucumán, Argentina.*

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ABSTRACT

The effect of two species of lactobacilli, *Lactobacillus casei* CRL 431 and *Lactobacillus rhamnosus* CRL 1224, on growth of different *Aspergillus flavus* strains was determined. *A. flavus* strains (Ap, TR_2, or CF_{80}) were grown in LAPTg broth at 37°C for 7 days as a single culture and in association with *L. casei* CRL 431 or *L. rhamnosus* CRL 1224 at initial inoculum ratios of 1:1, 1:10, and 1:100. In most cases, the mixed cultures had a lower fungal growth and a lower pH than the control cultures. Mycelial dry weight was reduced to 73 and 85% using *L. casei* CRL 431 and *L. rhamnosus* CRL 1224, respectively. The pH decrease in mixed cultures when the fungal mycelial dry weight is reduced may play an important role in inhibition. The number of viable bacteria was variably affected by fungal growth. These results indicate that *L. casei* CRL 431 and *L. rhamnosus* CRL 1224 may be useful as potential biocontrol agent against *A. flavus.*

Spoilage of food products pre- and postharvest involves a wide range of fungi that differ greatly in their ecological determinants. They damage foods causing losses in dry matter and quality. Some species can produce toxic metabolites (mycotoxins) of great concern to consumers with outbreaks of disease affecting both animal and human health *(3, 21, 33)*.

Aspergillus is a genus described 300 years ago. It occurs in a wide variety of habitats, particularly in tropical and subtropical regions, and is very important in foods. Most *Aspergillus* species are present in foods as spoilage or biodeterioration fungi and they compete with *Penicillium* and *Fusarium* species for dominance among the fungal flora *(20, 32).* In nature, these microorganisms are regularly found as saprophytes growing on inadequately dried products like cereal grains and groundnuts or derived formulations such as animal feedstuffs *(21, 38). Aspergillus flavus* is an economically important fungus. Different reports indicated that this fungus can produce mycotoxins, including aflatoxins, aflatrem, aflavinin, aspergillic acids, cyclopiazonic acid, kojic acid, etc. *(16–18, 38). Aspergillus* grows at 6 to 45°C with optimal growth occurring at 25 to 37°C *(20).*

The most effective means of preventing contamination of food with mycotoxins is to prevent the growth of mycotoxicogenic fungi *(4).* The development of biocompetitive microorganisms to control *A. flavus* has been investigated as an alternative approach for controlling mycotoxin contamination. Several studies have reported the inhibitory effects of lactic acid bacteria (LAB) on the growth of *Aspergillus,* and particularly *A. parasiticus,* with different mechanisms suggested for these observations *(10).* Competition for space, nutrients, and synthesis of antifungal compounds by biocompetitive agents is attributed to the effects on fungi growth.

The LAB possess nutritional and therapeutic benefits with various *Lactobacillus* strains commonly used as probiotic microorganisms. Furthermore, LAB have several properties of economic importance such as lactose utilization, proteinase activity, bacteriophage defense mechanisms, and bacteriocin production *(11, 26). Lactobacillus casei* CRL 431 is a very important probiotic strain. Fermented milk containing this strain and *Lactobacillus acidophilus* CRL 730 is consumed by many individuals in South America *(27).* When used in combination, these two bacteria can prevent gastrointestinal infection with *Salmonella enterica* subspecies *enterica* serovar Typhimurium *(29). L. casei* can reportedly inhibit the growth of different pathogens, including *Escherichia coli* and *Klebsiella pneumoniae (12)*; however, there is no similar work assessing the inhibition of fungal growth. This organism can also stimulate the immune system, which could be partly explained by the presence of lectinlike substances in the cell wall *(25)* and an increase in the number of cells producing immunoglobulin (Ig) A and IgM *(1, 30).* On the other hand, previous studies related to *L. rhamnosus* CRL 1224 are not known.

The aim of this work was to evaluate the effect of two different *Lactobacillus* strains, *L. casei* CRL 431 and *L.*

* Author for correspondence. Tel and Fax: (054) (3442) 425561/78; E-mail: dantejb@yahoo.com.ar.

rhamnosus CRL 1224, on the growth of *A. flavus* isolated from different sources.

MATERIALS AND METHODS

Microorganisms and culture conditions. *A. flavus* strains Ap, TR_2, and CF_{80} were isolated from animal, poultry, and cat foods, respectively. These strains were identified according to the method proposed by Pitt and Hocking *(32)* and were maintained under refrigeration (4°C) on potato dextrose agar (PDA) slants.

Cultures for inoculation were obtained by growing *A. flavus* on PDA slants at 28°C. After 7 days, spores were harvested in 0.05% Tween 80 (vol/vol) wetting solution. Spore counts were then determined using a haemocytometer and the suspensions were standardized to a final concentration of ca. 3×10^6 spores per ml.

L. casei CRL 431 (isolated from human feces) and *L. rhamnosus* CRL 1224 (isolated from yogurt) were obtained from the CERELA Culture Collection (Culture Collection of Centro de Referencia para Lactobacilos, Tucumán, Argentina). These bacteria were maintained frozen (−20°C) in milk–yeast extract. Working cultures were prepared by subculturing at 37°C in LAPTg broth *(34)* followed by overnight incubation at the same temperature.

Inhibition assays in broth medium. Two LAB cultures were added separately to 250-ml Erlenmeyer flasks containing 100 ml of LAPTg broth to obtain populations of 10^4, 10^5, and 10^6 CFU/ml. Thereafter, conidial suspensions of each *A. flavus* strain were added separately to each flask at a level of ca. 10^4 spores per ml to give *Lactobacillus*:spore ratios of 1:1, 10:1, and 100:1. Control flasks were separately inoculated with each organism at a level of ca. 10^4/ml. All flasks were incubated quiescently for 7 days at 37°C. Numbers of viable bacteria were determined *(24)* using LBS agar (ROGOSA agar, Merck, Darmstadt, Germany). The pH of the medium (Altronix model TPX-1 digital pH meter, Buenos Aires, Argentina) and growth of fungi expressed as dry weight of mycelium were determined at the end of each experiment. Inhibition of mold growth was calculated using the equation: [1 − (fungi growth in mixed culture/fungi growth in control culture)] × 100.

Inhibition assays on an agar medium. The antifungal activity of *L. casei* CRL 431 and *L. rhamnosus* CRL 1224 was determined by the well agar diffusion method *(36)*. An aliquot of spore suspension was mixed with PDA, LAPTg, and Czapek-Dox agar (Merck). Four wells (10-mm diameter) were made in each agar plate, where the LAB had been added.

The bacterial cultures (bacteria grown overnight at 37°C) were pelleted by centrifugation at 1,200 × *g* for 15 min. The supernatant was separated and the cell pellet was washed twice with peptone water. A portion of the supernatant was heated to 70°C for 1 h before assessing the interaction between LAB and *A. flavus* Ap. Treated and untreated supernatants were placed separately into the wells.

The wells were filled separately with 150 μl of the bacterial cell suspension in peptone water or the corresponding supernatants. The plates were preincubated at 4°C for 24 h to stop fungal growth and facilitate diffusion of the cell suspension and supernatant into the agar medium and then incubated for 4 days at 28°C.

Statistical analysis. The bacterial counts were transformed to log counts and subjected to a balanced analysis of variance test to determine the effect of treatment on bacterial populations. Dry weight of mycelium and the pH were analyzed by the same test. Variable means showing statistical significance were compared using Tukey's test (Minitab Student R12). All statements of significance are based on 0.05 level of probability *(35)*. Values are means of experiments conducted in duplicate and replicated three times.

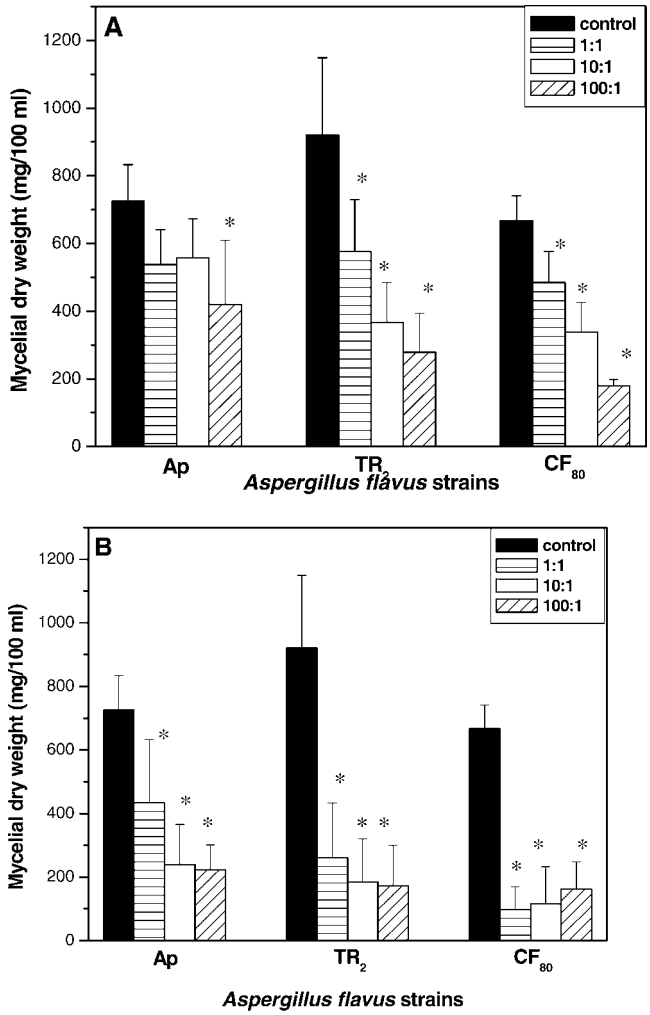

FIGURE 1. *Effect of* L. casei *CRL 431 (A) and* L. rhamnosus *CRL 1224 (B) on mycelial dry weight from different* A. flavus *strains. Vertical bars indicate the standard deviation* (n = 6). *Bars marked with an asterisk (*) showed significant reduction (*$P < 0.05$*) in mycelial dry weight from the control.*

RESULTS AND DISCUSSION

The effect of *L. casei* CRL 431 on mycelial dry weight for different *A. flavus* strains is shown in Figure 1A. Growth of *A. flavus* Ap, TR_2, and CF_{80} strains, measured by mycelial dry weight, was reduced from 725 to 420 mg, 920 to 278 mg, and 668 to 180 mg, respectively. The largest reduction in fungal growth (73%) was obtained from mixed cultures containing *L. casei* and *A. flavus* CF_{80} at an initial inoculum ratio of 100:1. Percent reduction in growth of the *Aspergillus* strains ranged from 26 to 37%, 23 to 60%, and 42 to 73% for bacteria:fungi ratios of 1:1, 10:1, and 100:1, respectively. Growth of *A. flavus* Ap strain was least affected with the only significant difference from the control seen at an initial bacteria:fungi inoculation ratio of 100:1.

The pH values in the different broth cultures decreased from 8.1 to 4.8, 7.7 to 4.3, and 8.0 to 3.9 for Ap, TR_2, and

CF80 strains respectively, when the bacteria:fungi ratio was increased. After incubation of control assays, the pH values in the fungi and bacteria controls increased and decreased, respectively, following incubation. At a 1:1 ratio, the *L. casei* CRL 431 population was higher at day 7 as compared with day 0 for *A. flavus* CF_{80}. At an initial bacteria:fungi ratio of 10:1, bacterial populations in mixed cultures with all *A. flavus* strains were not statistically different ($P < 0.05$). However, at an initial bacteria:fungi ratio of 100:1, bacterial populations in mixed cultures with *A. flavus* Ap were significantly lower ($P < 0.05$) after 7 days of incubation (Table 1).

The results of mixed cultures containing *L. rhamnosus* CRL 1224 and *A. flavus* strains are shown in Figure 1B and Table 2. During the interaction assays, fungal growth was significantly lower ($P < 0.05$) in all mixed cultures as compared with the controls. Mycelial dry weight decreased from 725 to 223 mg, 920 to 172 mg, and 668 to 98 mg, for mixed cultures with *A. flavus* Ap, TR_2, and CF_{80} strains, respectively. Thus, mold growth decreased by 40 to 85%, 67 to 82%, and 69 to 81% for initial bacteria:fungi inoculum ratios of 1:1, 10:1, and 100:1, respectively. Greatest fungal inhibition was seen in mixed culture containing *A. flavus* CF_{80} with those containing *A. flavus* Ap being least affected (Fig. 1B). Similar results were also obtained using *A. flavus* and *L. casei* CRL 431.

The pH of the mixed culture was statistically different ($P < 0.05$) from the fungal control and decreased in the presence of *A. flavus* Ap when the initial bacterial numbers increased. The pH reductions in mixed cultures compared with the fungal control were from 8.10 to 4.06, 7.69 to 4.02, and 7.97 to 3.85 for *A. flavus* Ap, TR_2, and CF_{80}, respectively. However, the population of *L. rhamnosus* CRL 1224 was significantly greater on day 7 than on day 0 for the control and at a bacteria:fungi ratio of 1:1. Populations were variable at a ratio of 10:1 and remained unchanged at 100:1 (Table 2).

Fungal growth can be improved or retarded as a result of interactions with other microorganisms present in the environment. El-Gendy and Marth *(8)* reported that the addition of *Lactococcus lactis* (ATCC 11454) and *L. casei* (ATCC 393) to *Aspergillus* cultures (toxigenic and nontoxigenic strains) reduced fungal growth after 2 weeks of incubation at 15°C. Furthermore, they demonstrated that the presence of LAB inhibited the growth of several *Penicillium* strains. Other authors have studied interactions between LAB and fungi in broth media, but they only found encouraging results when the bacteria grew before fungal spores inoculation *(6, 8, 9, 13–15, 19, 22).* In these studies, LAPTg was not used as a culture medium with 25 to 28°C selected as the incubation temperature; however, they were unable to obtain similar results to our work when *A. flavus* and LAB were grown simultaneously. Recently, Xu et al. *(39)* studied fungi-bacteria interactions in MRS medium at 28°C over 15 days and reported that growth of *Aspergillus parasiticus* NRRL 2999 was inhibited when spores were added to a 24-h *Lactobacillus plantarum* ATC8014 culture and when both organisms were added simultaneously. These findings are similar to ours in that the growth of *A.*

TABLE 1. *Bacterial population and pH from single and mixed cultures of* L. casei *CRL 431 and* A. flavus *strains*[a]

Treatment	Incubation (days)	Bacterial growth (log CFU per milliliter)			pH values		
		Ap	TR_2	CF_{80}	Ap	TR_2	CF_{80}
A. flavus	0	—	—	—	6.45 ± 0.23	6.61 ± 0.04	6.53 ± 0.07
	7	—	—	—	8.10 ± 0.21 A	7.69 ± 0.14 A	7.97 ± 0.18 A
L. casei CRL 431	0	4.59 ± 0.07 A	4.47 ± 0.24 A	4.07 ± 0.19 A	6.45 ± 0.23	6.61 ± 0.04	6.53 ± 0.07
	7	5.47 ± 0.72 AB	5.19 ± 0.77 AB	5.80 ± 0.33 AB	3.61 ± 0.08 D	3.68 ± 0.01 B	3.64 ± 0.05 B
L. casei CRL 431 + *A. flavus* (1:1)	0	4.59 ± 0.07 A	4.47 ± 0.24 A	4.07 ± 0.19 A	6.45 ± 0.23	6.61 ± 0.04	6.53 ± 0.07
	7	4.53 ± 1.30 A	5.06 ± 0.70 AB	6.53 ± 0.63 B	6.68 ± 0.84 B	5.29 ± 0.75 C	5.19 ± 0.53 C
L. casei CRL 431 + *A. flavus* (10:1)	0	5.59 ± 0.07 AB	5.47 ± 0.24 AB	5.06 ± 0.13 B	6.45 ± 0.23	6.61 ± 0.04	6.53 ± 0.07
	7	4.62 ± 0.96 A	5.79 ± 0.86 AB	6.16 ± 0.83 B	5.77 ± 1.20 BC	4.43 ± 0.33 D	4.42 ± 0.35 D
L. casei CRL 431 + *A. flavus* (100:1)	0	6.59 ± 0.07 B	6.47 ± 0.24 B	6.06 ± 0.13 B	6.45 ± 0.23	6.61 ± 0.04	6.53 ± 0.07
	7	4.56 ± 1.20 A	5.13 ± 0.92 AB	5.90 ± 0.82 B	4.78 ± 0.86 C	4.26 ± 0.33 BD	3.92 ± 0.03 BD

[a] Means with different letters in the same column are significantly different ($P < 0.05$). Each value is a mean ± standard deviation, $n = 6$.

TABLE 2. *Bacterial population and pH from single and mixed cultures of* L. rhamnosus *CRL 1224 and* A. flavus *strains*[a]

Treatment	Incubation (days)	Bacterial growth (log CFU per milliliter)			pH values		
		Ap	TR_2	CF_{80}	Ap	TR_2	CF_{80}
A. flavus	0	—	—	—	6.45 ± 0.23	6.61 ± 0.04	6.53 ± 0.07
	7	—	—	—	8.10 ± 0.21 A	7.69 ± 0.14 A	7.97 ± 0.18 A
L. rhamnosus CRL 1224	0	4.31 ± 0.27 A	4.48 ± 0.04 A	4.58 ± 0.06 A	6.45 ± 0.23	6.61 ± 0.04	6.53 ± 0.07
	7	6.84 ± 0.44 B	6.97 ± 0.38 B	6.93 ± 0.44 B	3.67 ± 0.06 B	3.72 ± 0.02 B	3.68 ± 0.02 B
L. rhamnosus CRL 1224 + *A. flavus* (1:1)	0	4.31 ± 0.27 A	4.48 ± 0.04 A	4.58 ± 0.06 A	6.45 ± 0.23	6.61 ± 0.04	6.53 ± 0.07
	7	6.16 ± 0.59 BC	6.88 ± 0.49 BC	6.22 ± 0.77 BC	5.50 ± 1.34 C	4.28 ± 0.42 C	3.85 ± 0.21 B
L. rhamnosus CRL 1224 + *A. flavus* (10:1)	0	5.31 ± 0.27 AC	5.48 ± 0.04 AC	5.58 ± 0.06 AC	6.45 ± 0.23	6.61 ± 0.04	6.53 ± 0.07
	7	6.87 ± 0.30 B	6.91 ± 0.43 BC	6.70 ± 0.37 BC	4.11 ± 0.27 B	4.09 ± 0.35 BC	3.93 ± 0.38 B
L. rhamnosus CRL 1224 + *A. flavus* (100:1)	0	6.31 ± 0.27 BC	6.48 ± 0.04 BC	6.58 ± 0.06 BC	6.45 ± 0.23	6.61 ± 0.04	6.53 ± 0.07
	7	6.50 ± 0.84 B	6.13 ± 1.25 BC	6.24 ± 0.84 BC	4.06 ± 0.10 B	4.02 ± 0.16 BC	3.91 ± 0.16 B

[a] Means with different letters in the same column are significantly different ($P < 0.05$). Each value is a mean ± standard deviation, $n = 6$.

flavus strains was inhibited by single *Lactobacillus* strains when simultaneously inoculated.

Different mechanisms have been proposed to explain the inhibitory effects of LAB on *Aspergillus* growth, including competitive inhibition, bacterial metabolites, pH, or a combination of these factors *(10).* Generally, fungal growth does not parallel mycotoxin production. Aflatoxin production was influenced by the lactic acid concentration, initial pH, and extent of mycelial growth *(7).* In our experiments, pH was a very important parameter because it was near to those of the bacterial control, when fungal growth was inhibited in mixed cultures. Furthermore, the lowest pH in mixed cultures corresponded to the greatest reduction in mycelial dry weight.

The pH increase could be the result of high levels of nitrogen in the medium and/or autolysis of fungal cells in those cultures where the fungi were present *(5).* The influence of the pH is dependent on many factors such as the substrate, incubation temperature, mold strain, and the presence of competing microflora *(14).*

Both *L. casei* CRL 431 and *L. rhamnosus* CRL 1224 supernatants (with or without heat treatment) and cells in peptone water did not inhibit any strains of *A. flavus* in Czapek-Dox agar, LAPTg agar, or PDA after 5 days of incubation. In the presence of *A. flavus* Ap, the bacterial supernatant changed the color of the spores from green to yellow around the wells on Czapek-Dox agar; however, this yellow zone decreased in size after the supernatant was heated at 70°C for 1 h. Microscope observations showed that the yellow zone was characterized by low spore concentration and pigmentation. No such changes were seen on PDA or LAPTg agar where this mold grew faster than on Czapek-Dox agar. Although the three *A. flavus* strains sporulated poorly on LAPTg agar, *A. flavus* TR_2 and CF_{80} produced sclerotia on PDA (data not shown).

The LAB produce a wide variety of antimicrobial substances, including organic acids, hydrogen peroxide, carbon dioxide, diacetyl, and low-molecular-weight antimicrobial compounds *(28, 31).* Several reports have described antifungal characteristics of these bacteria, including one account by Roy et al. *(36)* in which *Lactococcus lactis* subsp. *lactis* CHD-28.3 exhibited antifungical activity against *A. flavus, A. parasiticus,* and *Fusarium* spp.

The antifungal attributes of LAB have been reviewed by Batish et al. *(2).* Many variables including the culture medium, incubation temperature, incubation period, pH, and nutritional factors can affect the production of antifungal substances. It is well known that the growth medium for indicator organisms can influence the activity of antimicrobial substances *(37).* In our study, sporulation was only inhibited using supernatants from both bacteria, where *A. flavus* Ap grew more slowly.

Reduction of fungal growth alone cannot explain the decrease or absence of mycotoxin in mixed culture *(22, 23),* but the fact that a strain reduced the mycelial dry weight could be a good way to perform certain antitoxin assessments.

This investigation demonstrated that two LAB, namely *L. rhamnosus* CRL 1224 and *L. casei* CRL 431, may be

useful in inhibiting the growth of *A. flavus*. *L. rhamnosus* CRL 1224 was more inhibitory toward *A. flavus* than *L. casei* CRL 431. The pH can also play an important role in inhibition since the greatest reduction in fungal mycelial dry weight occurred at low pH values. Future studies should include the effects of these two LAB alone or together on aflatoxin production.

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REFERENCES

1. Agüero, G., S. Sánchez, S. Fernández, C. Allori, A. P. Pesce de Ruiz Holgado, and G. Perdigón. 1996. Administration of yogurt or *Lactobacillus casei* to malnourished mice: comparative effect on lymphoid cells and mucosal reconditioning of the intestine. *Food Agr. Immun.* 8:229–238.
2. Batish, V. K., U. Roy, R. Lal, and S. Grover. 1997. Antifungal attributes of lactic acid bacteria—a review. *Crit. Rev. Biotechnol.* 17: 209–225.
3. Beardall, J. M., and J. D. Miller. 1994. Diseases in human with mycotoxins as possible causes, p. 486–539. *In* J. D. Miller and H. L. Trenholm (ed.), Mycotoxins in grain. Eagan Press, St. Paul, Minn.
4. Bullerman, L. B. 1979. Significance of mycotoxins to food safety and human health. *J. Food Prot.* 42:65–86.
5. Ciegler, A., R. E. Peterson, A. A. Lagoda, and H. H. Hall. 1966. Aflatoxin production and degradation by *Aspergillus flavus* in 20-liter fermentors. *Appl. Microbiol.* 14:826–833.
6. Coallier-Ascah, J., and E. S. Idziak. 1985. Interaction between *Streptococcus lactis* and *Aspergillus flavus* on production of aflatoxin. *Appl. Environ. Microbiol.* 49:161–167.
7. El Gazzar, F. E., G. Rusul, and E. H. Marth. 1987. Growth and aflatoxins production by *Aspergillus parasiticus* NRRL 2999 in the presence of lactic acid and at different initial pH values. *J. Food Prot.* 50:940–944.
8. 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.
9. El-Gendy, S. M., and E. H. Marth. 1981. Growth and aflatoxin production by *Aspergillus parasiticus* in the presence of *Lactobacillus casei*. *J. Food Prot.* 44:211–212.
10. El-Nezami, H. S., and J. T. Ahokas. 1998. Lactic acid bacteria: an approach for detoxification of aflatoxins, p. 359–367. *In* S. Salminen and A. von Wright (ed.), Lactic acid bacteria. Marcel Dekker, New York.
11. Goldin, B. R. 1989. Lactic acid bacteria: implications for health. *Les laits fermentés*:95–104.
12. González, S. N., M. C. Apella, N. C. Romero, M. E. Nader de Macías, and G. Oliver. 1993. Inhibition of enteropathogens by *Lactobacilli* strains used in fermented milk. *J. Food Prot.* 56:773–776.
13. Gourama, H., and L. B. Bullerman. 1995. Antimycotic and antiaflatoxigenic effect of lactic acid bacteria: a review. *J. Food Prot.* 57: 1275–1280.
14. Gourama, H., and L. B. Bullerman. 1995. Inhibition of growth and aflatoxin production of *Aspergillus flavus* by *Lactobacillus* species. *J. Food Prot.* 58:1249–1256.
15. Gourama, H., and L. B. Bullerman. 1997. Anti-aflatoxigenic activity of *Lactobacillus casei pseudoplantarum*. *Int. J. Food Microbiol.* 34: 131–143.
16. Hocking, A. D. 1997. Toxigenic *Aspergillus* species, p. 393–405. *In* M. P. Doyle, L. R. Beuchat, and T. J. Montville (ed.), Food microbiology. ASM Press, Washington, D.C.
17. Hoerr, F. J. 2003. Mycotoxicoses, p. 1103–1132. *In* Y. M. Saif, H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. McDougald, and D. E. Swayne (ed.), Diseases of poultry. Iowa State University Press, Ames.
18. Horn, B. W., and J. W. Dorner. 1999. Regional difference in production of aflatoxin B_1 and cyclopiazonic acid by soil isolates of *Aspergillus flavus* along a transect within the United States. *Appl. Environ. Microbiol.* 65:1444–1449.
19. Karunaratne, A., E. Wezenberg, and L. B. Bullerman. 1990. Inhibition of mold growth and aflatoxin production by *Lactobacillus* spp. *J. Food Prot.* 53:230–236.
20. Kozakiewicz, Z., and D. Smith. 1994. Physiology of *Aspergillus,* p. 23–40. *In* J. E. Smith (ed.), *Aspergillus.* Plenum Press, New York.
21. Lacey, J. 1989. Pre- and post-harvest ecology of fungi causing spoilage of foods and other stored products. *Soc. Appl. Bacteriol. Symp. Ser.* 18:11S–25S.
22. Luchese, R. H., and W. F. Harrigan. 1990. Growth of, and aflatoxin production by *Aspergillus parasiticus* when in the presence of either *Lactococcus lactis* or lactic acid and at different initial pH values. *J. Appl. Bacteriol.* 69:512–519.
23. Luchese, R. H., J. F. P. Martins, and W. F. Harrigan. 1992. Aflatoxin production in a meat mix model system in the presence of *Pediococcus* and *Lactobacillus*. *J. Food Prot.* 55:583–587.
24. McKane, L., and J. Kandel. 1985. Microbiology essentials and applications. McGraw-Hill, New York.
25. Morata de Ambrosini, V. I., S. N. González, and G. Oliver. 1999. Study of adhesion of *Lactobacillus casei* CRL 431 to ileal intestinal cells of mice. *J. Food Prot.* 62:1430–1434.
26. Naidu, A. S., W. R. Bidlack, and R. A. Clemens. 1999. Probiotic spectra of lactic acid bacteria (LAB). *Crit. Rev. Food Sci. Nutr.* 38: 13–126.
27. Oliver, G., S. González, and R. Moreno. 1999. Fermented milk for biotherapy. *Interciencia* 24:177–181.
28. Ouwehand, A. C. 1998. Antimicrobial components from lactic acid bacteria, p. 139–159. *In* S. Salminen and A. von Wright (ed.), Lactic acid bacteria. Marcel Dekker, New York.
29. Perdigón, G., M. E. Nader de Macías, S. Alvarez, G. Oliver, and A. A. Pesce de Ruiz Holgado. 1990. Prevention of gastrointestinal infection using immunobiological methods with milk fermented with *Lactobacillus casei* and *Lactobacillus acidophilus*. *J. Dairy Res.* 57: 255–264.
30. Perdigón, G., E. Vintiñi, S. Alvarez, M. Medina, and M. Medici. 1999. Study of the possible mechanisms involved in the mucosal inmune system activation by lactic acid bacteria. *J. Dairy Sci.* 82: 1108–1144.
31. Piard, J. C., and M. Desmazeaud. 1992. Inhibiting factors produced by lactic acid bacteria. 2. Bacteriocins and other antibacterial substances. *Lait* 72:113–142.
32. Pitt, J. I., and A. Hocking. 1997. Fungi and food spoilage. Blackie Academic and Professional, London.
33. Preluski, D. B., B. A. Rotter, and R. G. Rotter. 1994. Toxicology of mycotoxins, p. 359–403. *In* J. D. Miller and H. L. Trenholm (ed.), Mycotoxins in grain. Eagan Press, St. Paul, Minn.
34. Raibaud, P., J. V. Galpin, R. Ducluzeau, G. Mocquot, and G. Oliver. 1973. Le genere *Lactobacillus* dans le tube digestif du rat. I.- Caractères des souches homofermentaires isolées de rats holo- et gnotoxéniques. *Ann. Microbiol.* 124 A:83–109.
35. Rossman, A. J., and B. L. Chance. 1998. Workshop statistics: discovery with data and Minitab. Springer-Verlag, New York.
36. Roy, U., V. K. Batish, S. Grover, and S. Neelakantan. 1997. Production of antifungal substance by *Lactococcus lactis* subsp. *lactis* CHD-28.3. *Int. J. Food Microbiol.* 32:27–34.
37. Salama, M. S., W. E. Sandine, and S. J. Giovannoni. 1995. A milk-based method for detecting antimicrobial substances produced by lactic acid bacteria. *J. Dairy Sci.* 78:1219–1223.
38. Smith, J. E., and K. Ross. 1991. The toxigenic *Aspergilli,* p. 101–118. *In* J. E. Smith and R. S. Henderson (ed.), Mycotoxins and animal foods. CRC Press, Boca Raton, Fla.
39. Xu, J., H. Wang, R. Ji, and X. Luo. 2003. Study on the effect of the growth and aflatoxin production by *Aspergillus flavus parasiticus* NRRL 2999 in the present of *Lactobacillus plantarum* ATCC 8014. *Wei Sheng Yan Jiu* 32:334–338.