Journal of Food Protection, Vol. 65, No. 8, 2002, Pages 1309–1316

Apparent Antifungal Activity of Several Lactic Acid Bacteria against *Penicillium discolor* Is Due to Acetic Acid in the Medium

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MS 01-319: Received 28 August 2001/Accepted 4 March 2002

ABSTRACT

Fifty-six dairy bacteria belonging to the genera *Lactococcus, Lactobacillus, Pediococcus, Propionibacterium, Streptococcus, Enterococcus, Leuconostoc,* and *Brevibacterium* were screened for antifungal activity against four species of fungi relevant to the cheese industry (*Penicillium discolor, Penicillium commune, Penicillium roqueforti,* and *Aspergillus vesicolor*). Most of the active strains belonged to the genus *Lactobacillus,* whereas *Penicillium discolor* was found to be the most sensitive of the four fungi investigated. Further studies on *P. discolor* showed antifungal activity only below pH 5. This effect of pH suggests that organic acids present in the culture could be involved in the detected activity. Determination of acid composition revealed lactic acid production for active dairy strains and the presence of acetic acid in active as well as inactive strains. It was demonstrated that the undissociated acetic acid originates from the bacterial growth medium. The synergistic effect of the acetic acid present and the lactic acid produced was likely the main factor responsible for the antifungal properties of the selected bacteria. These results could explain some discrepancies in reports of the antifungal properties of lactic acid bacteria, since the role of acetic acid has not been considered in previous studies.

Some species of molds are intentionally used for ripening purposes in cheese production. Nonetheless, many of these molds are considered undesirable contaminants of cheese during storage, even at refrigeration temperatures. Several studies on the distribution of fungal species on cheeses have shown that *Penicillium* is one of the predominant genera in the fungal mycoflora of cheese *(8, 24, 33).* Lund et al. *(21)* found that *Penicillium commune* was the fungus most frequently observed in semihard and semisoft cheeses collected from different European countries. Also, a high incidence of *Aspergillus versicolor* was found in Dutch cheese warehouses between 1975 and 1980, even though this species is not a frequent cheese contaminant *(28).*

The classical antifungal strategies to extend the shelf lives of food products are heat treatment to inactivate spores, the addition of weak acids as preservatives (mainly sorbic, benzoic, and propionic acids), and the application of antimicrobial agents such as natamycin *(5).* However, it is known that some fungi are able to adapt to the presence of sorbic acid *(6).* Also, *Penicillium discolor,* a fungus occurring in hard cheeses, has been found to be able to grow in the presence of natamycin, which can be a serious problem for the cheese industry *(13, 18).* The resistance of fungi to chemical preservatives and more severe legal limitations ensure an increasing demand for naturally preserved products.

Interactions between molds and different genera of bacteria have been extensively described. Several investigators have reported the production of antifungal compounds by *Bacillus* spp. *(26, 29), Pseudomonas (35),* and different genera of lactic acid bacteria *(3, 33).* The latter are of the greatest interest for industrial purposes, since lactic acid bacteria are food-grade microorganisms. These lactic acid bacteria have been shown to inhibit both the growth of molds *(2, 30, 33)* and the production of toxins *(2, 8, 14).* However, the results of these studies are contradictory. While the inhibition of mold growth in the presence of lactic acid bacteria has been reported by some authors *(12, 17, 36),* a stimulatory effect of several lactic acid bacteria on mold growth *(33, 36)* and toxin production *(39)* has also been described. In some of these studies, a loss of antifungal activity brought about by the addition of proteases indicates the proteinaceous nature of the antifungal compound *(14, 27, 34),* but the identity of these antifungal substances has not been fully investigated.

The purpose of this work was to study in more detail the antifungal properties of different dairy microorganisms, mainly lactic acid bacteria, in order to explain these contradictory reports.

MATERIALS AND METHODS

Microorganisms. *Penicillium discolor* NIZO F486, *Penicillium commune* NIZO F914, *Penicillium roqueforti* NIZO F487, and *Aspergillus versicolor* NIZO F491 were obtained from the Netherlands Institute for Dairy Research (NIZO) collection. The inoculum was prepared by growing the fungi on malt extract agar (Oxoid, Hampshire, England) at 25°C for 5 to 7 days until sporulation was observed. Spores were harvested by washing the agar surface with a sterile solution containing 15% (wt/vol) glycerol and 0.025% (wt/vol) Tween 20. Ten milliliters of this solution

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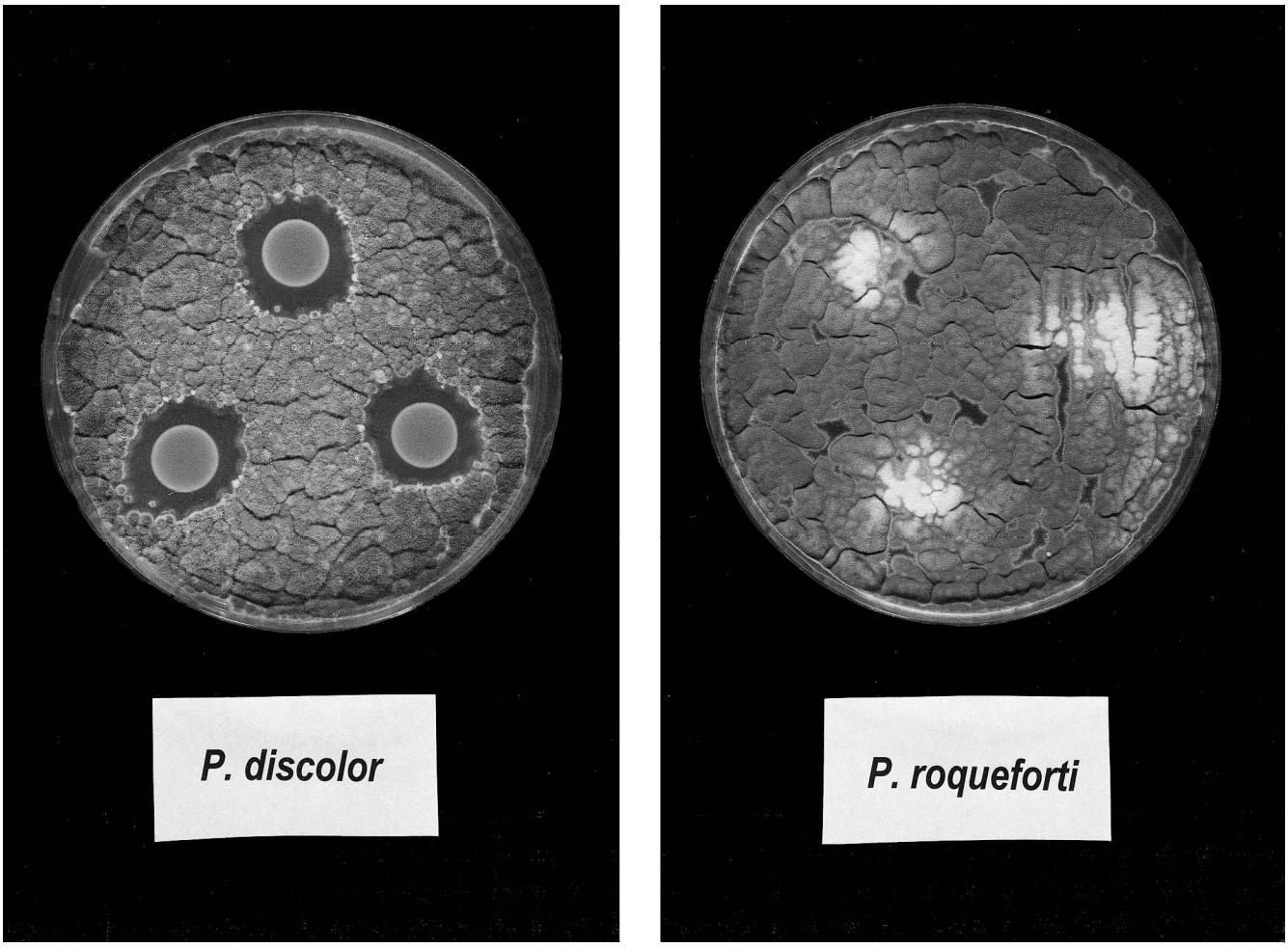


FIGURE 1. *(A) Inhibition (*I*) of* P. discolor *growth by* Lactococcus plantarum *NZ B249. (B) Inhibition of* P. roqueforti *spore formation by* Lactobacillus casei *subsp.* casei *NZ B889.*

was put on top of the agar, and the spores were loosened by gentle brushing with a sterile spatula. Spore counts in the resulting suspension were determined by microscopy with a hemocytometer chamber. Spore suspensions were finally adjusted to 10^7 spores of each fungus genus per ml and stored at −40°C.

Bacterial strains were obtained from the NIZO Culture Collection. Lactic acid bacteria were cultured in deMan Rogosa Sharpe (MRS) medium (Merck, Darmstadt, Germany) at 30°C and in MRS medium without acetate to check the effect of this organic acid (see "Discussion"). Strains of propionibacteria were grown in lactate broth (tryptone [5 g/liter], yeast extract [10 g/liter], sodium lactate [15 g/liter], and sodium acetate [8 g/liter]) and incubated in anaerobic jars at 30°C. *Streptococcus thermophilus* was grown in Tomaat Gist Vlees bouillon (a medium developed by NIZO food research; tryptone [10 g/liter], meat extract [3 g/liter], yeast extract [5 g/liter], tomato juice [40 ml/liter], glucose [20 g/liter], Tween 80 [1 ml/liter], and K_2HPO_4 [2 g/liter]) incubated at 42°C. Brevibacteria were cultivated aerobically in Difco nutrient broth (Difco Laboratories, Detroit, Mich.) at 25°C. *Debaromyces hansenii* was grown aerobically in malt extract broth (Oxoid) incubated at 25°C.

Frozen stock cultures of lactic acid bacteria were prepared in litmus milk with 15 g of yeast extract per liter inoculated with 1% fresh culture. Other genera were inoculated in the same proportion and maintained in the above-described media supplemented with 10% glycerol. These cultures were subsequently stored at −40°C. Active cultures were prepared by thawing a frozen vial, incubating it overnight under optimal conditions to enhance the growth, and subculturing it twice in fresh medium under optimal conditions before use.

Screening bacteria for antifungal activity. Three 10-μl drops from an active culture of each bacterial species tested were spotted onto agar plates and incubated until well-grown colonies could be observed (approximately 48 h). The plates were then overlaid with about 10 ml of GYA (glucose [20 g/liter], yeast extract [5 g/liter]), on which 0.1 ml of a mold spore suspension (10^5 spores per ml) was finally spread out. After incubation for up to 5 days at 25°C, the plates were examined for halo formation around the bacterial colonies. The growth of the fungi and especially the extent of sporulation were visually evaluated by comparing the color of the colonies with that of colonies on the control plates. Sporulated colonies differ significantly in color from unsporulated colonies (compare Fig. 1A and 1B). These experiments were performed in triplicate.

Preparation of culture supernatants. The selected bacteria were cultured in 100-ml Erlenmeyer flasks containing 50 ml of modified Rogosa broth (Merck) and incubated at 30°C. The inoculum contained 1% (vol/vol) fresh culture. After 72 h of incubation, the stationary-phase culture was centrifuged at 10,000 × *g* for 15 min. The supernatant was filtered through 0.22-μm sterile filters (Millipore SA, Madrid, Spain), and the cell-free extract obtained was used for determination of the antifungal activity.

Determination of the antifungal activity of bacterial supernatants and organic acids. A 10-ml sample of the solution to be tested (culture supernatant or organic acid solution) was mixed with the same volume of double-concentrated malt extract agar (Oxoid) and poured onto a plate. After drying, 10 μl of a mold spore suspension (10^6 spores per ml) was dropped on the agar, and the plates were incubated at 25°C for 5 days. The diameter of the fungal colony was measured and compared with that of a control, in which supernatant or the organic acid solution was replaced by medium or water, respectively. The antifungal activity was expressed in terms of inhibition of colony growth, defined as $I = 1 - (D_s/D_c)$, where D_s is the colony diameter of the sample and D_c is the colony diameter of the control. These experiments were performed in triplicate at pHs of 3, 4, 4.5, 5, and 6. Acidification of the supernatants was achieved by adding 1 M NaOH or HCl.

Ethanol and organic acid analyses. Lactic, acetic, and formic acids and ethanol were determined in culture supernatants by cation-exchange (Rezex Organic Acid column, Phenomenex, Aschaffenburg, Germany) high-performance liquid chromatography with 0.005 M H_2SO_4 as an effluent. The temperature and flow rate were set at 65°C and 0.6 ml/min, respectively. Detection was carried out with a differential refractometer (Waters 410, Waters, Etten-Leur, The Netherlands) at 40°C. Lactic acid (Fluka, Nulltarif, Germany), formic acid (Analar, Florida), acetic acid (Analar), propionic acid (Analar), and ethanol (Baker, London, UK) were used as standards.

The effect of organic acids. Different concentrations of lactic acid and acetic acid (Sigma, Barcelona, Spain) were prepared in 0.1 M biphtalate buffer (adjusted with 0.1 M NaOH or HCl) at pH values ranging from 3.5 to 6.0. This buffer system was chosen because it is not easily metabolized by microorganisms, and thus nutrients that could interfere with fungal growth during the bioassay were avoided. The inhibitory effects of these acids alone and in combination were tested in triplicate as described above.

Statistical analysis. A Student's t test ($\alpha = 0.05$) was used to test the significance of the differences between means of the dimensionless inhibition factor I.

RESULTS

Screening for antifungal activity. In a first screening, 56 dairy strains, predominantly lactic acid bacteria strains, were tested for antifungal activity against *P. discolor, P. commune, P. roqueforti,* and *A. versicolor.* As can be seen in Table 1, the majority of strains showing antifungal activity belonged to the genus *Lactobacillus,* especially *Lactobacillus casei* and *Lactobacillus plantarum.* Some *Lactococcus* and *Pediococcus* strains also showed antifungal activity. The inhibition of *P. discolor* by *Lactococcus plantarum* (NZ B249) and the inhibition of the sporulation of *P. roqueforti* by *Lactobacillus casei* subsp. *casei* (NZ B889) are illustrated in Figure 1. No antifungal activity was observed in propionibacteria, brevibacteria, or the yeast *D. hansenii.*

Both the mycelium growth and the sporulation of *A. versicolor* and *P. discolor* were inhibited by the active lactic acid bacteria. *P. roqueforti* was less sensitive, as only sporulation was inhibited. One bacterial strain of each genus with antifungal activity (Table 1) and one inactive strain (NZ B1; negative control) were selected for further studies. Because of the broad sensitivity observed for *P. discolor* and because *P. discolor* is a relevant cheese spoilage organism *(18),* this species was used as an indicator.

Effect of pH on antifungal activity. In preliminary experiments, it was observed that antifungal activity was always associated with the low pH of a culture. Therefore, the antifungal activity of the supernatants obtained from stationary-phase cultures (after 72 h of incubation) of the selected strains was tested at different pH values. The results are expressed as I. As can be seen in Figure 2, antifungal activity was observed only at pHs of <5. Moreover, the supernatant of the strain NZ B1 (negative control) became active against *P. discolor* when the pH was adjusted to ≤ 4.5.

The observed effect of the pH suggested that organic acids present in the supernatants could be involved in the detected antifungal activity. Therefore, the types of alcohols and organic acids produced after 9 and 72 h of incubation were determined (Table 2). The concentration of lactic and acetic acid increased during incubation. Furthermore, the dairy strains with observed antifungal activity produced more lactic acid than the negative control NZ B1.

The levels of lactic acid produced by the various strains ranged from 5.32 to 18.3 g/liter within 72 h of culturing (Table 2). Acetic acid was also found in all supernatants except for strains B261, B888, and B250 at almost the same concentration as that of the sodium acetate in MRS broth (3.57 g/liter). These three strains were probably more active. This finding indicated that most of the acetic acid originated from MRS broth.

Individual and combined effects of acetic and lactic acid at different pHs. In order to determine the extent to which the observed antifungal activity was due to the organic acids produced by the lactic acid bacteria and to the acetic acid in MRS, several concentrations of lactic acid (2 to 20 g/liter) and acetic acid (1 to 6 g/liter) were tested.

The effects of pH and acid concentration were determined at pHs of 3.5, 4, 4.5, 5, and 6. Additionally, the possibility of a synergistic effect of both factors was also tested by combining various lactic acid concentrations with 4 g of acetic acid per liter (the average concentration found in the cultures). The results are expressed as I. As shown in Figure 3, a combination of pH and acetic acid was responsible for the inhibition of the growth of *P. discolor.* Once the slight effect of the pH was eliminated, the lactic acid did not show any significant inhibitory effect, even at maximum concentration (Fig. 3).

A strong inhibitory effect of acetic acid on the growth of *P. discolor* was observed, and this effect was found to be extremely dependent on pH (Fig. 4). In fact, the 50% lethal dose (LD_{50}) for acetic acid (calculated as reported by Cabo et al. *(7)*) increased from 2.5 g/liter at pH 4 to 4.4 g/liter at pH 4.5. From Figures 3 and 4, it can be concluded that lactic acid and acetic acid act synergistically.

Antifungal activity in the absence of acetate. Although the results obtained clearly show a strong effect of

TABLE 1. *Antifungal activity of different bacterial strains*[a]

NIZO strain	Bacterium	Activity against: *P. discolor*	*P. commune*	*P. roqueforti*	*A. versicolor*
B692	*Lactobacillus acidophilus*	−	−	−	−
B229	*L. acidophilus*	−	−	−	−
B262	*Lactobacillus casei* subsp. *rhamnosus*	++	±, less spores	−, NSF (colony)	NT
B263	*L. casei* subsp. *rhamnosus*	++	±, less spores	−, NSF	NT
B264	*L. casei* subsp. *rhamnosus*	++	±, less spores	−, NSF	+++
B261	*L. casei* subsp. *casei*	+++	−, less spores	−, NSF	++
B889	*L. casei* subsp. *casei*	++	+, less spores	−, NSF	NT
B931	*L. casei* subsp. *casei*	++	±	−	+++
B282	*L. casei* subsp. *pseudoplantarum*	++	−, less spores	−, NSF	NT
B888	*L. casei* subsp. *pseudoplantarum*	+++	±, less spores	−, NSF	+++
B243	*L. casei* subsp. *tolerans*	++	±, less spores	−, NSF	++
B238	*L. casei* subsp. *tolerans*	++	−, less spores	−, NSF	++
B696	*Lactobacillus brevis*	+++	NT	−, NSF (colony)	+++
B193	*Lactobacillus delbrueki* subsp. *lactis*	−	NT	−	NT
B198	*L. delbrueki* subsp. *vulgaricus*	−	−	−	−
B294	*Lactobacillus fementum*	++	NT	−, NSF	NT
B307	*L. fermentum*	++	NT	−	NT
B887	*Lactobacillus plantarum*	+++	+, NSF	−, NSF	+++
3Kf1	*L. plantarum*	++	±	−, NSF	++
LWkf1	*L. plantarum*	++	±, less spores	+++, NSF (colony)	NT
B237	*L. plantarum*	++	±, NSF	−	+++
B635	*L. plantarum*	++	±, NSF	−	+++
B1	*Lactococcus lactis* subsp. *lactis*	−	NT	−	NT
B3	*L. lactis* subsp. *lactis*	−	NT	−	NT
B851	*L. lactis* subsp. *lactis*	−	−	−	−
B855	*L. lactis* subsp. *lactis*	−	−	−	−
B100	*Lactococcus plantarum*	−	−	−	NT
B250	*L. plantarum*	+++	++	−, NSF	+++
B253	*L. plantarum*	+++	NT	−, NSF (colony)	+++
B249	*L. plantarum*	+++	NT	−, NSF	+++
B252	*L. plantarum*	+	NT	−, NSF	NT
B314	*Pediococcus pentosaceus*	++	+	−, NSF (colony)	+++
B321	*P. pentosaceus*	+++	+	−, NSF	+++
B316	*Pediococcus* sp.	±	+	−, NSF	+
B363	*Propionibacterium freudenreichii freudenreichii*	−	NT	−	NT
B360	*P. freudenreichii shermanii*	±, NSF	NT	±	NT
B365	*P. freudenreichii shermanii*	±	NT	−, NSF (colony)	NT
B368	*P. freudenreichii freudenreichii*	−, NSF	−	−, NSF	−
B374	*P. freudenreichii freudenreichii*	−	NT	−, NSF (colony)	NT
B364	*Propionibacterium densenii*	±	NT	+	NT
B361	*Propionibacterium thoeni shermanii*	−, NSF (colony)	NT	−, NSF (colony)	NT
B380	*Propionibacterium* subsp.	±	NT	±	NT
B376	*Propionibacterium* subsp.	−, NSF (colony)	NT	−, NSF	NT
B362	*Propionibacterium acidipropionici*	±	NT	+	NT
B372	*P. freudenreichii shermanii*	±, NSF (colony)	NT	−, NSF (colony)	NT
B373	*P. freudenreichii shermanii*	−, NSF (colony)	NT	−, NSF (colony)	NT
B123	*Streptococcus termophillus*	−	−	−	−
B128	*S. termophillus*	−	−	−	−
B156	*Enterococcus durans*	−	NT	−	NT
B921	*Enterococcus faecium*	−	NT	−	NT
B161	*Leuconostoc mesenteroides*	++	+	−	++
B162	*Leuconostoc lactis*	−	±	−	−
NS 180	*Brevibacterium linens*	−	−	−	−
NS 195	*B. linens*	−	−	−	−
F937	*Debaromyces hansenii*	−	±	−	−

[a] −, no inhibition; ±, inhibition on the surface of the colony; +, irregular halo; ++, a small halo; +++, a very clear and large halo; NSF, no spore formation observed throughout the upper agar; NSF (colony), no spore formation in the upper agar surrounding a lactic acid bacterial colony; NT, not tested.

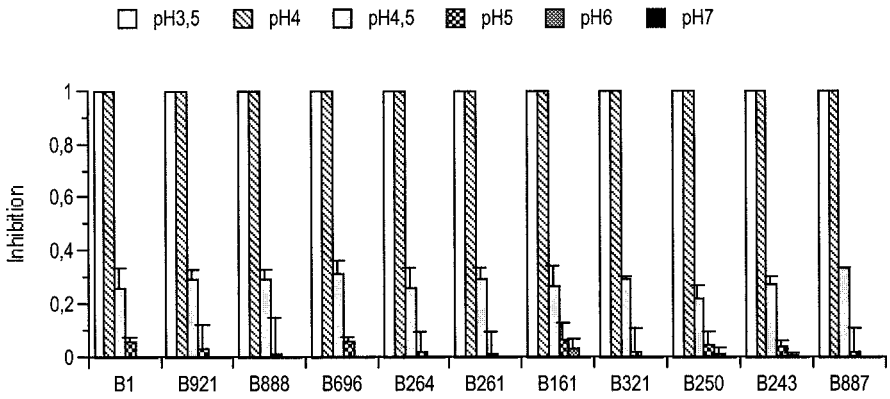


FIGURE 2. *Inhibition (I) of* P. discolor *growth by the supernatants of 72-h cultures of different lactic acid bacteria adjusted to pHs of 3, 4, 4.5, 5, 6, and 7.*

acetic acid, it is also possible that there is another compound in the culture that is partially responsible for the observed antifungal activity. This possibility was examined by comparing the antifungal activity of the supernatants of the selected bacteria in normal MRS with that of the same bacteria in MRS without sodium acetate. The antifungal activity levels (*I*) observed in the two media are shown in Figure 5. No differences were observed between growth levels in the two media. Strains B921 and B161 showed lower inhibition. Their metabolism was probably less active, because the lactic acid concentration after 72 h was lower for these strains than for the other strains (Table 2). Consequently, the final pHs of the supernatants were higher (4.43 and 4.45 in MRS and 4.17 and 4.11 in MRS without acetate for B921 and B1161, respectively, compared with 3 to 3.5 for the rest of the strains). This implies a lower concentration of undissociated acetic acid and thus lower inhibition.

Significant differences ($P < 0.01$) were found between the inhibition levels in MRS with and without sodium acetate. In fact, the antifungal activity almost disappeared in the absence of acetate, with the remaining activity being the result of both experimental errors and slight differences in the final pHs of the cultures. This finding indicates that there was no active compound present in the media besides the acetic acid.

TABLE 2. *Concentrations of lactic acid, acetic acid, and ethanol (g/liter) in cultures after 9 h of incubation*

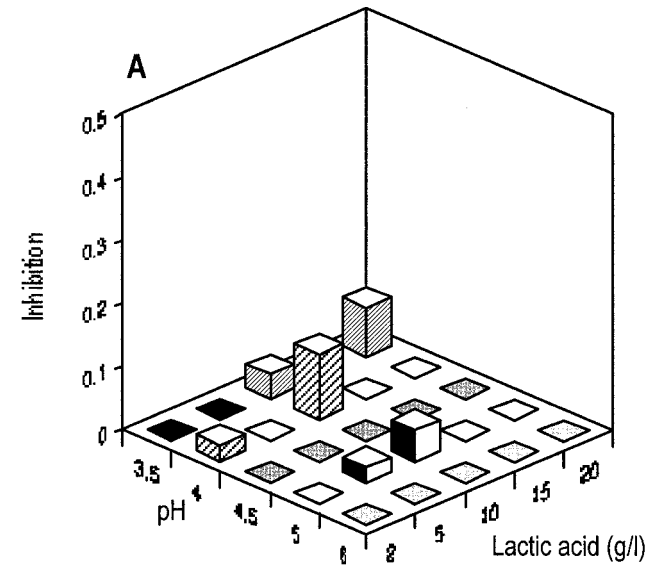
Strain	Lactic acid	Acetic acid	Ethanol
MRS	0.08	3.57	—
Lactobacillus casei subsp. *casei*	1.89	3.81	—
L. casei subsp. *pseudoplantarum*	4.09	3.66	—
Lactobacillus plantarum	4.25	3.32	—
L. casei subsp. *tolerans*	2.16	4.02	—
Lactococcus plantarum	2.81	3.94	—
Enterococcus faecium	1.16	3.94	—
Leuconostoc mesenteroides	0.86	4.01	—
Pediococcus pentosaceus	3.01	3.52	—
Lactobacillus brevis	0.60	4.04	0.2
L. casei subsp. *rhamnosus*	2.29	3.84	—
Lactococcus lactis subsp. *lactis* NZ B1[a]	0.19	3.90	—

[a] NZ B1 was used as a negative control.

DISCUSSION

Fifty-six dairy strains of bacteria were checked for antifungal activity, and then several of these strains were selected for further study. The results obtained indicate that acetic acid present in MRS medium was largely responsible for the observed antifungal activity of those strains. Thus, acidification due to lactic acid would appear to enhance the inhibitory effect of acetic acid. This finding would clearly explain the relationship found between the pH of a culture and antifungal activity: The higher the lactic acid production, the lower the final pH and therefore the higher the amount of acetic acid in undissociated form. This effect is particularly significant when the pH drops below the pK_a of acetic acid (4.75), since the percentage of undissociated acetic acid increases from 34.9 to 84.5% when the pH decreases from 5 to 4 *(16).* The combined effect of acetic acid and lactic acid was also cited by other authors to explain the antibacterial effect of four lactobacilli *(20).* Suzuki et al. *(33)* also pointed out that the antifungal activity observed in several bacterial cheese starters consisted mainly of lactic and acetic acids; this antifungal activity was lost when the pH was adjusted to 7. The inhibitory activity of acids may be due to either a specific effect on some metabolic activity or the acidification of the cytoplasm *(38).* The antimicrobial activity of the acids depends strongly on the pH, since they are active only in the undissociated form, that is, at $pH < pK_a$. In this form, their lipophilic condition permits them to penetrate across the membrane. At a higher intracellular pH, the acid dissociates to release protons and conjugate bases, which disrupts the membrane proton motive force *(1).*

The ineffectiveness of lactic acid in the inhibition of the growth of yeasts and molds has previously been reported *(37).* Not only did *Aspergillus parasiticus* grow in the presence of up to 20 g of lactic acid per liter, but the biosynthesis of aflatoxin was even stimulated at certain concentrations *(11).* Coallier-Ascah and Idziak *(8)* pointed out that the production of aflatoxin by *Aspergillus flavus* was not affected by lactic acid. Niku-Paavola et al. *(25)* found that lactic acid had no effect on the growth of *Fusarium avenaceum.* In contrast, Vandenberg *(34)* described a new antifungal compound produced by *Pediococcus,* consisting mainly of lactic acid and valine, which inhibited the growth of different fungal species. Also, Lavermicocca et al. *(19)*



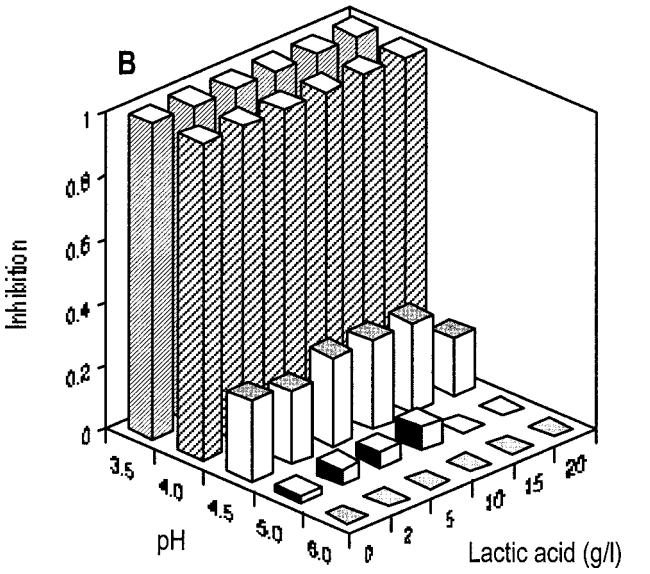


FIGURE 3. *Inhibition* (I) *of* P. discolor *by lactic acid (A) alone and (B) combined with 4 g of acetic acid per liter at different pH values.*

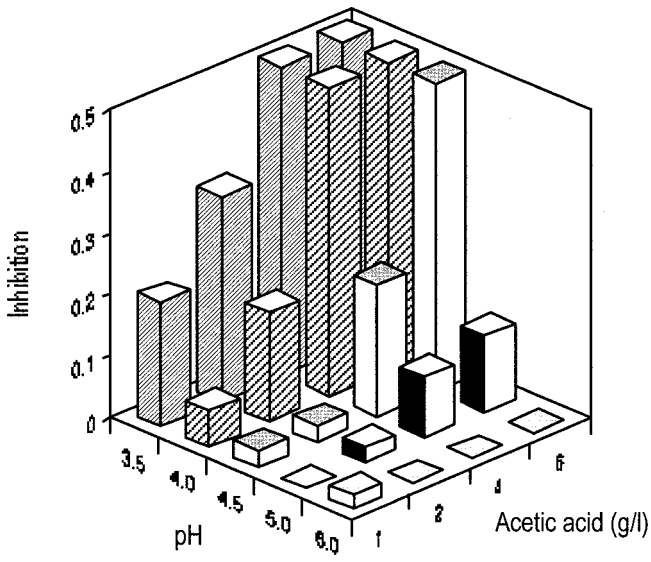


FIGURE 4. *Inhibition* (I) *of* P. discolor *by acetic acid at different pH values.*

have recently shown that phenyllactic and 4-hydroxy-phenyllactic were responsible for the antifungal activity of *Lactobacillus plantarum* 21B. The effect of pH is a matter of controversy between the different reports, but it is generally accepted that a low pH favors aflatoxin production and hinders mold growth *(15).*

Acetic acid is a generally recognized food preservative *(1),* since it inhibits both bacteria and molds *(1, 22, 32, 38).* Recently, Stratford *(31)* found acetic acid to be more toxic than fumaric, citric, malic, tartaric, lactic, or succinic acid against *Saccharomyces cerevisiae.* Driehuis et al. *(10)* showed that maize silage was more stable when inoculated with *Lactobacillus buchneri,* as a result of the production of acetic and propionic acid. Also, it was demonstrated that acetic and caproic acids were responsible for the inhibitory activity of *Lactobacillus sanfrancisco* CB1 against *Fusarium graminearum (9).* Moon *(23)* found that acetic acid decreased the growth rate of *Saccharomyces uvarum, Geotrichum candidum, Endomycopsis burtonii,* and *Hansenula canadensis* and that lactate and acetate acted synergistically against the latter two strains. However, whereas the lowest LD_{50} value observed for acetic acid in that study was 9 g/liter (against *Saccharomyces*), an LD_{50} value of 4.38 g of acetic acid per liter against *P. discolor* was found in the present study, which indicates a stronger sensitivity of this species to acetic acid.

Although much effort has been devoted to the study of the antifungal potential of lactic acid bacteria in recent years, no attention has been paid to the effect of the acetic acid content of MRS broth or Elliker broth. Karunaratne et al. *(17)* stated that the effects of several species of lactobacilli against *A. flavus* were greatly influenced by the substrate: while the growth of *A. flavus* was totally inhibited at pH 4 in MRS–corn meal media, it could tolerate the same pH in rice and corn. It was recently observed *(30)* that the effectiveness of different lactobacilli against *Fusarium* sp. increased when the organisms were cultured in MRS instead of Elliker broth (containing 3.57 and 1.5 g of acetic acid per liter, respectively). Additionally, whereas whole cells of lactobacilli inhibited the growth of *Fusarium* in MRS, the activity of their supernatants adjusted to pH 6 could be observed for only three of five strains after 1 week of incubation. Considering that lactic acid bacteria reach the stationary phase after only 24 h of incubation, the latter result could also be related to the release of the intracellular components through cellular lysis *(15).*

The present study has shown that the dairy microorganisms tested have no significant antifungal activity on *P. discolor, P. commune,* and *A. versicolor.* The acetic acid content of MRS largely accounted for the inhibition observed. This medium is commonly used for the growth of lactic acid bacteria, so acetic acid could also be at least partially responsible for the antifungal activity observed in

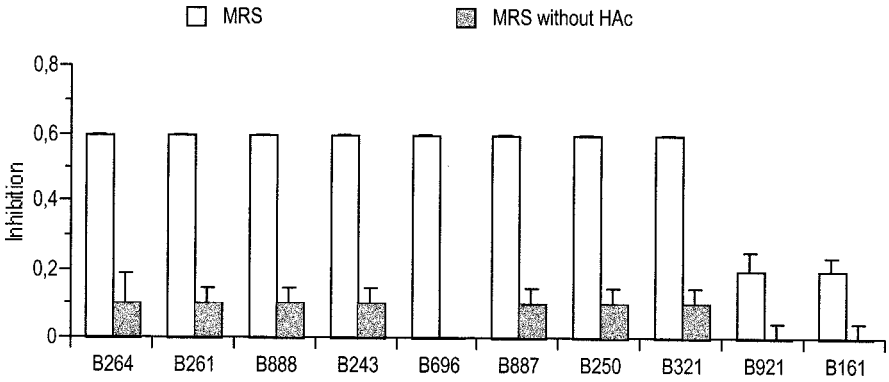


FIGURE 5. *Inhibition (I) of* P. discolor *by the selected lactic acid bacteria cultured for 72 h in MRS broth with (gray bars) and without (white bars) sodium acetate as an ingredient.*

many other studies. The presence of acetic acid in the medium, either as an ingredient or as a metabolite, should be considered in subsequent studies. These results also indicate a potential application of acetic acid to the prevention of the outgrowth of *P. discolor* in cheeses. The low LD_{50} of acetic acid for this species suggests that it should be possible to apply this acid as a preservative without producing off-flavors.

ACKNOWLEDGMENTS

The present work was supported by Caixa Galicia. We thank Roelie Holleman for the chromatographic analyses.

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