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Antibacterial Activity of *Lactobacillus sake* Isolated from Meat

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A total of 221 strains of *Lactobacillus* isolated from meat and meat products were screened for antagonistic activities under conditions that eliminated the effects of organic acids and hydrogen peroxide. Nineteen strains of *Lactobacillus sake*, three strains of *Lactobacillus plantarum*, and one strain of *Lactobacillus curvatus* were shown to inhibit the growth of some other lactobacilli in an agar spot test; and cell-free supernatants from 6 of the 19 strains of *L. sake* exhibited inhibitory activity against indicator organisms. Comparison of the antimicrobial spectra of the supernatants suggested that the inhibitory compounds were not identical. One of the six strains, *L. sake* Lb 706, was chosen for further study. The compound excreted by *L. sake* Lb 706 was active against various lactic acid bacteria and *Listeria monocytogenes*. Its proteinaceous nature, narrow inhibitory spectrum, and bactericidal mode of action indicated that this substance is a bacteriocin, which we designated sakacin A. Curing experiments with two bacteriocin-producing strains of *L. sake* resulted in mutants that lacked both bacteriocin activity and immunity to the bacteriocin. Plasmid profile analysis of *L. sake* Lb 706 and two bacteriocin-negative variants of this strain indicated that a plasmid of about 18 megadaltons may be involved in the formation of bacteriocin and immunity to this antibacterial compound. In mixed culture, the bacteriocin-sensitive organisms were killed after the bacteriocin-producing strain reached maximal cell density, whereas there was no decrease in cell number in the presence of the bacteriocin-negative variant.

In the meat industry, lactic acid bacteria are widely used as starter cultures for sausage fermentation (9, 12). They contribute to flavor development as well as to the preservation of fermented sausages. For the production of fermented sausage, naturally contaminated raw material is used. Therefore, an important role of the lactic acid bacteria is to inhibit the competing natural flora, which includes spoilage bacteria and, occasionally, pathogens such as *Staphylococcus aureus* and *Listeria monocytogenes*. In most cases, the formation of lactic and acetic acids from carbohydrates and the resulting pH decrease, respectively, are responsible for the antagonistic effect. However, in many foods, higher levels of acids are undesirable, and there are several meat products—for instance, fresh *mettwurst*—that contain only little, if any, added sugar. For these products, the inhibition of the undesired organisms must be achieved at least partially by means other than acidification. Lactic acid bacteria may inhibit other bacteria by the formation of antimicrobial compounds. There are several reports on the production of bacteriocins or bacteriocinlike substances by lactobacilli such as lactocin 27 (21), lactacin B (1), helveticin J (10), plantacin B (22), and plantaricin A (M. A. Daeschel, M. C. McKenney, and L. C. McDonald, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, P13, p. 277). Daeschel and colleagues (3, 4) have suggested the use of bacteriocin-producing lactic acid bacteria as starter cultures in vegetable fermentations.

In this study, 221 strains of *Lactobacillus sake*, *Lactobacillus curvatus*, and *Lactobacillus plantarum*, with potential use as starter cultures in meat products, were screened for antagonistic activities. Six strains of *L. sake* were found to excrete antimicrobial compounds into the broth.

MATERIALS AND METHODS

Strains and culture conditions. The 221 strains of *Lactobacillus* included in this study were isolated from fresh meat and different meat products and were identified in a previous study (17). The lactic acid bacteria were propagated and maintained in MRS medium (5), unless stated otherwise. The bacterial strains used as indicator organisms are given in Tables 1 and 2. The non-lactic acid bacteria were cultivated in standard I medium (E. Merck AG, Darmstadt, Federal Republic of Germany). For the detection of antagonistic activity, MRS containing only 0.2% glucose (MRS-0.2) was used. For differentiation between maltose-positive and maltose-negative strains of *L. sake*, a modified MRS agar supplemented with Chinablue (375 mg/liter) was used. In this Chinablue-MRS agar, K_2HPO_4 was substituted with a 5-ml/liter phosphate buffer (1 M, pH 6.8) and glucose was substituted with maltose (1%), which was added as a filter-sterilized solution.

Detection of antagonistic activity. For detection of antagonistic activity, an agar spot test and a well diffusion assay were used. The agar spot test was a modification of that described by Fleming et al. (6). Overnight cultures of the strains to be tested for production of an antimicrobial compound were spotted onto the surface of agar plates (MRS-0.2 with 1.2% agar) and incubated for 20 to 24 h at 25°C to allow colonies to develop. Anaerobic conditions were used to minimize the formation of hydrogen peroxide and acetic acid. Approximately 5×10^7 cells of the strains to be tested for sensitivity (indicator strains) were inoculated into 7 ml of soft MRS-0.2 agar (containing 0.7% agar) and poured over the plate on which the producer was grown. After anaerobic incubation for 24 h at 25°C, the plates were checked for inhibition zones. Inhibition was scored positive if the width of the clear zone around the colonies of the producer strain was 0.5 mm or larger. For the well diffusion assay, MRS-0.2 agar plates were overlaid with 7 ml of soft MRS-0.2 agar inoculated with 0.3 ml of an overnight culture of the indicator strain. Wells of 3 mm in diameter were cut into these agar plates, and 0.03 ml of the culture supernatant of the potential producer strain was placed into each well. The plates, with their wells unsealed, were incubated anaerobically for 24 h at 25°C and were subsequently examined for

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zones of inhibition. For a semiquantitative assay, twofold serial dilutions of the supernatant were used, as described by Barefoot and Klaenhammer (1).

Preparation of culture supernatants. The bacteriocin-producing strain was grown in MRS broth for 24 h at 25°C. A cell-free solution was obtained by centrifuging the culture, followed by filtration of the supernatant through a 0.2-μm-pore-size cellulose acetate filter. The supernatant was adjusted to pH 6.5 or dialyzed for 24 h against MRS broth without glucose (pH 6.5) at 4°C. Inhibitory activity from the hydrogen peroxide was eliminated by the addition of catalase (5 mg/ml). For some experiments, the culture supernatant was concentrated 10-fold with a rotary evaporator.

Sensitivity to heat and proteolytic enzymes. Concentrated cell-free culture supernatants were heated at 100°C for 20 min, and the remaining activity was assayed (1). To test the sensitivity to proteases, concentrated culture supernatants were treated with trypsin or pepsin, each at a final concentration of 0.5 mg/ml. Samples with and without proteases were filter sterilized and incubated at 37°C for 12 h. Residual activity was determined by the well diffusion assay.

Mode of action. To study the effect of the antibacterial compound on sensitive cells, 2 ml of the neutralized and 10-fold-concentrated culture supernatant of *L. sake* Lb 706 was added to 10 ml of a culture of *L. sake* Lb 790 (3×10^5/ml) in MRS broth. At appropriate intervals, the number of viable cells was determined. As a control, the effect of 2 ml of the neutralized and 10-fold-concentrated supernatant of a bacteriocin-negative variant of *L. sake* Lb 706 was examined.

Plasmid curing experiments. Plasmid curing was accomplished by growing cells at an elevated temperature (37°C) and, after treatment with acriflavine (20 μg/ml), with three consecutive transfers every 24 h. Bacteriocin-negative (Bac^-) mutants were detected by their inability to form inhibition zones in the agar spot test.

Plasmid isolation. Cells were grown to the late exponential phase in MRS broth containing 0.5% glucose, and plasmid DNA was extracted by the method of LeBlanc and Lee (11). Electrophoresis was conducted on 0.7% agarose gels in Tris acetate buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA [pH 8.0]), using a constant voltage of 100 V for 1 h. Purified supercoiled ladder DNA (Bethesda Research Laboratories, Gaithersburg, Md.), and the plasmids extracted from *Lactococcus lactis* subsp. *cremoris* AC-1 (7) were used as standards for molecular weight approximations.

Growth of an indicator strain in the presence of a bacteriocin producer. To demonstrate the effect of a bacteriocin-producing strain on a growing culture of an indicator strain, *L. sake* Lb 790 was grown together with *L. sake* Lb 706 and a bacteriocin-negative variant of this strain (Lb 706-B), respectively, in MRS broth at 15°C. As a control, the three strains were also grown separately. At appropriate intervals, the cell density was determined by plating the samples onto agar plates. Chinablue-MRS agar containing maltose was used to distinguish colonies of *L. sake* Lb 706 and Lb 706-B (maltose-negative) from those of Lb 790. Cell-free supernatants of the mixed cultures were also prepared to determine the inhibitory activity by the well diffusion assay.

RESULTS

Screening of lactobacilli from meats for production of antibacterial compounds. An agar spot test was used to screen 221 *Lactobacillus* isolates from meat and meat products for antagonistic activity. The indicator organisms used are listed in Table 1. To restrict the extent of acid production, low-glucose MRS medium (MRS-0.2) was used. A total of 19 of 142 strains of *L. sake* and 3 of 4 strains of *L. plantarum* were found to produce inhibition zones against some other lactobacilli, whereas of 75 strains of *L. curvatus* examined, only 1 strain was inhibitory against some indicator organisms. Sensitivity to the antibacterial activity of the 19 strains of *L. sake* was variable within the same species. For instance, *L. sake* Lb 699 was inhibited by *L. sake* Lb 796, whereas strains Lb 688 and Lb 693 were unaffected (Table 1).

Culture filtrates of the 19 strains of *L. sake* that were positive in the agar spot test (Table 1) were then checked by a well diffusion assay. The culture supernatants of only six strains of *L. sake* (Lb 706, Lb 796, Lb 972, Lb 975, Lb 977, and Lb 979) produced inhibition zones on agar. When the pH of the supernatants was readjusted to 6.5 and catalase was added, there was no reduction of inhibition (Fig. 1). This indicates that the inhibitory activity is not due to hydrogen peroxide or acid production but to an antimicrobial compound excreted into the broth. The inhibitory spectra of these six strains differed significantly (Table 1), suggesting that the antibacterial substances produced may be heterogenous. This was confirmed by checking the sensitivity of a derivative of *L. sake* Lb 706 (obtained by curing Lb 706 with acriflavine as described below) to the substances of *L. sake* Lb 706 and Lb 796. This variant (Lb 706-B) was Bac^- and was fully sensitive to the compound produced by the parent strain but was not inhibited by strain Lb 796. Moreover, the producer strain Lb 796 was sensitive to the compound excreted by *L. sake* Lb 706.

Protease and heat sensitivity of the antibacterial compound produced by *L. sake* Lb 706. The activity of the antibacterial compound produced by *L. sake* Lb 706 was destroyed by protease treatment (trypsin or pepsin) but was resistant to heat (Fig. 1). There was no reduction of the antibacterial titer after heating for 20 min at 100°C. These results demonstrate that the antibacterial compound produced by *L. sake* Lb 706 is a heat-stable protein or peptide. It was stable during frozen storage (data not shown).

Bactericidal action. The addition of the concentrated neutralized culture supernatant of *L. sake* Lb 706 to a culture of *L. sake* Lb 790 in MRS broth resulted in a rapid inactivation of sensitive cells. The number of viable cells per milliliter declined from 3×10^5 to 130 after 3 h of incubation (Fig. 2) and to below 30 cells per ml after 5 h; cells did not regrow within 24 h. In contrast, the culture of *L. sake* Lb 790 was unaffected by the addition of a concentrated supernatant of the bacteriocin-negative variant of *L. sake* Lb 706 (Lb 706-B). These results indicate a bactericidal mode of action of the antibacterial compound.

Spectrum of inhibitory activity. The antagonistic effect of the neutralized culture supernatant of *L. sake* Lb 706 on various gram-positive and gram-negative bacteria was tested by the well diffusion assay (Table 2). The supernatant was active against strains of *L. sake*, *L. curvatus*, *Carnobacterium piscicola*, *Leuconostoc paramesenteroides*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Listeria monocytogenes*. Weak activity against *Lactobacillus alimentarius* was also detected. The other gram-positive and gram-negative organisms tested were not inhibited. These included the food-borne pathogens *Staphylococcus aureus* and *Salmonella typhimurium*.

After Tagg et al. (20), compounds of a protein nature with a bactericidal mode of action restricted to bacteria closely related to the producers are called bacteriocins. In consequence, the antibacterial compound produced by *L. sake* Lb 706 is bacteriocinlike and is designated sakacin A.

TABLE 1. Inhibition of various lactobacilli and *Listeria monocytogenes* by strains of *L. sake*, *L. curvatus*, and *L. plantarum* on agar plates[a]

Producer strain	Inhibition of indicator strains[b]								
	L. sake				*L. curvatus* Lb 730	*L. plantarum* Lb 828	*L. divergens* Lb 836	*Listeria monocytogenes*	
	Lb 688	Lb 693	Lb 699	Lb 790				8732	17a
L. sake									
Lb 225	−	−	(+)	+	(+)	−	+	(+)	(+)
Lb 584	−	−	−	+	(+)	−	+	−	−
Lb 686	+	+	+	+	(+)	+	−	−	−
Lb 696	−	(+)	(+)	+	(+)	+	−	−	−
Lb 699	−	−	−	−	+	−	−	−	−
Lb 706[c]	+	+	+	+	(+)	−	+	+	+
Lb 713	+	(+)	−	+	+	+	−	−	−
Lb 723	(+)	−	−	−	−	+	−	−	−
Lb 796[c]	−	−	+	+	+	−	+	+	+
Lb 797	(+)	(+)	−	+	(+)	−	−	−	−
Lb 808	−	+	−	−	+	−	−	−	−
Lb 829	−	+	+	+	+	−	+	−	−
Lb 839	−	(+)	(+)	+	(+)	+	−	−	−
Lb 908	−	−	(+)	−	−	−	+	(+)	(+)
Lb 970	−	−	−	+	−	−	+	(+)	(+)
Lb 972[c]	−	−	+	+	+	−	−	−	−
Lb 975[c]	+	+	+	+	−	−	+	+	+
Lb 977[c]	+	+	+	+	(+)	−	+	+	+
Lb 979[c]	−	+	+	+	(+)	−	+	+	+
L. plantarum									
Lb 75	+	+	+	+	+	−	+	+	+
Lb 592	+	+	+	+	+	−	+	(+)	+
Lb 598	+	+	+	(+)	+	−	+	−	−
L. curvatus Lb 830	−	+	−	(+)	−	−	0	+	+

[a] The conditions used for the agar spot test are described in the text.
[b] Symbols: +, Large inhibition zone (>1.0 mm); (+), small inhibition zone (0.5 to 1.0 mm); −, no inhibition zone; 0, not determined.
[c] Strains producing an inhibitory substance in the culture supernatant.

Production of bacteriocin in broth. Cultures of *L. sake* Lb 706 were grown at 7, 15, and 25°C; and samples were taken at different time intervals. Their supernatants were examined for inhibitory activity. Furthermore, the production of bacteriocin in MRS and low-glucose MRS (MRS-0.2) medium was compared. The culture supernatant was dialyzed to eliminate the effect of higher amounts of acid resulting from the higher glucose content of the MRS medium. No activity was detected during the first 8 h of incubation. However, significant activity was detected after 23 h at 25°C and after 23 or 47 h at 15°C, when the bacteria were in the mid- or late-logarithmic growth phase. A loss of activity of the Lb 706 supernatant was observed after 47 h at 25°C.

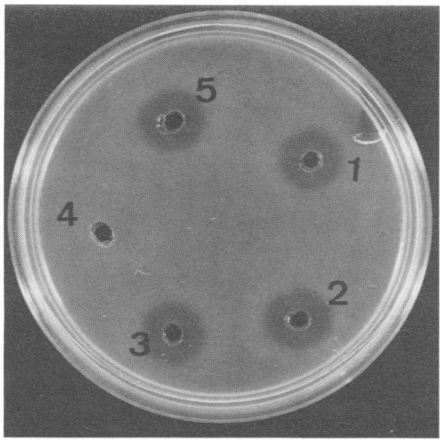

FIG. 1. Inhibition of *L. sake* Lb 790 by *L. sake* Lb 706 culture supernatant by the agar well diffusion assay. Wells contained cell-free, 10-fold-concentrated supernatants which were treated by sterile filtration only (well 1), adjusting the pH from 4.5 to 6.5 (well 2), boiling for 20 min (well 3), adding trypsin (0.5 mg/ml) (well 4), or adding catalase (5 mg/ml) (well 5).

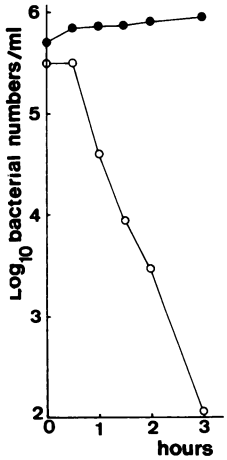

FIG. 2. Bactericidal effect of the bacteriocin produced by *L. sake* Lb 706 on a sensitive strain (*L. sake* Lb 790). Symbols: ○, Cell-free concentrated supernatant from *L. sake* Lb 706 added; ●, cell-free concentrated supernatant from *L. sake* Lb 706-B added.

TABLE 2. Inhibitory spectrum of the bacteriocin produced by *L. sake* Lb 706

Indicator species	Strain no.	Origin[a]	Inhibition by the culture supernatant from *L. sake* Lb 706[b]
Gram-positive bacteria			
Bacillus cereus	9634	ATCC	−
Bacillus licheniformis	B 26	Our strain collection	−
Bacillus subtilis	6633	ATCC	−
Bronchothrix thermosphacta	Mb 1	Our strain collection	−
Carnobacterium piscicola	Lb 914	Our strain collection	+
Enterococcus faecalis	Lb 785	Our strain collection	+
Enterococcus faecium	Lb 912	Our strain collection	+
Lactobacillus alimentarius	20249^T	DSM	(+)
Lactobacillus brevis	Lb 992	Our strain collection	−
Lactobacillus casei	Lb 803	Our strain collection	−
Lactobacillus curvatus	20010	DSM	+
Lactobacillus halotolerans	20190^T	DSM	−
Lactobacillus plantarum	20174^T	DSM	−
Lactobacillus sake	15521^T	ATCC	+
Lactobacillus sake	Lb 680	Commercial meat starter culture	+
Lactobacillus viridescens	20410^T	DSM	−
Lactococcus lactis	Lb 787	Commercial meat starter culture	−
Leuconostoc lactis	20192	DSM	−
Leuconostoc paramesenteroides	20288^T	DSM	+
Listeria monocytogenes	17 a	Our strain collection	+
Pediococcus acidilactici	Lb 628	Commercial meat starter culture	−
Pediococcus pentosaceus	Lb 987	Our strain collection	−
Staphylococcus aureus	14458	ATCC	−
Staphylococcus carnosus	St 38	Our strain collection	−
Gram-negative bacteria			
Pseudomonas aeruginosa	Ps 3	Our strain collection	−
Pseudomonas fluorescens	13525^T	ATCC	−
Citrobacter freundii	Ci 27	Our strain collection	−
Proteus vulgaris	Pr 14	Our strain collection	−
Salmonella derby	S 410	Our strain collection	−
Salmonella typhimurium	S 414	Our strain collection	−
Serratia liquefaciens	En 72	Our strain collection	−

[a] Abbreviations: ATCC, American Type Culture Collection (Rockville, Md.); DSM, Deutsche Sammlung von Mikroorganismen (Braunschweig, Federal Republic of Germany).

[b] Symbols: −, No inhibition zone; (+), small inhibition zone (0.5 to 1.0 mm); +, large inhibition zone (>1.0 mm).

Supernatants resulting from cultures grown in MRS broth generally produced larger inhibition zones than did those resulting from cultures grown in low-glucose medium. For these reasons, we routinely used cultures grown for 24 h in MRS broth at 25°C for the preparation of culture supernatants.

Plasmid curing experiments. *L. sake* Lb 706, Lb 796, and Lb 979 were screened for the occurrence of Bac$^-$ variants following repetitive transfers at 37°C and in MRS broth containing acriflavine (20 μg/ml). We failed to isolate Bac$^-$ variants from cultures grown at a temperature of 37°C, which is above the growth optimum of the psychrotrophic strains of *L. sake*. All 6,579 colonies examined were still able to produce inhibition zones. However, after three consecutive transfers (every 24 h) in the presence of acriflavine, 11 and 8 Bac$^-$ variants were detected among 256 and 356 bacteriocin-producing colonies of strains Lb 706 and Lb 796, respectively. These Bac$^-$ variants did not significantly differ from the bacteriocin-producing parent strains in rates of growth or acid formation or in their sugar fermentation pattern. They were sensitive to the bacteriocin produced by the parent strain, which indicates that they also lost their immunity to the bacteriocin. Two representative Bac$^-$ variants derived from Lb 706 (named Lb 706-B and Lb 706-M) were chosen for plasmid isolation. Figure 3 shows that the parent strain Lb 706 contained a plasmid of about 18 megadaltons which was absent in both Bac$^-$ variants.

Inhibition of *L. sake* Lb 790 by *L. sake* Lb 706 in MRS broth. The inhibition of a growing culture of the indicator strain Lb 790 by adding a bacteriocin-producing strain (Lb 706) in MRS broth was examined and compared with the effect of a Bac$^-$ variant of Lb 706 (Lb 706-B). During the first 15 h at 15°C, Lb 706 and Lb 706-B did not affect the growth rate of Lb 790 (Fig. 4). After 23 h, however, the cell density of Lb 790 grown with Lb 706 was somewhat lower than that of Lb 790 grown with Lb 706-B. After 26 h, when *L. sake* Lb 706 had reached a cell density of about 1×10^8/ml, there was a rapid decline from 2×10^6 to less than 1×10^4 viable cells of Lb 790 and the cell number remained below 1×10^4 during the whole experiment, whereas in the presence of the Bac$^-$ variant Lb 706-B, the cell density of Lb 790 continued to increase. More than 99% of the indicator cells were killed by the bacteriocin producer, whereas in the presence of the Bac$^-$ variant, Lb 790 reached a cell density of more than 10^8/ml. This shows that the decrease in cell number is caused by the antibacterial compound produced by *L. sake* Lb 706. It was also confirmed that in the course of the experiment there was no difference in the growth rates of Lb 706 and Lb 706-B. When the neutralized supernatants of the cultures containing Lb 706 were checked, a small zone of inhibition was first observed after 26 h at 15°C; and larger zones of inhibition were detected after 35, 47, and 64 h (Fig. 5). The inhibitory activity was unaffected by treatment with cata-

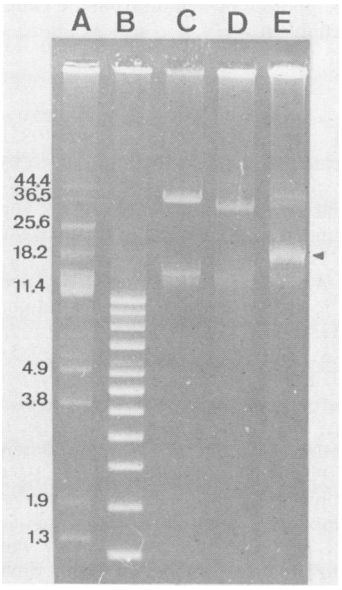

FIG. 3. Agarose gel electrophoresis of plasmid DNA from *L. sake* Lb 706 and derivative strains. Electrophoresis of DNA was conducted in 0.7% agarose at 100 V for 1 h. Lanes: A, *Lactococcus lactis* subsp. *cremoris* AC-1 (9) reference plasmids; B, supercoiled ladder DNA (Bethesda Research Laboratories) reference plasmids; C, *L. sake* Lb 706-B (Bac^-); D, *L. sake* Lb 706-M (Bac^-); E, *L. sake* Lb 706 (Bac^+). Numbers on the left indicate the molecular mass of standard plasmids (in megadaltons). The arrowhead designates the position of the plasmid that was probably involved in bacteriocin production.

lase. The supernatants of the control cultures containing Lb 706-B did not show any inhibitory activity.

DISCUSSION

Lactic acid bacteria originally isolated from meat and meat products are probably the best candidates for improving the microbiological safety of these foods, because they are well adapted to the conditions in meats and should therefore be more competitive than lactic acid bacteria from other sources. Therefore, we looked for antagonistic activities of 221 isolates from fresh meat, fermented sausages, and other meat products. A total of 19 of 142 strains of *L. sake*, 3 of 4 strains of *L. plantarum*, but only 1 of 75 strains of *L. curvatus* exhibited antagonistic effects on solid agar medium under conditions that reduced the effect of organic acids and hydrogen peroxide. However, only six strains of *L. sake* were shown to excrete an antibacterial compound into the liquid medium under test conditions. Similar results were obtained by Geis et al. (7), who tested 93 strains of *Streptococcus lactis* subsp. *cremoris* (*Lactococcus lactis* subsp. *cremoris*). They found that 36 strains exhibited antagonistic effects on agar, but only one of them produced an antibacterial substance in the liquid medium.

Different strains of *L. sake* appear to produce slightly different antibacterial compounds. We chose the compound produced by *L. sake* Lb 706 (designated sakacin A) for detailed characterization. The bactericidal mode of action and the proteinaceous nature of this substance are typical characteristics of a bacteriocin (20). As reported for the *L. acidophilus* bacteriocins (1, 14) and many other bacteriocins of gram-positive bacteria (20), sakacin A is heat stable. Like other bacteriocins from lactic acid bacteria, it was not active against gram-negative organisms but inhibited strains of some species closely related to the producer. With the exception of *Listeria monocytogenes*, the inhibitory activity of sakacin A was restricted to lactic acid bacteria. Most of the bacteriocin-sensitive strains belonged to the genus *Lactobacillus*. The sensitivity was strain specific rather than species specific: Not all strains of *L. sake* were sensitive to the agent. Similar results were reported for the bacteriocins produced by *Lactobacillus acidophilus* (1), *Lactobacillus helveticus* (10), and *L. plantarum* (M. A. Daeschel, M. C. McKenney, and L. C. McDonald, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, P13, p. 277). The taxonomic position of the genus *Listeria* is not quite clear, but there are indications that this genus is closely associated to the genus *Lactobacillus* (13, 16, 23). Therefore, the sensitivity of *Listeria* species to a bacteriocin produced by a *Lactobacillus* species is not surprising. Recently, Daeschel et al. (M. A. Daeschel, L. J. Harris, M. E. Stiles, and T. R. Klaenhammer, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, P43, p. 281) screened many bacteriocin-producing lactic acid bacteria for inhibition of *Listeria* species and found that some of them were able to produce an antimicrobial substance that was active against *Listeria monocytogenes*.

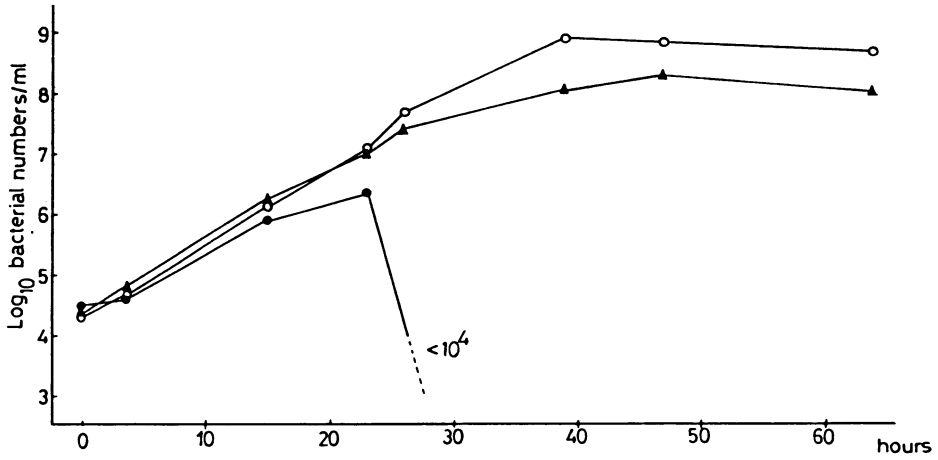

FIG. 4. Growth of *L. sake* Lb 790 in the presence of *L. sake* Lb 706 and Lb 706-B (Bac^-) in MRS broth at 15°C. Symbols: ○, Lb 790; ●, Lb 790 in a mixed culture with Lb 706; ▲, Lb 790 in a mixed culture with Lb 706-B.

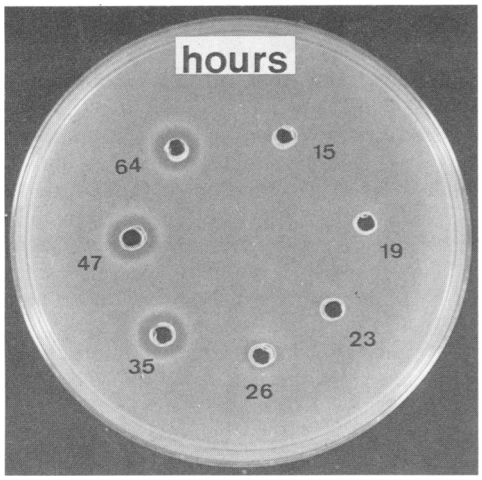

FIG. 5. Detection of bacteriocin activity in the neutralized supernatant from a mixed culture of *L. sake* Lb 706 and *L. sake* Lb 790 after different times of incubation (15, 19, 23, 26, 35, 47, and 64 h) at 15°C.

Curing studies with acriflavine resulted in mutants that were unable to produce inhibition zones on agar and that had become sensitive to the parent bacteriocin. These Bac^- variants of *L. sake* Lb 706 did not differ from the Bac^+ parent strains in their sugar fermentation patterns or growth rates. Plasmid profile analysis of two Bac^- variants revealed a loss of about 18 megadaltons of plasmid material. This plasmid may therefore encode for both bacteriocin production and resistance to the bacteriocin but does not appear to carry any information related to the fermentation of the sugars tested. However, further studies are required to establish whether this plasmid is responsible for bacteriocin production and immunity. Evidence for plasmid linkage of bacteriocin activity and immunity to the same plasmid has also been found for bacteriocin-producing strains of *L. acidophilus* (14), lactococci (15), and pediococci (4; S. K. Ray, M. C. Johnson, and B. Ray, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, H9, p. 146). In contrast, the pediocin PA-1-producing strain of *Pediococcus acidilactici* (8) did not show plasmid-encoded immunity. For many other *Lactobacillus* bacteriocins (1, 10; Daeschel et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, P13, p. 277), efforts to link production of inhibitory substances to plasmid DNA were unsuccessful.

We were able to demonstrate the killing of a bacteriocin-sensitive strain of *L. sake* in MRS broth by *L. sake* Lb 706. There was a rapid decrease in the number of viable cells after the bacteriocin producer reached a cell density of about 10^8/ml. Thus, a large number of cells is necessary to obtain bacteriocin concentrations sufficient for effective inhibition of the bacteriocin-sensitive organism. Similar results have been obtained with the facultative pathogen *Listeria monocytogenes* (U. Schillinger, unpublished data), which is a common contaminant of meat and meat products (2, 18, 19). Bacteriocin-producing *L. sake* strains may be useful for the inactivation of *Listeria monocytogenes*. However, the formation and the effect of the bacteriocin in a meat system remains to be examined. The conditions in a solid substrate like meat are quite different from those in liquid medium like MRS broth. Experiments with inoculated meat and meat products are now being carried out.

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