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Inhibition of Food-Borne Bacterial Pathogens by Bacteriocins from Lactic Acid Bacteria Isolated from Meat†

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Ten strains of bacteriocin-producing lactic acid bacteria were isolated from retail cuts of meat. These 10 strains along with 11 other bacteriocin-producing lactic acid bacteria were tested for inhibitory activity against psychrotrophic pathogens, including four strains of *Listeria monocytogenes*, two strains of *Aeromonas hydrophila*, and two strains of *Staphylococcus aureus*. Inhibition due to acid, hydrogen peroxide, and lytic bacteriophage were excluded. The proteinaceous nature of the inhibitory substance was confirmed by demonstration of its sensitivity to proteolytic enzymes. Eight of the meat isolates had inhibitory activity against all four *L. monocytogenes* strains. Bacteriocin activity against *L. monocytogenes* was found in all of the strains obtained from other sources. Activity against *A. hydrophila* and *S. aureus* was also common.

Lactic acid bacteria are commonly isolated from meats (7, 31, 32). These organisms can inhibit the natural microflora of meat, which include spoilage bacteria and, if present, pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus* (3). Lactic acid bacteria can produce a variety of antimicrobial agents, including organic acids, diacetyl, and hydrogen peroxide (15). Minimally processed meat products which rely solely on refrigeration for preservation are particularly susceptible to growth by spoilage bacteria and pathogens that are psychrotrophic. Preservation of these products by production of organic acids or diacetyl would be organoleptically unacceptable.

While it is not a highly conserved characteristic, some lactic acid bacteria produce bacteriocins (21). Bacteriocins produced by gram-positive bacteria are biologically active proteins demonstrating a bactericidal mode of action (34). Certain bacteriocins produced by lactic acid bacteria inhibit a variety of food-borne pathogens, including *Bacillus cereus*, *Clostridium perfringens*, *Listeria* species, and *S. aureus* (9, 17, 32, 33). This suggests that bacteriocin-producing lactic acid bacteria may be useful as natural preservatives. However, there have been no studies comparing the activity of these bacteriocins against the same panel of pathogens under well-controlled experimental conditions.

L. monocytogenes and *Aeromonas hydrophila* grow at temperatures of <5°C, and *S. aureus* grows at temperatures between 5 and 12°C (28). Refrigeration is inadequate to control their growth. *Listeria* spp. are highly associated with fresh meat (19) and raw fermented sausage (12). *L. monocytogenes* has been recovered from 12 to 18% of precooked, ready-to-eat chilled foods in England (14) and about one-third of ready-to-eat meat products sampled from Europe and Canada (19). Due to the ability of *L. monocytogenes* to grow at refrigeration temperatures, the U.S. Department of Agriculture Food Safety and Inspection Service has set a zero tolerance limit for the organism in ready-to-eat foods. *S. aureus* can grow slowly at refrigeration temperatures and produce enterotoxin. The organism has been implicated in food poisoning outbreaks involving a number of foods (6). *A. hydrophila* is a frequent isolate from retail meat products (27). While no confirmed cases of food poisoning due to *A. hydrophila* have been reported, it has been associated with gastrointestinal illness and does grow at refrigeration temperatures. Golden et al. (16) showed that incubation under N_2 at 5°C favored growth of *Aeromonas* spp., whereas viability under CO_2 decreased with increased incubation time.

The purposes of this study were to isolate bacteriocin-producing lactic acid bacteria from meat samples and to determine their spectrum of activity against psychrotrophic food-borne pathogens. The antimicrobial activity of previously identified bacteriocin-producing lactic acid bacteria against these pathogens was also determined. We report that several strains produce bacteriocins active against *L. monocytogenes*, *S. aureus*, and *A. hydrophila*.

MATERIALS AND METHODS

Bacterial strains and growth media. The strains used in this study are listed in Table 1. The stock culture collection was maintained at −80°C in 20% glycerol. From these, cultures of working lactic acid bacteria were made as stabs on lactobacilli MRS broth (Difco Laboratories, Detroit, Mich.) supplemented with 1.5% Bacto-Agar (Difco). Working pathogen cultures were made as stabs on Trypticase soy agar (BBL, Microbiology Systems, Cockeysville, Md.) with a 0.6% yeast extract (Difco) supplement. These were maintained as stab cultures and transferred bimonthly for a maximum of six transfers before a new working culture was made.

Isolation of bacteriocin-producing lactic acid bacteria from meat. Eleven different cuts of raw meat, including cuts of beef, lamb, and pork, were purchased from a local supermarket. Samples, 50 g, were weighed aseptically into sterile stomacher bags, sealed, and placed at 4°C for up to 3 weeks. At weekly intervals, a sample was added to sterile 0.1% peptone to obtain a 1:10 dilution and placed in a stomacher for 2 min. Serial dilutions were made in 0.1% peptone, spread plated onto MRS agar in quadruplicate, and incubated anaerobically at 25°C for 48 to 72 h until growth was evident. Anaerobic incubation (GasPak; BBL) was used to

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TABLE 1. Strains used in this study and their sources

Species	Strain and source (reference)[a]
Lactobacillus sake	ATCC 15521
Lactobacillus delbrueckii subsp. *lactis*	ATCC 12315
Lactobacillus plantarum	ATCC 10241
Lactobacillus casei	ATCC 7469
Lactobacillus brevis	ATCC B155, ATCC 4006, and ATCC 13648
Leuconostoc cremoris	ATCC 19254
Leuconostoc blayaisense	ATCC 27309
Lactococcus lactis subsp. *lactis*	ATCC 15346
Lactococcus lactis	ATCC 11454 and ATCC 21053
Lactobacillus acidophilus	N2; Barefoot and Klaenhammer[b] (5)
Lactobacillus acidophilus	88; Muriana and Klaenhammer[b] (24)
Lactobacillus sake	Lb796 and Lb706; Schillinger[b] and Lucke (32)
Lactobacillus plantarum	LB592 and LB75; Schillinger[b] and Lucke (32)
Lactobacillus sp.	LV69 (17)[c]
Pediococcus pentosaceus	ATCC 43201 (11)
Pediococcus pentosaceus	ATCC 43200 (11)
Pediococcus acidilactici	H; Bhunia, Johnson, and Ray[b] (8)
Aeromonas hydrophila	ATCC 7965 and K144[d]
Listeria monocytogenes	Scott A and V7 (13), LM 101m and 103m[e]
Staphylococcus aureus	ATCC 25923 and 196E

[a] All ATCC strains were obtained from the American Type Culture Collection.
[b] Source of strain.
[c] Obtained from C. D. Harding, Institute of Food Research, Bristol, England.
[d] *A. hydrophila* and *S. aureus* were the gift of S. A. Palumbo, Eastern Regional Research Center, USDA, Philadelphia, Pa.
[e] These listeria were from M. Doyle, University of Wisconsin, Madison.

rule out any inhibition due to hydrogen peroxide production. Three plates from the two dilutions having 30 to 300 CFU were overlaid with approximately 8 ml of brain heart infusion (BHI; Difco) which contained 1% agar. The overlay agar was seeded with *S. aureus* 25923, *L. monocytogenes* Scott A, or *L. sake* 15521 at a level of 10^5 to 10^6 organisms per ml. A fourth plate from these dilutions was saved as a master control plate (no indicator overlay) for use in future replicate plating. The plates with the overlay were incubated anaerobically overnight at 37°C. Replica plates of those plates with inhibition zones were made from the master control plate onto Trypticase soy agar (without glucose) with a 0.5% yeast extract supplement (TSAYE). TSAYE plates were used to eliminate acid production due to glucose present in MRS. The indicator overlay was repeated. Colonies showing zones of inhibition were picked from the master control plate with no indicator overlay into MRS broth or diluted into peptone diluent and plated on TSAYE and MRS and incubated at 30°C. The cultures were streaked onto MRS agar plates, Gram stained, and assayed for bacteriocin activity by using the spot deferred antagonism assay described by Harris et al. (17). Activity against each of the three indicator organisms was determined. TSAYE was used as the base agar, and BHI with 1% agar was the overlay agar. Plates were incubated anaerobically at 30°C.

Identification of meat isolates to species level. Bacteriocin-producing meat isolates were identified to the species level by the scheme devised by Schillinger and Lucke (31) for lactobacilli, with several modifications. The configuration of the lactic acid enantiomers was determined enzymatically, using lactate dehydrogenase from beef heart (Sigma Chemical Co., St. Louis, Mo.) and D-(−)-lactic dehydrogenase from *Lactobacillus leichmannii* (Sigma). The morphology of the isolates was determined under ×1,000 magnification, using phase-contrast microscopy.

Sensitivity of bacteriocin to enzyme. The protein nature of the bacteriocin was confirmed by a modification of the method of vanBelkum et al. (35). A 2-μl amount of an overnight MRS broth culture was spotted onto a TSAYE plate and incubated anaerobically overnight at 30°C. A 2-μl portion of the enzyme (10 mg/ml) was spotted adjacent to the producer spot, and the plates were incubated at 30°C for 2 h to allow diffusion of the protease. Proteases studied were protease (*Streptomyces griseus* type XIV), α-chymotrypsin (bovine pancreas, type II), proteinase K (fungal type XI), pepsin (porcine stomach mucosa), and trypsin (bovine pancreas type XIII), all from Sigma. The plates were overlaid with 5 ml of BHI-1% agar seeded with 10^4 *L. monocytogenes* LM 103m per ml. A negation of the zone of inhibition in the region of the protease indicated inhibitor sensitivity to that particular protease and confirmed that the inhibitor was a protein.

Bacteriocin spectrum of activity. All of the lactic acid bacteria were screened for activity against a pathogen panel containing four strains of *L. monocytogenes* and two strains each of *S. aureus* and *A. hydrophila*. The spot deferred antagonism test was used as described above with TSAYE or BHI as top and bottom agars. The incubation periods were 24 h for the bacteriocin producers, 24 h for *A. hydrophila* and *S. aureus*, and 48 h for the listeria at 30°C.

Detection of lytic bacteriophage. To detect the presence of lytic bacteriophage, a portion of the clearing zone was cut from a spot deferred antagonism assay plate. The agar plug was added to 3 ml of BHI broth and macerated with a sterile glass rod. The mixture was held at room temperature for 1 h. A 100-μl amount of the suspension and 100 μl of an indicator organism (grown overnight at 30°C in BHI broth) were suspended in 4 ml of soft (0.8%) agar. The soft-agar suspension was poured evenly over a BHI agar plate and incubated overnight at 30°C. The formation of plaques was indicative of phage activity. Retention of inhibitory activity in a flip plate assay (20) was also used to rule out phage-mediated lysis.

Plasmid isolation. A modification of the screening method designed by Anderson and McKay (2) for the isolation of plasmid DNA was followed. The plasmid profiles of *L. plantarum* BN, *L. bavaricus* MN, and *Leuconostoc* strain OX were examined. Strains were grown in APT broth (Difco). The cells were washed once with 1.0 ml of 6.7% sucrose–50 mM Tris–1 mM EDTA, pH 8.0. The preparations were not vortexed. After the addition of 3% NaCl-saturated phenol, 250 μl of chloroform was added and the preparation was centrifuged. The preparation was also centrifuged for 5 min after the addition of chloroform-isoamyl alcohol (24:1). The plasmid DNA was allowed to precipitate overnight at −20°C. Plasmid sizes were estimated by using the mobility plasmids of *Escherichia coli* V517 (23).

Agarose gel electrophoresis. Agarose gel electrophoresis was performed in Tris-acetate buffer, pH 8.0. Gels contained 0.7% agarose, and electrophoresis was performed at 75 V for

TABLE 2. Physiological characteristics of the lactic acid bacteria isolates[a]

Characteristic	Strain designation									
	BN	GN	MN	DX	JX	OX	PX	QX	TX	VX
Fermentation of:										
Arabinose	−	−	−	−	−	−	−	−	−	−
Cellobiose	+	+	+	+	+	+	+	+	−	+
Esculin	+	+	+	+	+	−	−	−	−	−
Galactose	+	+	−	+	+	−	−	−	−	−
Gluconate	−	+	−	+	−	−	−	−	−	−
Glycerol	−	+	+	+	−	−	−	−	−	−
Inulin	−	+	−	+	−	−	−	−	−	−
Lactose	+	+	−	+	+	−	−	−	+	−
Maltose	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	−	+	−	−	−	−	−	−
Melezitose	+	−	−	−	−	−	−	−	−	−
Melibiose	+	−	+	−	+	−	−	−	−	−
Raffinose	+	−	−	−	−	−	−	−	+	−
Rhamnose	+	−	−	−	+	−	−	−	−	−
Ribose	+	+	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	−	−	−	−	−
Sorbitol	+	−	+	+	−	−	−	−	−	−
Sucrose	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+
Xylose	−	−	−	−	−	−	−	−	−	−
Gas from glucose	−	−	−	−	−	+	+	+	−	+
NH_3 from arginine	−	+	−	+	−	−	−	−	−	−
Growth at:										
4°C	−	+	−	−	+	+	+	+	−	−
8°C	−	+	+	+	+	+	+	+	−	+
10°C	+	+	+	+	+	+	+	+	−	+
15°C	+	+	+	+	+	+	+	+	−	+
45°C	−	−	−	−	−	−	−	−	−	−
pH 3.9	+	−	−	−	+	+	−	−	−	−
Growth in:										
6.5% NaCl	+	+	+	+	+	+	+	+	−	+
7.0% NaCl	+	−	+	+	+	+	+	+	−	+
10.0% NaCl	−	−	−	−	+	−	−	−	−	−
Slime from sucrose	−	−	−	−	−	+	+	+	+	−
Voges-Proskauer	+	+	+	+	+	+	−	−	+	−
Formation of:										
H_2S	+	+	+	+	+	−	−	+	−	−
DL-Lactic acid	n	L	L	L	L	n	DL	DL	n	n
Gram stain	+	+	+	+	+	+	+	+	+	+
Morphology	r	r	r	r	r	c	r	r	c	c
Catalase test	−	−	−	−	−	−	−	−	−	−

[a] +, positive; −, negative. r, Rod; c, cocci; n, not performed; L, 90% or more of the total lactic acid is of the L-configuration; DL, 25 to 75% of the total lactic acid is of the L-configuration.

6 h. Gels were stained with ethidium bromide (0.5 μg/ml) and photographed on Polaroid 55 film.

RESULTS

Ten bacteriocin-producing lactic acid bacteria were isolated from the meat samples. The physiological characteristics important to the identification of these meat isolates to the species level are presented in Table 2. Based on these results and the schemes for identifying species developed by Schillinger and Lucke (32), two strains were identified as *L. bavaricus*, two were identified as *Carnobacterium piscicola* (formerly classified as *L. carnis*), and one was identified as *L. plantarum* (Table 3). Two leuconostoc isolates and one streptococcus isolate were also identified. These were not identified to species level.

The sensitivities of the inhibitory substances produced by the lactic acid bacteria to various proteolytic enzymes are demonstrated in Table 4. All of the inhibitory substances were inactivated by one or more proteases, except for the inhibitory substances produced by *P. pentosaceus* 43200 and *P. pentosaceus* 43201. *L. bavaricus* MN was not sensitive to trypsin, while *L. bavaricus* JX was sensitive to trypsin action. The same can be seen for *L. plantarum* BN versus *L. plantarum* Lb75. Likewise, the same pattern of protease sensitivity between *L. viridescens* PX and QX was observed.

TABLE 3. Identification of meat isolates

Isolate	Genus and species	Source and comment
BN	*Lactobacillus plantarum*	Beef
GN	*Carnobacterium piscicola*	Beef
DX	*Carnobacterium piscicola*	Beef
JX	*Lactobacillus bavaricus*	Lamb
MN	*Lactobacillus bavaricus*	Beef
OX	*Leuconostoc* sp.	Lamb
PX	*Lactobacillus viridescens*	Beef; same as QX
QX	*Lactobacillus viridescens*	Pork; same as PX
TX	*Streptococcus* sp.	Beef
VX	*Leuconostoc* sp.	Pork

The spectrum of activity of the bacteriocin-producing lactic acid bacteria is shown in Table 5. Eight of our meat isolates had inhibitory activity against all four *L. monocytogenes* strains. Seven of the meat isolates which inhibited *L. monocytogenes* also inhibited *A. hydrophila*. Both *S. aureus* strains were inhibited by *L. bavaricus* JX, while *Leuconostoc* strain OX and *L. viridescens* PX and QX inhibited only *S. aureus* 196E. Bacteriocin activity against *L. monocytogenes* was found in all of the strains obtained from other sources. Activity against *S. aureus* was also common in these strains. In all cases in which the producer demonstrated activity against *S. aureus*, strain 196E was inhibited. Strain 25923 was not always inhibited.

No clearing zones were formed from homogenates of the clearing zones from the putative bacteriocin producers on lawns containing sensitive indicator strains. This indicated that the lysis in the overlay plates was not caused by lytic bacteriophage. In addition, clearing zones were observed in flip plates, which exclude lysis by lytic phage by putting an agar barrier between the bacteriocin-producing cells and the indicator cells.

By using the plasmid isolation technique described above, the plasmid profiles of the three strains examined can be seen in Fig. 1. Plasmid DNA was not detected in *L. plantarum* BN (lane B). *L. bavaricus* MN (lane C) harbors two plasmids, of 22.6 and 15.5 MDa. *Leuconostoc* OX (lane D) harbors one plasmid, of 11.0 MDa. The chromosomal DNA band can be seen in all three strains.

DISCUSSION

The inhibitory agents produced by all of the lactic acid bacteria examined in this study can be characterized as bacteriocins, since inhibition due to acid, hydrogen peroxide, and bacteriophage have been excluded. Also, the proteinaceous nature of the inhibitory substances produced by the laboratory isolates was confirmed by their protease sensitivity.

Certain strains of lactobacilli are more commonly isolated from meats (32). Among our isolates these include *L. plan-*

TABLE 4. Sensitivity of bacteriocins to proteolytic enzymes with *L. monocytogenes* as the indicator organism[a]

Strain	Chymotrypsin	Trypsin	Proteinase K	Pronase E	Pepsin
L. lactis 11454	−	−	−	+	−
L. acidophilus N2	−	−	−	+	−
L. sake Lb706	+	+	+	+	−
L. plantarum Lb592	+	ND	ND	ND	ND
L. plantarum Lb75	ND	+	ND	ND	ND
Lactobacillus strain LV69	+	+	+	+	−
P. pentosaceus 43201	−	−	−	−	−
P. pentosaceus 43200	−	−	−	−	−
P. acidilactici H	+	ND	ND	ND	ND
L. plantarum BN	+	−	ND	−	−
C. piscicola GN	−	−	+	+	−
C. piscicola DX	+	−	+	+	−
L. bavaricus MN	+	−	+	+	−
L. bavaricus JX	ND	+	ND	ND	ND
Leuconostoc strain OX	+	+	+	+	−
L. viridescens PX	+	+	+	+	−
L. viridescens QX	+	+	+	+	−
Leuconostoc strain VX	+	ND	+	+	−

[a] +, sensitivity; −, no sensitivity. ND, not done.

tarum, *L. carnis*, and *L. viridescens*. *L. bavaricus* is less frequently isolated.

The proteinaceous nature of the inhibitory substances identified previously as bacteriocins was confirmed by demonstration of protease sensitivity, with the exceptions of *Pediococcus pentosaceus* 43200 and 43201. It is possible that these bacteriocins contain only a minor component of proteinaceous character, as do glycoprotein bacteriocins (29). Alternatively, the active domains of these substances may not be affected by these enzymes. Liu and Hansen (22) reported that cleavage of the nisin peptide into two fragments resulted in a 10-fold reduction in activity but not complete inactivation. Finally, Daeschel and Klaenhammer (11) cite unpublished research that the inhibitory substances produced by these two strains are indeed protease sensitive.

The pattern of protease sensitivity indicates the uniqueness of the bacteriocins produced by our meat isolates compared with previously identified strains of the same species. It also indicates that the strains are indeed different. Conversely, the same pattern of protease sensitivity between *L. viridescens* PX and QX coupled with an identical spectrum of activity leads to the conclusion that they represent multiple isolations of the same organism.

The high sensitivity of the *Listeria* strains to the bacteriocins produced by the meat isolates is not surprising, since the screening procedure was based on the inhibition of *L.*

TABLE 5. Sensitivity of pathogens to bacteriocin-producing strains

Producing strain	Indicator strain[a]							
	A. hydrophila		*L. monocytogenes*				*S. aureus*	
	7965	K144	Scott A	V7	101m	103m	25923	196E
L. lactis 11454	++	++	++	++	++	++	++	++
L. acidophilus N2	−	−	++	++	++	++	−	−
L. acidophilus 88	+	++	−	−	−	++	++	++
L. sake Lb796	++	++	+	−	++	++	++	++
L. sake Lb706	++	++	++	++	++	++	++	+
L. plantarum Lb592	++	++	+	+	+	+	++	++
L. plantarum Lb75	++	++	+	+	+	+	++	++
Lactobacillus strain LV69	+	+	+	+	+	++	+	+
P. pentosaceus 43201	++	++	+	+	+	+	+	+
P. pentosaceus 43200	++	++	+	+	+	+	++	++
P. acidilactici H	ND	++	+	+	+	+	−	+
L. plantarum BN	+	+	+	+	+	+	−	−
C. piscicola GN	+	+	++	++	++	++	−	−
C. piscicola DX	+	+	++	++	+++	++	−	−
L. bavaricus MN	−	+	+++	+++	+++	+++	−	−
L. bavaricus JX	++	++	−	−	−	−	++	+
Leuconostoc strain OX	+	+	++	++	+++	+++	−	+
L. viridescens PX	+	+	+++	+++	+++	+++	−	+
L. viridescens QX	+	+	+++	+++	+++	+++	−	+
Streptococcus strain TX	ND	ND	−	−	−	−	ND	ND
Leuconostoc strain VX	+	+	++	++	++	++	−	+

[a] ND, not done. −, no zone; +, zone < 1 mm; ++, 1 mm < zone < 10 mm; +++, zone > 10 mm.

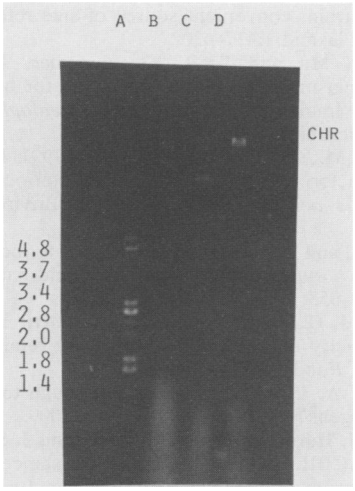

FIG. 1. Plasmid profiles. Lane A, *E. coli* V517; lane B, *L. plantarum* BN; lane C, *L. bavaricus* MN; lane D, *Leuconostoc* strain OX. CHR, Chromosomal band.

monocytogenes Scott A. On further testing, *Streptococcus* strain TX lost this characteristic, suggesting the possibility of a plasmid-borne determinant that may have been lost in the early stages of the research. *L. bavaricus* JX was never found to have activity against *L. monocytogenes* Scott A. It was active against *S. aureus* 25923 and *L. sake* 15521. The difference in sensitivity of the *Listeria* strains to the bacteriocins produced by *L. acidophilus* 88 and N2 is surprising, since their protein structure is very similar (25). Ahn and Stiles (1) report limited inhibitory activity against *L. monocytogenes* by bacteriocin-producing strains of lactic acid bacteria isolated from vacuum-packaged meats. These strains were screened as potential producers against a battery of lactic acid bacteria as indicator organisms. The indicator organism used in the initial screening needs to reflect the final or proposed application of the bacteriocin-producing strain. We are not the first to report bacteriocin activity against *L. monocytogenes*. Spelhaug and Harlander (33) also identified the sensitivity of *L. monocytogenes* V7 and Scott A and *S. aureus* 196E to bacteriocins produced by *P. pentosaceus* 43200 (FBB 61) and *L. lactis* 11454. Along with our studies, these results suggest that the inability of Roller et al. (30) to detect antimicrobial activity associated with *P. pentosaceus* 43200 was due to their use of a broth medium which did not support pediocin A production. Schillinger and Lucke (32) identified the sensitivity of *L. monocytogenes* 8732 and 17A to *L. sake* Lb 706 and Lb792, which are also meat isolates, and to *L. plantarum* Lb 75 and Lb 592. Harris et al. (17) tested the sensitivity of a battery of *Listeria* strains to the bacteriocin produced by *L. acidophilus* 88 and demonstrated a lack of activity. They also confirmed the activity of the bacteriocins produced by *L. lactis* 11454 and *P. pentosaceus* 43200 to *Listeria* spp. We only demonstrated activity by *L. acidophilus* 88 to *L. monocytogenes* 103m. On the basis of zone size, the laboratory meat isolates produce either more bacteriocin or a bacteriocin(s) with higher antilisterial activity compared with the other strains. The widespread inhibition of *A. hydrophila* 7965 and K144 by many of the bacteriocin producers is surprising and rather interesting. Previously, no activity against gram-negative strains by gram-positive bacteriocin producers has been reported. Okereke and Montville (26) report the inhibition of *Clostridium botulinum* spores by bacteriocins of *L. lactis* 11454, *L. acidophilus* N2, *P. pentosaceus* 43200 and 43201, and *L. plantarum* BN, Lb592, and Lb75.

Plasmid DNA in lactic acid bacteria is not always easily detected. This may be due to growth temperature, copy number, and isolation procedures (10). The three isolates we observed here demonstrated different plasmid patterns. Bacteriocin production can be both plasmid mediated and chromosomally linked (4, 11, 18). The association of the *L. bavaricus* MN and *Leuconostoc* strain OX plasmids with bacteriocin activity needs to be determined.

Due to the nature of the isolation procedure, the bacteriocin-producing laboratory isolates grow well at refrigeration temperatures, which could give them a competitive edge over spoilage organisms. The new class of minimally processed meat products emerging on the market relies solely on refrigeration as a means of preservation. However, if these products are contaminated with psychrotrophic foodborne pathogens, these products could pose a serious health threat. The use of bacteriocin-producing migroorganisms may provide a natural means of preservation. The ability of these organisms to inhibit these pathogens simultaneously in laboratory medium and in meat systems is under investigation.

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