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Isolation and Characterization of Pediocin L50, a New Bacteriocin from *Pediococcus acidilactici* with a Broad Inhibitory Spectrum

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Lactic acid bacteria were isolated from Spanish dry-fermented sausages and screened for bacteriocin production. About 10% of the isolates produced antimicrobial substances when grown on solid media, but only 2% produced detectable activity in liquid media. Strain L50, identified as *Pediococcus acidilactici*, showed the strongest inhibitory activity and was active against members of all of the gram-positive genera tested. The strain produced a heat-stable bacteriocin when grown at 8 to 32°C but not at 45°C. The bacteriocin was purified to homogeneity. Its mass was determined to be 5,250.11 ± 0.30 by electrospray mass spectrometry. The N terminus of the bacteriocin was blocked for sequencing by Edman degradation, but a partial sequence of 42 amino acids was obtained after cleavage of the peptide by cyanogen bromide. The sequence showed no similarity to those of other bacteriocins. Pediocin L50 appears to contain modified amino acids but not lanthionine or methyl-lanthionine.

Lactic acid bacteria are widely used as starter cultures for dairy, meat, and vegetable fermentations. *Lactobacillus* and *Pediococcus* strains are commonly used in the manufacture of fermented sausages (33), contributing to flavor and color development (27). The lactic acid bacteria also have the potential to inhibit the growth of pathogenic and spoilage bacteria, thereby improving the hygienic quality and extending the shelf life of different meats and meat products (2, 28, 33, 35). Although reduction of pH and removal of carbohydrates are the primary effects exerted by these bacteria (5), they also produce other substances such as hydrogen peroxide, carbon dioxide, diacetyl, and bacteriocins that are antagonistic toward other microorganisms (5, 13, 24, 33). The presence of pediococci producing bacteriocin-like substances was first observed in cucumber brines by Fleming et al. (7) and was later reported to occur in fermented vegetables (6, 12), in fresh and fermented sausages (3, 11, 30), and recently in wine (34).

In recent years, there have been several reports dealing with the antimicrobial spectrum, purification, and characterization of bacteriocins produced by pediococci. However, of these bacteriocins, only pediocin PA-1 has been described in detail at the molecular level. Pediocin PA-1 is a plasmid-encoded bacteriocin produced by *Pediococcus acidilactici* PAC 1.0 (11). The bacteriocin shows antimicrobial activity against a wide spectrum of lactic acid bacteria and *Listeria monocytogenes* strains (11, 26). Purification of the bacteriocin resulted in a 4,629-molecular-weight peptide with 44 amino acids (13, 22). This is the only bacteriocin from pediococci with a known primary structure. A bacteriocin active against several lactic acid bacteria, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Clostridium perfringens*, which was designated pediocin AcH, was isolated from *P. acidilactici* H (3) and was later shown to be identical to pediocin PA-1 (21). Pediocin SJ-1 from *P. acidilactici* SJ-1, with an apparent molecular weight of 4,000 (30), and pediocin A from *Pediococcus pentosaceus* FBB61 and L7230 (6) have been reported to be plasmid-encoded bacteriocins with antimicrobial spectra similar to that of pediocin AcH. A bacteriocin produced by *Pediococcus cerevisiae* FBB63 was tentatively linked to a plasmid (12). In this paper, we describe a new bacteriocin produced by *P. acidilactici* L50. The producing strain was isolated from Spanish dry-fermented sausages. Pediocin L50 is inhibitory to a number of spoilage and food-borne pathogenic bacteria, including *Listeria monocytogenes*, *S. aureus*, and *Clostridium botulinum* strains, and is thus a promising agent in food and feed preservation.

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MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains used as indicator microorganisms for bacteriocin screening and evaluation of antimicrobial activities are given in Table 1. The lactic acid bacteria were propagated in MRS broth (Oxoid Ltd., Basingstoke, United Kingdom) at 32 or 37°C. *Clostridium* strains were propagated anaerobically (Oxoid anaerobic system) in reinforced clostridial medium (Oxoid) at 32 or 37°C. *Propionibacterium* spp. were grown anaerobically in GYE medium containing peptone (Difco, Detroit, Mich.; 10 g/liter), Lab-Lemco powder (Oxoid; 10 g/liter), NaCl (5 g/liter), D-glucose (5 g/liter), and yeast extract (Oxoid; 3 g/liter [pH 7.0]). All other strains were grown in brain heart infusion medium (Oxoid) at 32 or 37°C.

Isolation of bacteriocin-producing lactic acid bacteria. Spanish dry-fermented sausages, manufactured with no added starter cultures, were used as the source of lactic acid bacteria. Samples from each sausage were homogenized in 1% peptone and 0.8% NaCl and incubated for 5 h at 32°C before being plated on MRS agar. Bacteria isolated from the sausages were stabbed onto MRS plates and incubated at 32°C for 3 h, and then 15 ml of soft agar containing about 10^6 CFU of the indicator strain was poured over the plates. *P. acidilactici* 347, *Lactobacillus sake* 148, and *Listeria monocytogenes* Scott A were used as indicators. After incubation for 24 h at 32°C, the plates were checked for inhibition zones.

Characterization of isolate L50. The isolate was subjected to identification tests as described by Garvie (9). Fermentation patterns were determined with API Rapid CH fermentation strips (API, Biomerieux) in CHL medium as specified by the manufacturer. Total proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the pattern obtained was compared with those of reference strains described by Kersters and de Ley (16) and Pot et al. (25) by B. Pot, University of Ghent, Ghent, Belgium.

Bacteriocin production in liquid media. Isolates showing antimicrobial activity were grown in MRS broth at 32°C until the stationary phase. The cultures were centrifuged at 12,000 × *g* for 10 min at 4°C. To eliminate growth inhibition caused by organic acids and hydrogen peroxide, the pH of the supernatants was adjusted to 6.2 with 1 M NaOH, and 130 U of catalase (Boehringer GmbH,

TABLE 1. Antimicrobial activity of *P. acidilactici* L50 and MICs of pediocin L50 against gram-positive bacteria

Indicator species	Strain	Source[a]	Inhibition by[b]:		Bacteriocin concn (ng/ml)[c]
			S	CS	
Lactobacillus acidophilus	4356	ATCC	13.4	17.8	
Lactobacillus bulgaricus	11842	ATCC	8.7	11.4	60
Lactobacillus casei	334	ATCC	13.5	17.5	13
Lactobacillus curvatus	NCFB2739	NCFB		7.7	114
Lactobacillus fermentum	9338	ATCC	15.4	18.1	260
Lactobacillus helveticus	15009	ATCC	8.0	13.0	57
Lactobacillus plantarum	NCDO1193	NCDO			
Lactobacillus reuteri	DSM20016	DSM			
Lactobacillus sake	NCFB2714	NCFB		8.3	77
	148	Our strain collection	12.2	17.0	3
Lactobacillus salivarius	NCFB2747	NCFB			
Pediococcus acidilactici	347	Our strain collection	15.5	18.8	4
Pediococcus pentosaceus	FBB61	TNO	14.2	17.4	7
	FBB63	TNO	12.5	16.5	120
	PC1	TNO	7.4	9.7	107
Leuconostoc cremoris	DB1275	TNO	8.0	12.0	109
Lactococcus cremoris	CNRZ117	INRA	7.3	9.4	103
Lactococcus lactis	CNRZ148	INRA	11.4	18.0	100
	CNRZ150	INRA	7.0	11.3	59
	BB24	Our strain collection		11.0	50
Enterococcus faecalis	EF	TNO	12.6	14.4	193
Staphylococcus carnosus	MC1	TNO	11.6	14.6	225
Listeria innocua	BL86/26	TNO	12.0	15.0	457
Bacillus cereus	9139	ATCC			187
Clostridium sporogenes	C22/10	TNO	13.0	16.2	475
Clostridium tyrobutyricum	3,5CT	TNO		12.0	443
	NCDO1754	NCDO		13.0	459
Propionibacterium acidipropionici	NCDO563	NCDO	13.0	16.3	213
Propionibacterium sp.	P4	TNO	13.2	17.0	189
	P6	TNO	15.0	18.6	218
Clostridium perfringens	376	CECT		11.7	475
Clostridium botulinum	551	CECT		11.2	471
Listeria monocytogenes	7973	NCTC	10.6	14.4	278
	LI5sv1/2	FVM	11.6	15.0	475
	5105	NCTC	10.7	14.6	377
	LI1sv4	FVM	11.0	14.6	151
	Scott A	FVM	11.3	15.1	463
Staphylococcus aureus	137	FRI	11.6	14.0	740
	196E	FRI	11.3	14.0	163
	349	FRI	11.6	16.0	404
	361	FRI	12.3	17.0	377
	472	FRI	12.0	14.0	470

[a] Abbreviations: ATCC, American Type Culture Collection (Rockville, Md.); CECT, Colección Española de Cultivos Tipo (Valencia, Spain); DSM, Deutsche Sammlung von Mikroorganismen und Zell kulturen, GmbH (Braunschweig, Germany); INRA, Station de Recherches Laitières (Jouy-en-Josas, France); FRI, Food Research Institute (Madison, Wis.); FVM, Facultad de Veterinaria (Madrid, Spain); NCDO, National Collection of Dairy Organisms (Reading, United Kingdom); NCFB, National Collection of Food Bacteria (Reading, United Kingdom); NCTC, National Collection of Type Cultures (London, United Kingdom); TNO, Nutrition and Food Research (Zeist, The Netherlands).

[b] Diameter of inhibition zone in millimeters. S, supernatant; CS, 20-fold-concentrated supernatant.

[c] Bacteriocin concentration inhibiting growth of indicator microorganisms by 50%.

Mannheim, Germany) per ml was added. The supernatants were further filter sterilized through 0.22-μm-pore-size filters (Millipore Corp., Bedford, Mass.). Concentrated preparations were obtained by lyophilization. The lyophilized samples were redissolved to 1/20 of their original volume in 4 mM phosphate buffer (pH 7.0). The samples were stored at −20°C until further use.

Bacteriocin activity assays. Antimicrobial activity secreted into liquid media was assayed by two methods. In method 1, 20 ml of soft agar was inoculated with 10^6 CFU of the indicator strain in a petri dish. After cooling, wells (6-mm diameter) were made in the agar, and 50 μl of bacteriocin solution was added to the wells. The plates were kept at 4°C for 2 h and subsequently incubated under optimal conditions for growth of the target strains. The antimicrobial activity was quantified by the diameter of the inhibition zones around the wells.

In method 2, a dilution assay in microtiter plates was performed essentially as described by Holo et al. (14). The bacteriocin was serially diluted in MRS broth, and an overnight culture of *P. acidilactici* 347 diluted in MRS to an optical density of 0.1 at 620 nm was used as an indicator. After 14 h of incubation at 32°C, the growth of the indicator was measured spectrophotometrically at 620 nm. One bacteriocin unit was defined as the amount of bacteriocin causing 50% growth inhibition (50% of the turbidity of the control culture without bacteriocin) in this assay. The microtiter plate assay was used to evaluate the MIC of purified bacteriocin. The assay was carried out as described above with the appropriate growth media and incubation conditions for each strain. The plates were incubated until the cultures in the wells containing no added bacteriocin had reached the stationary phase.

Bacteriocin purification. The bacteriocin was purified from a 1-liter culture of strain L50 grown in MRS broth at 32°C until the late logarithmic phase (A_{620}, approximately 0.6). After the cells were removed by centrifugation at 500 × *g*, ammonium sulfate was added to the supernatant. The mixture was stirred at 4°C for 30 min and then centrifuged at 12,000 × *g* for 20 min at 4°C. The pellet and floating solid material were combined and dissolved in 200 ml of 20 mM sodium phosphate buffer (pH 5.8). The bacteriocin preparation was then subjected to cation-exchange chromatography followed by hydrophobic-interaction and reverse-phase chromatographies (PepRPC HR5/5 fast-performance liquid chromatography system; Pharmacia-LKB, Uppsala, Sweden) essentially as described by Nissen-Meyer et al. (23). The bacteriocin was eluted from the reverse-phase column with a linear 2-propanol gradient in 0.1% trifluoroacetic acid (TFA) at

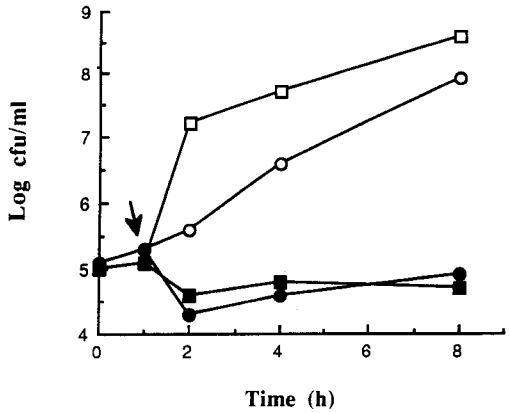

FIG. 1. Effect of a 20-fold-concentrated supernatant of *P. acidilactici* L50 on the viable count of *P. acidilactici* 347 (●) and *Listeria monocytogenes* Scott A (■) cultures. The arrow indicates when the bacteriocin was added. The viable counts of *P. acidilactici* 347 (○) and *L. monocytogenes* Scott A (□) with the addition of 20-fold-concentrated supernatant from *P. acidilactici* 144, a non-bacteriocin-producing strain, are shown as controls.

a flow rate of 0.5 ml min^{-1}. Fractions with high bacteriocin activity were mixed and rechromatographed on the reverse-phase column. Purified bacteriocin was stored in 50 to 60% 2-propanol and/or ethanol containing 0.1% TFA at −20°C.

Determination of protein. The protein concentration of purified bacteriocin was determined with the Coomassie protein assay reagent (Pierce Chemicals, Rockford, Ill.).

SDS-PAGE analysis. The purified bacteriocin was dried under a vacuum and analyzed by PAGE as described by Laemmli (19). After electrophoresis, the gel was stained with Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Richmond, Calif.).

Amino acid composition and sequence analysis. The purified bacteriocin was hydrolyzed and analyzed on an amino acid analyzer as described previously (8). The amino acid sequence was determined by Edman degradation with an Applied Biosystems model 477A automatic sequence analyzer with an on-line 120A phenylthiohydantoin amino acid analyzer (4). The C-terminal part of the sequence was obtained after cleavage of the bacteriocin with cyanogen bromide (CNBr) as described by Sletten and Husby (31).

Mass spectrometry analysis of pediocin L50 was performed by S. Bayne at Novo Nordisk A/S (Gentofte, Denmark) with a PE Sciex API1 electrospray mass spectrometer.

RESULTS

Screening for production of antibacterial compounds. A stab-on agar test was used to screen 500 lactic acid bacterial isolates from five Spanish dry-fermented sausages for antagonistic activity. A total of 55 isolates were found to produce clear inhibition zones against *P. acidilactici* 347, *Lactobacillus sake* 148, and *Listeria monocytogenes* Scott A. The culture supernatants of these isolates were tested for inhibitory activity by the agar well diffusion test, with 42 gram-positive and 6 gram-negative bacteria of interest in the food industry used as indicators. Twelve of the isolates were found to secrete antimicrobial substances against some of the indicator strains listed in Table 1. None of the gram-negative bacteria tested, *Salmonella typhimurium*, *Pseudomonas fluorescens*, *Yersinia enterocolitica*, *Escherichia coli*, *Aeromonas hydrophila*, or *Enterobacter aerogenes*, was inhibited by supernatants or 20-fold-concentrated supernatants of any of the isolates.

The antimicrobial activity spectrum of strain L50 (Table 1) was broader than that of any of the other isolates (results not shown), and this strain was chosen for further characterization. The strain was identified as *P. acidilactici* by biochemical tests and carbohydrate fermentation patterns. Comparison of its SDS-PAGE protein pattern with a database of more than 7,000 patterns from lactic acid bacteria supported this identification.

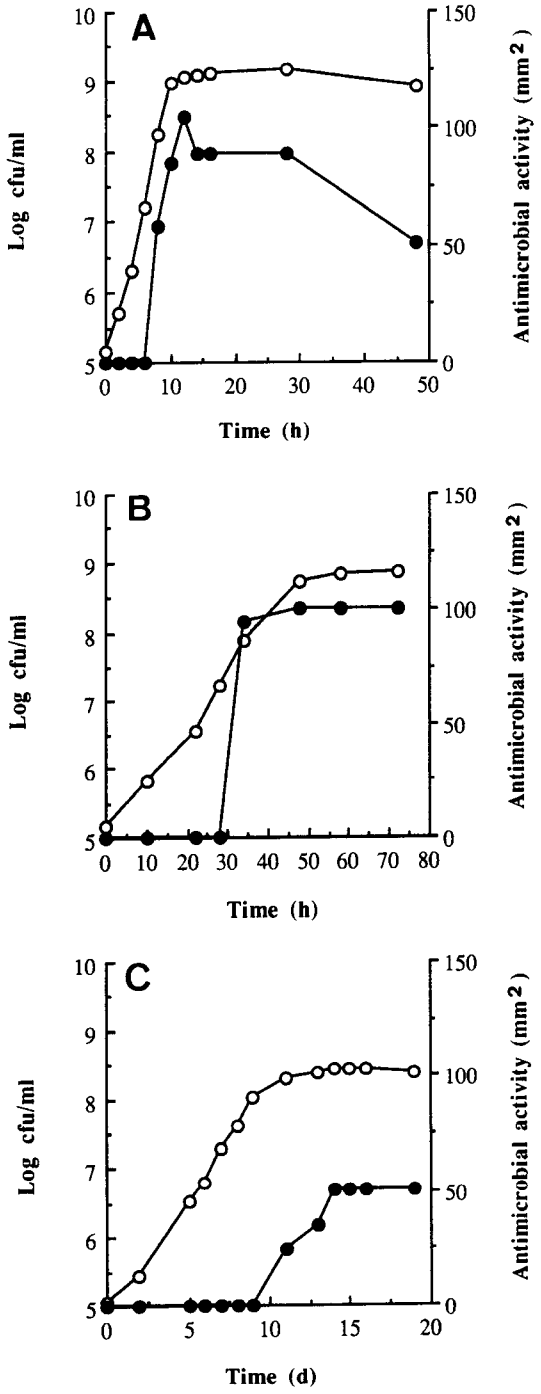

FIG. 2. Growth of *P. acidilactici* L50 (○) and bacteriocin production (●) at 32°C (A), 16°C (B), and 8°C (C). d, days.

Table 1 shows the effect of culture supernatants and concentrated culture supernatants of *P. acidilactici* L50 on the growth of the gram-positive bacteria in the agar well diffusion assay. Among spoilage and food-borne pathogenic bacteria, all of the *Listeria*, *Staphylococcus*, and *Enterococcus* strains tested were inhibited by *P. acidilactici* L50 supernatants. No halo was seen when the supernatant was tested against strains of *C. perfringens*, *C. botulinum*, or *Clostridium tyrobutyricum*, but when 20-fold-concentrated supernatants were used, inhibition

TABLE 2. Purification of pediocin L50

Purification stage	Vol (ml)	Total A_{254}[a]	Total activity (BU)	Sp act[b]	Increase in sp act (fold)	Yield (%)
Culture supernatant	1,000	34,200	130,000	3.8	1	100
Fraction						
I. Ammonium sulfate precipitation	200	3,620	117,000	32.3	9	90
II. Cation-exchange chromatography	50	33.5	160,500	4,791	1,261	124
III. Hydrophobic-interaction chromatography	10	10.2	192,450	18,868	4,965	148
IV. Reverse-phase chromatography	1.2	0.24	104,070	433,625	114,112	80

[a] Total A_{254} equals the optical density at 254 nm multiplied by the volume in milliliters.
[b] Specific activity is bacteriocin units (BU) per milliliter divided by the optical density at 254 nm.

zones of 11 to 13 mm were obtained. Only four of the strains tested, three lactobacilli and *Bacillus cereus*, were not inhibited by the preparations. No test was performed to determine whether the preparations could inhibit the outgrowth of spores.

The antimicrobial activity of the cell-free supernatants of strain L50 was sensitive to trypsin, papain, pepsin, protease II, protease VI, and protease XIV, with a time- and concentration-dependent inactivation kinetics (results not shown), showing that the inhibitory compound is proteinaceous, i.e., a bacteriocin. We have called this bacteriocin pediocin L50. Treatment with lipolytic or amylolytic enzymes had no effect on the bacteriocin activity. The antagonistic activity of 20-fold-concentrated culture supernatants from strain L50 was stable at pH levels between 2 and 11 for 24 h at 25°C. The preparations also showed detectable activity after 40 min of exposure to 100°C. At this temperature, the half-life was found to be about 5 min.

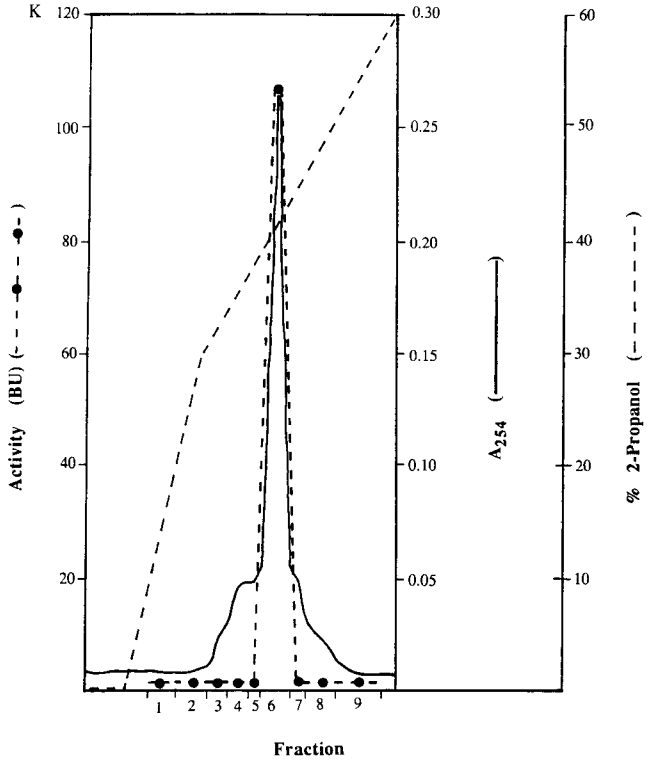

FIG. 3. Reverse-phase chromatography of pediocin L50. The amount applied to the column was obtained from a 1-liter culture. BU, bacteriocin units.

The effect of pediocin L50 on the viable count in cultures of *P. acidilactici* 347 and *Listeria monocytogenes* is shown in Fig. 1. The effect was bactericidal, as the viable count dropped by about 90% shortly after the addition of the preparation.

The antagonistic activity of *P. acidilactici* L50 was measured after 16 h of incubation at 32°C in several culture media. Bacteriocin production was seen in all media tested, but the highest activities were obtained in MRS broth. The production in MRS broth was measured at several growth temperatures. Figure 2 shows the growth and bacteriocin production at 8, 16, and 32°C. The amount formed at 16°C was comparable to that formed at 32°C, while considerably less was produced at 8°C. In all cases, the highest activity was found from the onset of the stationary phase. At 8 and 16°C, the activity was very stable, while at 32°C, a decrease in activity was seen throughout the stationary phase. No bacteriocin production was observed in cultures grown at 45°C.

Purification of the bacteriocin. Results from the purification of the bacteriocin from a late-logarithmic-phase culture of *P. acidilactici* L50 grown at 32°C in MRS broth are summarized in Table 2. The fractions from the first run on the reverse-phase column showing the highest activities were collected and rechromatographed on the same column. A single absorbance peak, coinciding with the activity peak, was obtained (Fig. 3). The final specific activity of the pure bacteriocin was approximately 115,000-fold greater than that in the culture supernatant, and the recovery was 80% (fraction IV). The protein concentration in this fraction was estimated to be 60 μg/ml.

Estimation of molecular weight. Purified bacteriocin from the reverse-phase chromatography step (fraction IV) was an-

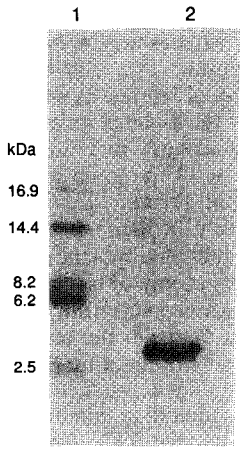

FIG. 4. SDS-PAGE analysis of pediocin L50. Lanes: 1, molecular mass standards; 2, pediocin L50.

TABLE 3. Amino acid composition of pediocin L50

Amino acid	Amt (nmol)	Residues/molecule	
		By hydrolysis[a]	Observed in sequence
Pro	4.2	1.1	1
Asx	4.8	1.4	1
Thr	5.2	1.5	1
Ser	Tr	Tr	0
Glx	20.0	5.6	3
Gly	16.0	4.5	4
Ala	13.2	3.7	3
Val	5.6	1.6	3 (4)[b]
Met	7.1	2	2
Ile	24.1	6.8	7 (8)
Leu	7.4	2.1	2
Tyr	5.9	1.7	2
Phe	8.6	2.4	3
His	4.6	1.3	0
Lys	23.4	6.6	4 (5)
Arg	1.8	0.5	0
Cys	0	Tr	0
Trp	ND[c]	ND	0
Xaa[d]	ND	ND	5
Total	151.9		42

[a] Assuming that the peptide contains 2 methionine residues.
[b] Values in parentheses include residue 17.
[c] ND, not determined.
[d] Xaa, unidentified residue.

alyzed by SDS-PAGE. A single protein band was detected, indicating that the bacteriocin had been purified to homogeneity. In SDS-PAGE, the bacteriocin migrated with an apparent molecular weight of about 3,600 (Fig. 4). By mass spectrometry, the mass was found to be 5,250.11 ± 0.30.

Amino acid composition and sequence analysis. The amino acid composition of pure pediocin L50 from fraction IV was determined (Table 3). In addition to normal amino acids, an unidentified compound was observed in the acid hydrolysate. The signal from this compound was weak, however, with an intensity of about the same magnitude as that of the arginine signal. Lanthionine or methyl-lanthionine was not detected. The N terminus of the bacteriocin was blocked for sequencing by Edman degradation. From the amino acid composition and the size of the peptide, it could be deduced that the bacteriocin contains two methionine residues. Prior to sequencing, the bacteriocin was therefore subjected to CNBr cleavage. Thus, we obtained the sequence of two C-terminal CNBr cleavage fragments. Because the cleavage was incomplete, it was possible to determine the order of the two fragments in the peptide. Altogether, the partial sequence of 42 amino acid residues was obtained (Fig. 5). Some of the positions could not be identified. Furthermore, in the Edman degradation, the lysine residues gave signals of about half the intensity expected and were always accompanied by another, unidentified compound giving a signal of similar intensity.

Determination of MIC values of pediocin L50. The MICs of purified pediocin L50 determined by a microtiter plate assay against selected indicator microorganisms are given in Table 1. Most of the lactic acid bacteria tested were inhibited by the purified bacteriocin, with MICs ranging from 3 to 260 ng/ml. All of the spoilage and food-borne pathogenic bacteria tested were also inhibited by pediocin L50, with MICs ranging from 163 to 740 ng/ml. Purified pediocin L50 also inhibited the growth of *B. cereus* ATCC 9139, although this strain was not inhibited by crude preparations in the agar well diffusion test (Table 1).

DISCUSSION

Fifty-five of the 500 isolates of lactic acid bacteria from Spanish dry-fermented sausages exerted antagonistic activity in the stab-on agar test against the three indicator microorganisms tested. However, in liquid culture grown to the stationary phase, only 12 of them showed detectable antimicrobial activity. Exponential-phase cultures were not tested. Similar frequencies have been noted by Geis et al. (10) with lactococci and by Schillinger and Lücke (27) and Sobrino et al. (32) with lactobacilli. The production of antagonistic activities in solid media only has also been reported for lactacin B (1) and propionicin PLG-1 (20). The antimicrobial spectra of the 12 isolates tested differed significantly (results not shown), suggesting that the antimicrobial substances produced may be heterogeneous, as described previously for lactobacilli isolated from Spanish dry-fermented sausages (32). The antimicrobial activity of *P. acidilactici* L50 was selected for further characterization on the basis of its broad inhibitory spectrum. Pediocin L50 inhibited members of all of the gram-positive genera tested, although resistant lactobacilli were found. Like other bacteriocins from lactic acid bacteria (17), pediocin L50 was not active against gram-negative microorganisms.

A number of characteristics of pediocin L50 make it interesting as a food preservative. First, it inhibited the growth of all of the gram-positive food spoilage bacteria tested as well as pathogens such as *Listeria monocytogenes*. Second, the bacteriocin appears to be stable under a wide variety of conditions. Its thermostability indicates that it can be used in pasteurized products. The bacteriocin produced by *P. acidilactici* L50 is also stable over a wide pH range, indicating that it may be useful in acidic as well as nonacidic foods. This is different from nisin, which is unstable at natural and alkaline pH values (15). Furthermore, strain L50 is able to produce bacteriocin at low temperatures. The inclusion of this strain may therefore protect refrigerated foods from the growth of psychrotrophic spoilage and food-borne pathogenic bacteria such as *Listeria monocytogenes*.

The procedure described for the purification of pediocin L50 resulted in a single absorbance peak containing about 80% of the bacteriocin activity of the starting material. An increase in bacteriocin activity was seen after the cation-exchange and hydrophobic-interaction chromatography steps. An increase in total activity of the bacteriocin during purification was also seen with pediocin PA-1 (22). We did not examine whether the

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  1                                                                  (Lys+)
N-Met Gly Ala Ile Ala Lys+ Leu Val Ala Lys+ Phe Gly Xaa Xaa Ile Val (VAL) Lys+ Tyr Tyr
                                                                   (Ile)
  21
  Lys+ Gln Ile Met Gln Phe Ile Gly Gln (Gly) (Val) Thr Ile Asn Xaa Ile Pro Leu Ile Xaa Phe-C
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FIG. 5. Partial amino acid sequence of the C-terminal part of pediocin L50 obtained after CNBr cleavage. Methionine at position 1 was not seen in the sequence analyzed but was inferred from the cleavage method. Parentheses indicate that the residue was not determined with certainty. Xaa indicates that the residue could not be identified. Lys+ indicates the presence of an unidentified residue in addition to Lys.

apparent activation was due to the removal of inhibitors of bacteriocin activity. It is, however, noteworthy that *B. cereus* was sensitive to the purified bacteriocin, while crude preparations appeared to be inactive against this species. The mass of pediocin L50 was found to be 5,250 by mass spectrometry. The calculated mass of the C-terminal part that was sequenced is about 4,500 to 4,700. Thus, pediocin L50 contains about 46 to 48 amino acid residues. The amino acid sequence obtained by Edman degradation of CNBr-cleaved pediocin L50 does not resemble other known bacteriocin sequences. However, pediocin L50 is small, heat stable, hydrophobic, and cationic, and these features are common for most of the known bacteriocins from lactic acid bacteria (18). To our knowledge, all modified bacteriocins from lactic acid bacteria contain lanthionine or methyl-lanthionine and thus belong to the lantibiotics (29). Pediocin L50 appears to be an exception to this rule, being a nonlantibiotic containing modified amino acid residues. Isolation of the genes necessary for pediocin L50 production and sequencing of the structural gene will be useful in the determination of the complete primary structure of pediocin L50. This work is in progress.

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