JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY



# Bacteriocins from *Pediococcus pentosaceus* L and S from Pork Meat

LI-JUNG YIN, CHIEN-WEI WU, AND SHANN-TZONG JIANG*

Department of Food Science, National Taiwan Ocean University, Keelung, Taiwan 202, Republic of China

Two strains of *Pediococcus pentosaceus* were isolated from refrigerated pork and found to produce antimicrobial substances that may inhibit foodborne pathogens and have potential as natural food preservatives. They were named *P. pentosaceus* L and S. The antimicrobial substances were purified to electrophoretical homogeneity by chloroform extraction and designated pentocins L and S with molecular masses (*M*) of 27 and 25 kDa, respectively. Both pentocins also had broad inhibition spectra and were thermostable. They inhibit the growth of tested spore-forming G (+) and G (−) strains and the germination of *Bacillus subtilis* ATCC 10225, *B. subtilis* ATCC 10254, and *Bacillus cereus* ATCC 11778 spores. The inhibition activities decreased as the glucose in the medium decreased from 8.0 to 2.0%.

**KEYWORDS: Pentocins; *Pediococcus pentosaceus*; bacteriocin; lactic acid bacteria**

## INTRODUCTION

Lactic acid bacteria, isolated from meats (*1*−*3*), can produce a variety of antimicrobial substances including organic acids, diacetyl compounds, and hydrogen peroxide (*4*). They can consequently inhibit the natural microflora of meats including spoilage bacteria and pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus* (*5*−*8*). The processed meat products that rely solely on refrigeration for preservation are particularly susceptible to the growth of psychrophilic spoilage bacteria and pathogens. However, preservation of these products by the production of organic acids and diacetyl compounds is usually organoleptically unacceptable.

*L. monocytogenes* and *Aeromonas hydrophila* can grow at temperatures lower than 5 °C, and *S. aureus* grows at 5−12 °C (*9*), suggesting that refrigerated storage cannot control their growth. Furthermore, *Listeria* spp. are highly associated with fresh meat (*10*) and raw fermented sausage (*11*). *L. monocytogenes* has been recovered from 12−18% of precooked, ready-to-eat chilled foods in England (*12*) and from about one-third of ready-to-eat meat products sampled from Europe and Canada (*10*). The U.S. Department of Agriculture Food Safety and Inspection Service has determined that *S. aureus* can grow slowly at refrigerated temperatures and produce enterotoxin. This organism has been implicated in food poisoning outbreaks involving a number of foods (*13*).

Many strains of lactic acid bacteria produce bacteriocins (*7*, *14*, *15*). According to Jack et al. (*16*) and Tagg et al. (*17*), bacteriocins produced by Gram-positive bacteria are biologically active proteins demonstrating a bactericidal mode of action. Certain bacteriocins produced by lactic acid bacteria inhibit foodborne pathogens, including *Bacillus cereus*, *Clostridium perfringens*, *Listeria* species, and *S. aureus* (*3*, *18*−*20*). These data suggest that bacteriocin-producing lactic acid bacteria have high potential as natural preservatives. Bacteriocins with wide spectra of antimicrobial activity showed good potential for food preservation.

This study was to isolate lactic acid bacteria (LAB) from pork and to further purify and characterize the bacteriocins produced by the isolated LAB.

## 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 −70 °C in 50% glycerol. From these, cultures of LAB were made as stabs on lactobacilli MRS broth (Difco Laboratories, Detroit, MI) supplemented with 1.5% Bacto-Agar (Difco). Working pathogen cultures were made as stabs on Trypticase soy agar (BBL, Microbiology System, Cockeysville, MD) with 2.0% yeast extract (Difco) supplement. These were maintained as stab cultures and transferred bimonthly for a maximum of six transfers before a new culture was made.

**Isolation of Bacteriocin-Producing Lactic Acid Bacteria from Meat.** Ten different cuts of raw pork meat were purchased from a local supermarket. Samples, 50 g, were weighed aseptically into sterile stomacher bags, sealed, and placed at 5 °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 stomacher for 2 min. Serial dilutions were made in 0.1% peptone, spread plated onto MRS agar in quadruplicate, and incubated anaerobically at 37 °C for 48−72 h until growth was evident. Anaerobic incubation (GasPak; BBL) was used to rule out any inhibition due to hydrogen peroxide production. Three plates from the two dilutions having 30−300 colony-forming units (CFU) were overlaid with ∼8 mL of brain−heart infusion (BHI; Difco), which contained 1% agar. The overlay agar was seeded with *L. monocytogenes* CCRC 14845 at a level of $10^5$−$10^6$ organisms/ML. A fourth plate from these

* Author to whom correspondence should be addressed (fax 886-2-2462-0684; e-mail sjiang@mail.ntou.edu.tw).

10.1021/jf025838f CCC: $25.00 
Published on Web 01/18/2003

**Table 1.** Antibiotic Sensitivity of Meat Isolates I and II

| antibiotic | concn ($\mu$g) | R[a] | I[a] | MS[a] | S[a] | isolate I | isolate II |
|---|---|---|---|---|---|---|---|
| ampicillin | 10 | 21[b] | | 22–29 | 30 | MS | MS |
| cefotaxime | 30 | 14 | | 15–22 | 23 | MS | MS |
| ceftazidime | 30 | 14 | | 15–22 | 23 | R | MS |
| cefuroxime | 30 | 14 | | 15–22 | 23 | MS | S |
| clindamycin | 2.0 | 14 | 15–16 | | 17 | S | S |
| erythromycin | 15 | 13 | 14–17 | | 18 | S | S |
| gentamicin | 10 | 12 | 13–14 | | 15 | I | R |
| imipenem | 10 | | | | 16 | S | S |
| moxalactam | 30 | 14 | | 15–22 | 23 | R | R |
| nalidixic acid | 30 | 13 | 14–18 | | 19 | R | R |
| netilmicin | 30 | 12 | 13–14 | | 15 | S | S |
| penicillin | 10 | 19 | | 20–27 | 28 | MS | S |
| tetracyclin | 30 | 14 | 15–18 | | 19 | S | I |
| ticarcillin | 75 | 14 | | 15–19 | 20 | S | S |
| vancomycin | 30 | 9 | 10–11 | | 12 | S | R |

[a] R, resistant; I, intermediate; MS, moderately susceptible; S, susceptible. [b] Diameter of inhibition zone (mm).

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 with inhibition zones were made from the master control plate onto Trypticase soy agar (without glucose) with a 2.0% yeast extract supplement (TSAYE). TSAYE plates were used to eliminate acid production due to glucose present in MRS. The indicator overlay was repeated. Colonies revealed inhibition zones were picked from master control plate with no indicator overlay into MRS broth incubated at 37 °C.

**Identification of Meat Isolates.** Bacteriocin-producing meat isolates were identified to the species level according to the protocol suggested by Schillinger and Lucke (*3*) for lactobacilli. The morphology of the isolates was determined under ×10,000 magnification, using transmission electron microscopy (Hitachi, H-7000, Hitachi Co.).

**Sensitivity of Meat Isolates to Antibiotics.** After the cell density in MRS broth had been adjusted to ~$1.5 \times 10^8$ CFU/mL, the cultures of isolates were streaked onto MRS agar plates and then the paper susceptibility disks with a diameter of 6 mm were stuck on. The resulting samples were incubated at 37 °C for 12 h, and then the size of the inhibition zone was recorded (*21*). The antibiotics used in this study included ampicillin (10 $\mu$g), cefotaxime (30 $\mu$g), ceftazidime (30 $\mu$g), cefuroxime (30 $\mu$g), clindamycin (2 $\mu$g), erythromycin (15 $\mu$g), gentamicin (10 $\mu$g), imipenem (10 $\mu$g), moxalactam (30 $\mu$g), nalidixic acid (30 $\mu$g), netilmicin (30 $\mu$g), penicillin (10 $\mu$g), tetracyclin (30 $\mu$g), ticarcillin (75 $\mu$g), and vancomycin (30 $\mu$g), which were all from BBL.

**Effects of Glucose and Initial pH on Growth, pH of Medium, and Inhibition Activity of Meat Isolates.** After activation, the cultures of isolates were inoculated to MRS, M-17, or TSBYE broths with three different concentrations of glucose (2.0%, control; 4.0 and 8.0%, test groups). After the initial pH values had been adjusted to 4.0, 5.0, 6.0, 6.7 (original values of these broths), and 7.0, samples were incubated at 37 °C. After 0, 6, 12, 24, and 48 h of incubation, lactic acid bacteria count, pH of media, and inhibition ability were determined.

**Isolation of Bacteriocins.** After 48 h of incubation at 37 °C, the MRS broth was centrifuged at 5000*g* and 25 °C for 30 min and then filtered through a membrane (0.45 $\mu$m, no. 4654, Gelman) to remove the cells. The filtrates were further washed with 2 volumes of sterile distilled water and concentrated to ~30 mL using Amicon ultrafiltration (cutoff = 1,000, 180 mL, model 8400). *L. monocytogenes* CCRC 14845 was employed to detect the inhibition ability of the purified bacteriocins. The concentrated fractions were adjusted to pH 6.0 and used as crude bacteriocins for further purification. The filtrates after Amicon ultrafiltration had no inhibition ability against *L. monocytogenes* CCRC 14845.

**Chloroform Extraction.** Four hundred milliliters of MRS broth was inoculated with 0.1% of an overnight culture of *Pediococcus pentosaceus* L and *Pediococcus pentosaceus* S and incubated for 18 h at 37 °C. The culture was centrifuged at 9500*g* for 15 min (4 °C), and the bacteriocin-containing supernatant was filtered through a 0.45 $\mu$m filter. The filtrate was stirred vigorously using a magnetic stirrer for 20 min with 400 mL of chloroform. The mixture was then centrifuged at 10400*g* and 4°C for 20 min. Four phases were observed in the chloroform-containing mixture. The solvent−aqueous interface layer, which had high antibacterial activity, was collected and dissolved in 5−10 mL of buffer (0.1 M Tris-HCl, pH 7.0), because there was no activity in the aqueous phase, solvent phase, and precipitates. The bacteriocin-containing buffer was concentrated with a vacuum evaporator (40 °C) (Rotavapor R114, Büchi) to remove the residual chloroform. The undissolved substances were redissolved using the same buffer twice and subjected to ultrafiltration (cutoff = 50000 Da; Amicon ultrafiltration, XM50). The filtrates were concentrated using ultrafiltration (cutoff = 10000 Da; Amicon ultrafiltration, YM10). The residues were dissolved in a minimal volume of 0.1 M Tris-HCl buffer (pH 7.0). The final volume of the concentrated bacteriocin was 2−3 mL (*22*).

**Sodium Dodecyl Sulfate—Polyacrylamide Gel Electrophoresis (SDS-PAGE).** To confirm the purity of purified bacteriocin and to determine its molecular weight, purified bacteriocins in a dissociating buffer (62.5 mM Tris-HCl buffer, pH 6.8, containing 3% SDS and 0.002% bromophenol blue) were heated at 100 °C for 5 min. The purity of the purified bacteriocins was determined using an 8−17.5% gradient polyacrylamide SDS-PAGE, whereas the MW was determined by 15% SDS-PAGE according to the method of Laemmli (*23*). Albumin (66 kDa), glutamate dehydrogenase (53 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), and trypsin inhibitor (20.1 kDa) were used as protein markers.

**Protein Concentration.** Protein concentrations of purified bacteriocins during purification were determined by using the dye-binding method of Bradford (*24*). Bovine serum albumin was used as a standard protein.

**Inhibition Assay.** The agar diffusion method was used to assay the inhibition ability of bacteriocins according to the method of Piddock (*25*). The broth was inoculated with indicator organisms (**Table 1**) and incubated at 37 °C for 24 h. After the level of cells in the broth had been adjusted to ~$1.5 \times 10^8$ CFU/mL, ~0.1 mL of indicator broth was mixed uniformly with 15 mL of warm agar. The agar was poured into a Petri dish and allowed to stand at 5 °C for 1 h. After the agar had been punched with a stainless ring having a diameter of 6 mm, ~25 $\mu$L of sample was added into the hole and incubated at 5 °C for 24 h to allow bacteriocin diffusion. The resulting samples were incubated at 37 °C for 6 h, and then the size of the inhibition zone was recorded to qualify the inhibition ability.

**Biochemical Properties.** *Sensitivity of Bacteriocin to the Proteolytic Enzymes.* After the pH of the purified bacteriocins had been adjusted to 4.0, 5.0, 6.0, 7.0, and 8.0 using 1.0 N HCl or 1.0 N NaOH, proteases were added and incubated at 37 °C for 2 h. The reaction was stopped by heating in an 80 °C water bath for 15 min. After the mixture had cooled to room temperature, the inhibition ability of the resulting bacteriocins was determined according to the method of Piddock (*25*). Proteases used in this study included pepsin (from porcine stomach mucosa, Sigma), α-chymotrypsin (from bovine pancreas, Sigma), Pronase (from *Streptomyces griseus*, Sigma), and bromelain (from pineapple stem, Sigma).

*Thermostability of Purified Bacteriocins.* After the pH of purified bacteriocins had been adjusted to 4.0, 5.0, 6.0, 7.0, and 8.0 using 1.0 N HCl or 1.0 N NaOH, the resulting samples were incubated at 80 or 100 °C for 15, 30, 45, and 60 min or at 121 °C for 15 min. They were cooled to room temperature, and the inhibition ability was determined according to the method of Piddock (*25*).

*Bacteriocin Spectrum of Activity.* The isolated LAB were screened for activity against a pathogen panel and spoilage bacteria.

## RESULTS

Two bacteriocin-producing lactic acid bacteria were isolated from the meat samples. According to the physiological characteristics, including the Gram stain, catalase test, motility, growth temperature, growth pH, and resistance to NaCl, isolates I and II are similar, except that isolate I can grow at a lower

**Table 2.** Growth of *P. pentosaceus* L and S in MRS Broth with Different Glucose Contents and Initial pH at 37 °C

| glucose content (%) | pH | *P. pentosaceus* L | | | | | *P. pentosaceus* S | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 h | 6 h | 12 h | 24 h | 48 h | 0 h | 6 h | 12 h | 24 h | 48 h |
| 2 | 4.0 | 4.80[a] | 5.85 | 6.08 | 6.53 | 6.50 | 4.85 | 5.28 | 6.18 | 7.70 | 8.46 |
| | 5.0 | 4.85 | 7.32 | 8.60 | 8.04 | 8.41 | 4.86 | 7.46 | 8.48 | 8.30 | 8.40 |
| | 6.0 | 4.88 | 7.71 | 8.48 | 8.82 | 8.70 | 4.84 | 7.62 | 8.48 | 8.51 | 8.58 |
| | 6.7 | 4.90 | 7.48 | 8.91 | 9.20 | 9.04 | 4.91 | 6.54 | 8.54 | 8.45 | 8.83 |
| | 7.0 | 4.89 | 7.58 | 8.81 | 8.89 | 8.79 | 4.89 | 7.59 | 8.41 | 8.51 | 8.72 |
| 4 | 4.0 | 4.85 | 5.00 | 6.36 | 6.40 | 6.60 | 4.90 | 6.26 | 6.51 | 7.89. | 8.41 |
| | 5.0 | 4.88 | 7.36 | 8.67 | 8.79 | 9.57 | 4.87 | 7.32 | 8.58 | 9.28 | 8.72 |
| | 6.0 | 4.91 | 7.41 | 8.68 | 8.89 | 9.32 | 4.92 | 7.48 | 8.58 | 9.15 | 8.70 |
| | 6.7 | 4.89 | 7.67 | 8.60 | 8.83 | 9.08 | 4.93 | 7.74 | 8.41 | 9.32 | 8.85 |
| | 7.0 | 4.92 | 7.59 | 8.61 | 8.95 | 8.79 | 4.92 | 7.41 | 8.72 | 8.66 | 8.54 |
| 8 | 4.0 | 4.90 | 5.56 | 6.46 | 6.53 | 6.70 | 4.90 | 5.88 | 6.53 | 8.20 | 8.89 |
| | 5.0 | 4.89 | 6.65 | 8.52 | 8.60 | 9.64 | 4.89 | 7.32 | 8.57 | 8.90 | 8.95 |
| | 6.0 | 4.91 | 7.38 | 8.51 | 8.79 | 8.45 | 4.93 | 7.41 | 8.66 | 9.11 | 8.78 |
| | 6.7 | 4.90 | 7.20 | 8.71 | 8.72 | 8.86 | 4.91 | 7.49 | 9.48 | 8.46 | 8.85 |
| | 7.0 | 4.95 | 7.52 | 8.75 | 8.30 | 9.26 | 4.96 | 7.49 | 9.18 | 8.81 | 8.97 |

[a] Log CFU/mL.

temperature (down to 4 °C for 7 days) and isolate II can resist NaCl (10% NaCl for 3 days) (data not shown). From the API 50CHL identification system, these two isolates are highly similar to *P. pentosaceus* CCRC 14024, except for the utilization of D-xylose, salicine, and β-gentiobiose (data not shown). Transmission electron microscopy indicated both isolates with cocci morphology (data not shown). On the basis of these results and the schemes for identifying species developed by Schillinger and Lucke (*3*), isolates I and II are identified as *P. pentosaceus*. However, the sensitivity to antibiotics (**Table 1**) suggested that the physiological properties of these two strains were different. They were, therefore, denominated *P. pentosaceus* L and *P. pentosaceus* S, respectively.

**Effects of Glucose Content and Initial pH on Growth, pH of Medium, and Antagonistic Activity of *P. pentosaceus* L and S.** During 48 h of incubation, despite the glucose content in the MRS broth, the growth of *P. pentosaceus* L in broth with an initial pH of 4.0 was significantly slower than that in broths with other pH values ($p < 0.05$). However, this phenomenon was not observed in *P. pentosaceus* S, although the growth at pH 4.0 was much slower than that at higher pH values during the first 24 h of incubation (**Table 2**). This might be due to the growth suppressed by the acidic environment during the early stage of incubation. The highest growth of these two strains in broths with the tested initial pH of 5.0−7.0 was observed after 12 or 24 h of incubation, despite the glucose content (**Table 2**). The pH of the medium decreased during incubation of both *P. pentosaceus* L and S in MRS broth. After 48 h of incubation in those with an initial pH of 5.0−7.0, the pH of the medium decreased to ∼3.9 (data not shown). No significant difference in medium pH between these two strains was observed ($p < 0.05$) (data not shown). However, the pH of all media incubated with an initial pH of 4.0 decreased gradually during incubation and reached ∼3.5 after 48 h of incubation (data not shown).

To further confirm the effect of the initial pH and glucose content on the antagonistic activity, *P. pentosaceus* L, *P. pentosaceus* S, or both in MRS, M-17, or TSBYE broth with different initial pH values and glucose contents were cultivated with *L. monocytogenes* CCRC 14845. No antagonistic activity was observed on *P. pentosaceus* L grown in M-17 broth (**Table 3**). *P. pentosaceus* S grown in MRS, M-17, or TSBYE broth revealed antagonistic activity against *L. monocytogenes* CCRC 14845. The higher in glucose content or lower in initial pH, the greater the antagonistic activity during cultivation ($p < 0.05$) (**Table 3**). However, when they were incubated in TSBYE containing 0.25 or 5.25% glucose or 0.1% Tween 80 and 5.25% glucose, both strains revealed inhibition activity against *L. monocytogenes* CCRC 14845 despite individual or mixed cultivation. The higher the initial LAB count, the greater inhibition activity against *L. monocytogenes* CCRC 14845 was observed during incubation ($p < 0.05$) (**Table 4**).

**Table 3.** Antagonistic Activity of *P. pentosaceus* L and S against *L. monocytogenes* CCRC 14845 Grown in Broths with Different Glucose Contents and Initial pH Values

| medium | glucose content (%) | pH | *P. pentosaceus* L | | | *P. pentosaceus* S | | |
|---|---|---|---|---|---|---|---|---|
| | | | 12 h[a] | 24 h | 48 h | 12 h | 24 h | 48 h |
| MRS | 2.0 | 4.0 | 28.0[b] | 54.3 | 85.0 | 36.3 | 85.0 | 96.3 |
| | | 5.0 | 13.0 | 45.0 | 74.3 | 28.0 | 54.3 | 85.0 |
| | | 6.0 | 0 | 36.3 | 64.0 | 0 | 45.0 | 74.3 |
| | | 6.7 | 0 | 28.0 | 45.0 | 0 | 36.3 | 64.0 |
| | 4.0 | 4.0 | 36.3 | 74.3 | 108.0 | 45.0 | 96.3 | 108.0 |
| | | 5.0 | 28.0 | 64.3 | 96.3 | 36.3 | 85.0 | 96.3 |
| | | 6.0 | 0 | 54.3 | 85.0 | 28.0 | 74.3 | 85.0 |
| | | 6.7 | 0 | 45.0 | 74.3 | 13.0 | 64.0 | 85.0 |
| | 8.0 | 4.0 | 45.0 | 94.3 | 120.3 | 54.3 | 108.0 | 120.3 |
| | | 5.0 | 36.3 | 85.0 | 108.0 | 45.0 | 94.3 | 108.0 |
| | | 6.0 | 28.0 | 74.3 | 108.0 | 36.3 | 85.0 | 108.0 |
| | | 6.7 | 13.0 | 64.0 | 94.3 | 13.0 | 74.3 | 94.3 |
| M-17 | 2.0 | 4.0 | 0 | 0 | 0 | 45.0 | 64.0 | 85.0 |
| | | 5.0 | 0 | 0 | 0 | 45.0 | 64.0 | 85.0 |
| | | 6.0 | 0 | 0 | 0 | 36.3 | 64.0 | 74.3 |
| | | 6.7 | 0 | 0 | 0 | 36.3 | 54.3 | 64.0 |
| | 4.0 | 4.0 | 0 | 0 | 0 | 45.0 | 64.0 | 85.0 |
| | | 5.0 | 0 | 0 | 0 | 64.0 | 64.0 | 85.0 |
| | | 6.0 | 0 | 0 | 0 | 28.0 | 54.3 | 85.0 |
| | | 6.7 | 0 | 0 | 0 | 28.0 | 54.3 | 64.0 |
| | 8.0 | 4.0 | 0 | 0 | 0 | 45.0 | 64.0 | 85.0 |
| | | 5.0 | 0 | 0 | 0 | 64.0 | 64.0 | 85.0 |
| | | 6.0 | 0 | 0 | 0 | 28.0 | 54.3 | 85.0 |
| | | 6.7 | 0 | 0 | 0 | 28.0 | 54.3 | 64.0 |
| TSBYE | 0.25 | | | 54.3 | 36.3 | | 85.0 | 45.0 |
| | 5.25 | | | 74.3 | 45.0 | | 133.0 | 85.0 |
| | 5.25 (with 0.1% Tween 80) | | | 74.3 | 45.0 | | 146.3 | 85.0 |

[a] Incubation time. [b] Inhibition zone ($mm^2$).

**Bacteriocins Produced by *P. pentosaceus* L and *P. pentosaceus* S.** Following concentration by Amicon ultrafiltration, the inhibitory substances were found in the concentrated fraction with molecular mass ($M$) >1000 Da. The bacteriocins from *P. pentosaceus* L and S were isolated by chloroform extraction. After ultrafiltration to exclude the $M > 50$ kDa and $M < 10$ kDa fractions, the bacteriocins were purified to electrophoretical homogeneity (**Figure 1**) and named pentocins L and S, respectively. The $M$ of purified pentocins L and S were 27 and 25 kDa, respectively (**Figure 1**). Both pentocins L and S were inactivated by various proteolytic enzymes including pepsin, α-chymotrypsin, Pronase, and bromelain (data not shown). They were very stable at temperatures below 80 °C and pH 4.0−8.0 (**Tables 5** and **6**). There was >80% activity left even after 30 min of heating at 80 °C, pH 4.0−8.0, for pentocin L and pH 4.0−6.0 for pentocin S. About 41 and 37% activity of pentocin S was left even after 30 min of heating at 100 °C, pH 4.0 and 5.0, respectively, and 34% activity of pentocin L was left after 30 min of heating at 100 °C, pH 4.0. Both pentocins still had activity even after 15 min of heating at 100 °C, under the tested pH values. This phenomenon suggested the potential of these bacteriocins for use in the preservation of foods.

The spectrum of antimicrobial activity of the pentocins L and S is shown in **Tables 7** and **8**. Both pentocins inhibited both vegetative cells and spores of all tested spore-forming strains.

**Table 4.** Change in *L. monocytogenes* CCRC14845 Count When Cultivated with *P. pentosaceus* L or S in TSBYE Broth Containing 0.25 or 5.25% Glucose or 0.1% Tween 80 and 5.25% Glucose during Incubation at 37 °C

| | | incubation time | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | count of LAB | 0 h | | | 24 h | | | 48 h | | |
| strain | (CFU/mL) | A[a] | B | C | A | B | C | A | B | C |
| control[b] | | 4.28[c] | 3.58 | 3.48 | 6.02 | 5.94 | 5.33 | 7.86 | 7.84 | 7.46 |
| L | $5 \times 10^5$ | 3.96 | 3.90 | 3.98 | 3.96 | 2.70 | <2.30 | 3.23 | 3.20 | 3.19 |
| | $5 \times 10^4$ | 4.08 | 4.11 | 4.03 | 4.58 | 3.72 | <2.30 | 4.38 | 4.28 | 4.22 |
| | $5 \times 10^3$ | 4.08 | 4.03 | 4.13 | 4.70 | 3.77 | 3.78 | 5.52 | 5.15 | 5.13 |
| | $5 \times 10^2$ | 4.00 | 4.15 | 4.11 | 4.87 | 3.78 | 3.89 | 6.08 | 5.95 | 5.90 |
| | $5 \times 10^1$ | 3.95 | 4.33 | 4.35 | 4.90 | 4.06 | 4.26 | 6.98 | 6.18 | 6.72 |
| S | $5 \times 10^5$ | 3.99 | 3.06 | 3.11 | 3.19 | <2.30 | <2.30 | 3.30 | 3.25 | 3.22 |
| | $5 \times 10^4$ | 3.89 | 3.35 | 3.36 | 3.18 | 3.08 | 2.89 | 4.41 | 4.35 | 4.33 |
| | $5 \times 10^3$ | 4.04 | 4.06 | 4.03 | 4.15 | 3.25 | 3.33 | 5.40 | 5.16 | 5.10 |
| | $5 \times 10^2$ | 4.11 | 4.25 | 4.22 | 4.33 | 3.68 | 3.56 | 6.10 | 6.01 | 5.95 |
| | $5 \times 10^1$ | 4.20 | 2.50 | 2.66 | 4.61 | 4.11 | 4.06 | 6.62 | 6.55 | 6.43 |
| L + S[d] | $5 \times 10^5$ | 3.95 | <2.30 | <2.30 | 3.03 | <2.30 | <2.30 | 3.15 | 3.02 | 3.00 |
| | $5 \times 10^4$ | 3.87 | 3.15 | 3.06 | 3.23 | 3.05 | 2.44 | 3.28 | 3.23 | 3.45 |
| | $5 \times 10^3$ | 4.00 | 4.06 | 3.73 | 3.34 | 3.13 | 3.06 | 4.81 | 3.34 | 3.33 |
| | $5 \times 10^2$ | 4.15 | 4.25 | 4.12 | 3.64 | 3.22 | 3.19 | 5.38 | 4.64 | 3.52 |
| | $5 \times 10^1$ | 4.00 | 4.44 | 4.39 | 3.98 | 4.45 | 4.40 | 6.76 | 4.98 | 4.45 |

[a] A, TSBYE; B, TSBYE + 5.0% glucose; C, TSBYE + 5.0% glucose + 0.1% Tween 80; TSBYE originally contains 0.25% glucose. [b] Without LAB. [c] Log CFU/mL. [d] Mixed cultivation with *P. pentosaceus* L and S.

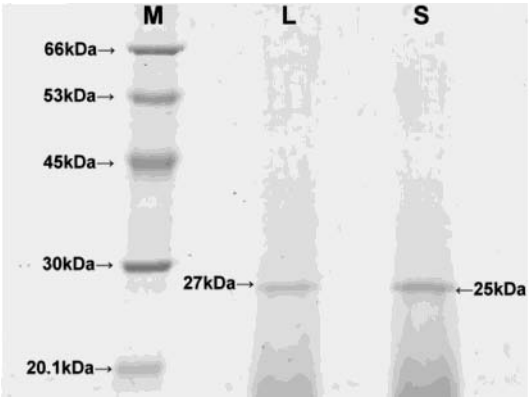


**Figure 1.** SDS-PAGE (8–17.5% gradient polyacrylamide) of the purified pentocins L and S.

**Table 5.** Effect of Heat Treatment on the Inhibition Activity of Pentocin L at Different pH Values against *L. monocytogenes* CCRC 14845

| | inhibition zone ($mm^2$)/inhibition activity (%) | | | | |
|---|---|---|---|---|---|
| heat treatment | pH 4.0 | pH 5.0 | pH 6.0 | pH 7.0 | pH 8.0 |
| 35 °C, 60 min | 108/100 | 96.3/100 | 85.0/100 | 74.3/100 | 64.0/100 |
| 80 °C, 15 min | 96.3/89 | 85.0/88 | 74.3/87 | 64.0/86 | 54.3/85 |
| 80 °C, 30 min | 96.3/89 | 85.0/88 | 74.3/87 | 64.0/86 | 54.3/85 |
| 80 °C, 45 min | 85.0/79 | 74.3/77 | 54.3/64 | 45.0/61 | 36.3/57 |
| 80 °C, 60 min | 74.3/69 | 64.0/66 | 45.0/53 | 28.0/38 | 13.0/20 |
| 100 °C, 15 min | 54.3/50 | 36.3/38 | 28.0/29 | 13.0/18 | 13.0/20 |
| 100 °C, 30 min | 36.3/34 | 0 | 0 | 0 | 0 |
| 100 °C, 45 min | 0 | 0 | 0 | 0 | 0 |
| 100 °C, 60 min | 0 | 0 | 0 | 0 | 0 |
| 121 °C, 15 min | 0 | 0 | 0 | 0 | 0 |

They also inhibited the growth of Gram-negative strains including *Klebsiella oxytoca* ATCC 13182, *Proteus vulgaris* ATCC 13315, *Shigella dysenteriae* ATCC 13983, and *Vibrio cholerae*, Gram-positive strains including *L. monocytogenes* RII, *L. monocytogenes* LM, *L. monocytogenes* CCRC 14845, and *Clostridium sporogenous* ATCC 11259. However, pentocin L could further inhibit the growth of *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC11303, and *Staphylococcus epidermidis* ATCC 14990.

**Table 6.** Effect of Heat Treatment on the Inhibition Activity of Pentocin S at Different pH Values against *L. monocytogenes* CCRC 14845

| | inhibition zone ($mm^2$)/inhibition activity (%) | | | | |
|---|---|---|---|---|---|
| heat treatment | pH 4.0 | pH 5.0 | pH 6.0 | pH 7.0 | pH 8.0 |
| 35 °C, 60 min | 133.0/100 | 120.3/100 | 108/100 | 85.0/100 | 74.3/100 |
| 80 °C, 15 min | 120.3/90 | 108.0/88 | 96.3/89 | 74.3/87 | 54.3/73 |
| 80 °C, 30 min | 120.3/90 | 108.0/88 | 96.3/89 | 54.3/64 | 45.0/61 |
| 80 °C, 45 min | 108.0/81 | 96.3/80 | 85.0/79 | 36.3/43 | 28.0/38 |
| 80 °C, 60 min | 96.3/72 | 85.0/71 | 54.3/50 | 28.0/33 | 13.0/18 |
| 100 °C, 15 min | 85.0/64 | 64.0/53 | 28.0/26 | 13.0/15 | 13.0/18 |
| 100 °C, 30 min | 54.3/41 | 45.0/37 | 0 | 0 | 0 |
| 100 °C, 45 min | 0 | 0 | 0 | 0 | 0 |
| 100 °C, 60 min | 0 | 0 | 0 | 0 | 0 |
| 121 °C, 15 min | 0 | 0 | 0 | 0 | 0 |

## DISCUSSION

The inhibitory substances produced by *P. pentosaceus* L and S can be characterized as bacteriocins, because inhibition due to acid, hydrogen peroxide, and bacteriophage has been excluded. Also, the proteinaceous nature of the pentocins L and S was confirmed by the sensitivity to proteases.

The sensitivity of *Listeria* strains to the bacteriocins produced by *P. pentosaceus* L and *P. pentosaceus* S is not surprising, because the screening procedure was based on the inhibition of *L. monocytogenes* CCRC 14845. Ahn and Stiles (*26*) reported the limited inhibitory activity against *L. monocytogenes* by bacteriocin-producing strains of LAB isolated from vacuum-packaged meats. These strains were screened as potential producers against the bacteria of LAB as indicator organisms. The indicator organism used in the initial screening needs to reflect the final or proposed application of the bacteriocin-producing strains. Spelhaug and Harlander (*20*) also identified the sensitivity of *L. monocytogenes* V7 and Scott A and *S. aureus* 196E to bacteriocins produced by *P. pentosaceus* 43200 (FBB61) and *L. lactis* 11454. Along with our studies, these results suggest that the inability to detect antimicrobial activity associated with *P. pentosaceus* 43200 by Roller et al. (*27*) might be due to the broth medium that did not support pediocin A production. Schillinger and Lucke (*3*) identified the sensitivity

**Table 7.** Antibacterial Spectrum of Pentocins L and S

| organism | pentocin L | pentocin S |
|---|---|---|
| G (–) | | |
| *Alcaligenes faecalis* ATCC 8750 | –[a] | – |
| *Aeromonas faecalis* | – | – |
| *Enterobacter aerogenes* ATCC 13048 | + | – |
| *Escherichia coli* ATCC 11229 | – | – |
| *E. coli* ATCC 11303 | + | – |
| *Klebsiella oxytoca* ATCC 13182 | + | + |
| *Pseudomonas fluorescens* ATCC 13523 | – | – |
| *Proteus vulgaris* ATCC 13315 | + | + |
| *Shigella dysenteriae* ATCC 13983 | + | + |
| *Vibrio cholerae* | + | + |
| G (+) | | |
| *Listeria monocytogenes* Ram II | + | + |
| *L. monocytogenes* LM | + | + |
| *L. monocytogenes* CCRC 14845 | + | + |
| *Staphylococcus aureus* ATCC 25923 | – | – |
| *Staphylococcus epidermidis* ATCC 14990 | + | – |
| *Streptococcus faecalis* DS-5 | – | – |
| spore-forming bacteria | | |
| *Bacillus circulans* ATCC 11059 | + | + |
| *Bacillus subtilis* ATCC 10225 | + | + |
| *B. subtilis* ATCC 10254 | + | + |
| *Bacillus cereus* ATCC 11778 | + | + |
| *Clostridium sporogenous* ATCC 11259 | + | + |

[a] –, inhibition zone <6 mm; +, inhibition zone >6 mm.

**Table 8.** Effect of Pentocins L and S on the Growth of Bacterial Vegetative Cell and Spores

| | inhibition zone ($mm^2$) | |
|---|---|---|
| bacterium | pentocin L | pentocin S |
| *B. subtilis* ATCC 10225 | | |
| vegetative cell | 120.3 | 189.0 |
| spore | 64.0 | 85.0 |
| *B. subtilis* ATCC 10254 | | |
| vegetative cell | 174.3 | 146.3 |
| spore | 96.3 | 108.0 |
| *B. cereus* ATCC 11778 | | |
| vegetative cell | 236.3 | 236.3 |
| spore | 189.0 | 189.0 |

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. (*19*) tested the sensitivity of *Listeria* strains to the bacteriocin produced by *L. acidophilus* 88 and did not demonstrate any activity. They also confirmed the activity of bacteriocins produced by *L. lactis* 11454 and *P. pentosaceus* 43200 to *Listeria* spp. Results obtained in this study also demonstrated good antimicrobial activity of *P. pentosaceus* L and S against *L. monocytogenes* RII, LM, and CCRC 14845. According to Okereke and Montville (*28*), bacteriocins from *L. lactis* 11454, *L. acidophilus* N2, *P. pentosaceus* 43200 and 43201, and *L. plantarum* BN, Lb592, and Lb75 could inhibit *Clostridium botulinum* spores. In this study, pentocins L and S were found to be able to inhibit *C. sporogenous* ATCC 11259.

Because of the nature of the isolation procedure, the bacteriocin-producing *P. pentosaceus* L and S grew well at refrigerated temperatures, which would consequently give them a competitive edge against the spoilage organisms and pathogens of refrigerated meats in markets. However, if these products were contaminated with psychrophilic foodborne pathogens, they would have a serious effect on consumers' health. The use of bacteriocin-producing microorganisms may provide a natural means of preservation. The ability of these organisms to inhibit pathogens and spoilage bacteria simultaneously in laboratory medium and in meat systems is under investigation.

## ACKNOWLEDGMENT

We sincerely thank Professors H. C. Chen, C. L. Pan, and G. J. Tsai, Department of Food Science, National Taiwan Ocean University, Taiwan, for their courtesy in providing the tested strains.

## LITERATURE CITED

(1) Berry, E. D.; Liewen, M. B.; Mandigo, R. W.; Hutkins, R. W. Inhibition of *Listeria monocytogenes* by bacteriocin-producing *Pediococcus* during the manufacture of fermented semidry sausage. *J. Food Prot.* **1990**, *53*, 194−197.

(2) Schillinger, U.; Lucke, F. K. Identification of *Lactobacillili* from meat and meat products. *Food Microbiol.* **1987**, *4*, 199−208.

(3) Schillinger U.; Lucke, F. K. Antibacterial activity of *Lactobacillus sake* isolated from meat. *Appl. Environ. Microbiol.* **1989**, *55*, 1901−1906.

(4) Gilliland, S. E. Role of starter culture bacteria in food preservation. In *Bacteria Starter Cultures for Foods*; Gilliland, S. E., Ed.; CRC Press: Boca Raton, FL, 1985; pp 176−185.

(5) Bacus, J. N. Update: meat fermentation. *Food Technol.* **1984**, *38*, 59−63.

(6) Contreras, B. G. L.; De Vuyst, L.; Devreese, B.; Busanyova, K.; Raymaeckers, J.; Bosman, F.; Sablon, E.; Vandamme, E. J. Isolation, purification and amino acid sequence of lactobin A, one of the two bacteriocins produced by *Lactobacillus amylovorus* LMGP-13139. *Appl. Environ. Microbiol.* **1997**, *63*, 13−20.

(7) De Vuyst, L.; Vandamme, E. J. Antimicrobial potential of lactic acid bacteria. In *Bacteriocins of Lactic Acid Bacteria: Microbiology, Genetics and Applications*; De Vuyst, L., Vandamme, E. J., Eds.; Chapman and Hall: London, U.K., 1994; pp 1−11 and 91−142.

(8) Ennahar, S.; Aoude-Werner, D.; Sorokine, O.; Van Dorsselaer, A.; Bringel, F.; Hubert, J. C.; Hasselmann, C. Production of pediocin AcH by *Lactobacillus plantarum* WHE 92 isolated from cheese. *Appl. Environ. Microbiol.* **1996**, *62*, 4381−4387.

(9) Palumbo, S. A. Is refrigeration enough to restrain food-borne pathogens. *J. Food Prot.* **1986**, *49*, 1003−1009.

(10) Johnson, J. L.; Doyle, M. P.; Cassens, P. G. *Listeria monocytoggenes* and other *Listeria* spp. in meat and meat products. A review. *J. Food Prot.* **1990**, *53*, 81−91.

(11) Farber, J. M.; Warburton, D. W.; Horu, L.; Tittiger, F. Surveillance of raw-fermented (dry-cured) sausages for the presence of *Listeria* spp. *Can. Inst. Food Sci. Technol. J.* **1988**, *21*, 430−434.

(12) Gilliland, R. J.; Miller, K. L.; Roberts, D. *Listeria monocytogenes* and chilled foods. *Lancet* **1989**, *1*, 383−384.

(13) Bean, N. H.; Griffin, P. M. Foodborne disease outbreaks in the United States 1973−1987: Pathogens, vehicles and trends. *J. Food Prot.* **1990**, *53*, 804−817.

(14) Jiménez-Diaz, R.; Rios-Sanchez, R. M.; Desmazeaud, M.; Ruiz-Barba, J. L.; Piard, J. C. Plantaricins S and T, two new bacteriocins produced by *Lactobacillus plantarum* LPCO 10 isolated from a green olive fermentation. *Appl. Environ. Microbiol.* **1993**, *59*, 1416−1424.

(15) Klaenhammer, T. R. Bacteriocins of lactic acid bacteria. *Biochimie* **1988**, *70*, 337−349.

(16) Jack, R. W.; Tagg, J. R.; Ray, B. Bacteriocins of gram-positive bacteria. *Microbiol. Rev.* **1995**, *59*, 171−200.

(17) Tagg, J. R.; Dajani, A. S.; Wannamaker, L. W. Bacteriocins of gram-positive bacteria. *Bacteriol. Rev.* **1976**, *40*, 722−756.

(18) Carminati, D.; Giraffa, G.; Bossi, M. G. Bacteriocinlike inhibitors of *Streptococcus lactis* against *Listeria monocytogenes*. *J. Food Prot.* **1989**, *52*, 614−617.

(19) Harris, L. J.; Daeschel, M. A.; Stiles, M. E.; Klaenhammer, T. R. Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes*. *J. Food Prot.* **1989**, *52*, 384−387.

(20) Spelhaug, S. R.; Harlander, S. K. Inhibition of foodborne bacterial pathogens by bacteriocins from *Lactobacillus lactis* and *Pediococcus pentosaceus*. *J. Food Prot.* **1989**, *52*, 856−862.

(21) Tsai, W. C. *Practical Clinical Microbial Diagnosis*; Tsai, W. C., Ed.; Jiu-Zhou Publishing: Taipei, Taiwan, 1993; pp 385−430.
(22) Burianek, L. L.; Yousef, A. E. Solvent extraction of bacteriocins from liquid cultures. *Lett. Appl. Microbiol.* **2000**, *31*, 193−197.
(23) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature* **1970**, *277* (259), 680−685.
(24) Bradford, M. M. A rapid and sensitive method for the quantition of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(25) Piddock, L. J. V. Techniques used for the determination of antimicrobial resistance and sensitivity in bacteria. *J. Appl. Bacterol.* **1990**, *68*, 307−318.
(26) Ahn, C.; Stiles, M. E. Antibacterial activity of lactic acid bacteria isolated from vacuum-packaged meats. *J. Appl. Bacteriol.* **1990**, *69*, 302−310.
(27) Roller, S. D.; Barnaby-Smith, F.; Dea, I. C. M.; Swift, R. *Inst. Food Technol. Annu. Meet. Prog. Abstr.* **1990**, *No. 64*, 115.
(28) Okereke, A.; Montville, T. J. Bacteriocin-mediated inhibition of *Clostridium botulinum* spores by lactic acid bacteria at refrigeration abuse temperatures. *Appl. Environ. Microbiol.* **1991**, *57*, 3423−3428.

**Received for review July 23, 2002. Revised manuscript received November 28, 2002. Accepted December 16, 2002.**

JF025838F