Comparative Study of the Antilisterial Activity of Nisin A and Pediocin AcH in Fresh Ground Pork Stored Aerobically at 5°C

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ABSTRACT

Listeria innocua strain Lin11 was used to compare the inhibitory activity of two bacteriocins (nisin A and pediocin AcH) in a decontamination process consisting of soaking artificially contaminated pieces of raw pork meat in a bacteriocin-containing solution before they were ground and stored aerobically at 5°C. Nisin A proved to be considerably more efficient than pediocin AcH, but generally after two days surviving bacteria in meat treated with each bacteriocin resumed growth at a rate similar to that of the control. Increasing the nisin concentration in the decontaminating bath resulted in greater loss of viability followed by regrowth of survivors. In addition, listeria cells surviving nisin action were found to have become resistant to nisin whereas survivors of pediocin AcH remained susceptible to this bacteriocin. The factors affecting bacteriocin activity in raw ground pork meat were then investigated. With the use of cold water and hot aqueous acid extraction to determine free (not bound) and total (bound to meat and free) bacteriocins, respectively, it was found that nisin was more stable than pediocin AcH. The loss of effectiveness, especially of pediocin AcH, was attributed to rapid degradation by meat proteases. It was concluded that nisin A is more appropriate than pediocin AcH for decontamination of this kind of meat but that routine use of nisin A at concentrations not high enough to eradicate all listerial cells could result in emergence of populations resistant not only to nisin A but to other bacteriocins.

Key words: Nisin, pediocin, fresh meat, listeria, decontamination

Refrigeration of fresh meat and storage in a vacuum or modified atmosphere can control microbial growth and extend its shelf life. However, these conditions facilitate growth of psychrotrophic anaerobic and facultative anaerobic bacteria such as some lactic acid bacteria and Enterobacteriaceae, as well as bacterial species such as Brochothrix thermosphaeta, Listeria monocytogenes, and Clostridium lariame (11, 16, 22). Because of its ubiquitous presence in the environment and resistance to adverse conditions, eradication of L. monocytogenes from meat processing facilities and in raw meat or meat products by physical and chemical antimicrobial treatments can be very difficult. Antibacterial compounds from different sources are now being studied to determine their efficiency in controlling Listeria in food systems. One such group is the small hydrophobic peptides or bacteriocins of some lactic acid bacteria (20). Several studies have shown the bactericidal effect of nisin and pediocins against L. monocytogenes in raw and heated meat (9, 15, 16, 26, 28, 29, 30) as well as in fermented and unfermented meat products (10, 14, 17, 25, 40). However, the results of these studies showed a great variation in the bactericidal activity of nisin and pediocins against Listeria spp. One reason for this variation could be that certain meat components could reduce the antilisterial effect of bacteriocins, but this aspect has not been addressed satisfactorily.

In this study we found that the listerial populations in fresh ground pork stored aerobically at 5°C can be appreciably reduced by soaking contaminated meat in a bacteriocin-containing solution before grinding. We compared the bactericidal effectiveness of two well-known bacteriocins, nisin A and pediocin AcH (which is identical to pediocin PA-1), to identify the importance of two factors that could adversely affect their efficient use in fresh meat systems, namely bacteriocin stability and the presence of resistant cells in sensitive listerial populations.

MATERIALS AND METHODS

Organisms and culture conditions
Listeria innocua Lin11 and L. ivanovii LIPE (from the Institut Pasteur, Paris, France) were grown for 16 h at 30°C in TSB (tryptic soy broth, Difco) containing 0.6% yeast extract and adjusted to pH 6.5 (TSBYE). Solid medium (TSYE) was prepared by admixing agar (Difco) into TSBYE at 12 g/liter. The pediocin AcH producer strain, Pediococcus acidilactici F (provided by B. Ray, University of Wyoming, Laramie, WY, USA) was grown in TGE broth (Trypticase glucose yeast extract (6)). Prior to utilization the strains from frozen stocks were subcultured twice in appropriate media.

Preparation and assay of bacteriocins
A 106-IU/ml stock solution of nisin A was prepared by dissolving pure nisin A (50 × 10^6 IU/g, from Aplin & Barrett Ltd., Bevermister, UK) in 0.02 N HCl. Pediocin AcH was purified essentially by the method of Yang et al. (39) with minor modifications. Briefly, P. acidilactici F was grown at 37°C in 1 liter of TGE...
broth to a final pH of 3.8. Cells were harvested by centrifugation, washed, and resuspended in 100 ml of sterile deionized water, the pH was adjusted to 2.0 with concentrated HCl, and the suspension was heated at 70°C for 1 h. Cells were removed by centrifugation, and the culture supernatant containing a high level of pediocin AcH was subjected to ultrafiltration against a membrane with a 1,000-Da cutoff (Millipore Corporation, Bedford, MA, USA) to concentrate pediocin AcH.

The titer of each of the two bacteriocin solutions was determined by the "spot-on-lawn" method. Briefly, serial twofold dilutions of a solution in 10 mM sodium phosphate buffer (pH 6.5) were prepared and 10 μl of each dilution transferred onto the surface of a TSAYE plate containing a lawn of about 1 × 10^7 L. innocua Lin11 per ml of agar medium. The agar plates were incubated for 18 h at 30°C and examined for clear zones of inhibition. The titer was defined as the reciprocal of the highest dilution showing inhibition of the indicator strain multiplied by 100 to express the result as activity units per milliliter (AU/ml) of a solution.

Preparation of meat

The shoulders of a pork carcass (surface pH between 5.7 and 6.2) were obtained from an abattoir (S.A. Prade, St Quentin en Yvelines, France) 24 h after slaughter. A particular muscle of the shoulder (caput longum musculi tricipitis brachii) was chosen throughout the study as it is largely used in France for preparing "chipolatas," a very popular French sausage variety. The muscle was aseptically separated, its surface being rapidly exposed to a flame before being removed with a sterile knife. The muscle was then cut with a sterile scalpel into cubes approximately 1 by 1 by 1 cm, resulting in surface areas of about 6 cm^2 each. After grinding and blending, the meat preparations generally had bacterial counts less than 10^2 CFU/g (enumeration on TSAYE either at 6°C for 14 days or at 30°C for 3 days).

Inoculation of meat with L. innocua Lin11 and effect of treatment with bacteriocins

Meat cubes were artificially contaminated by soaking for 20 min in 200 ml of 8.7-g/liter sodium chloride solution containing 10^7 CFU/ml of L. innocua Lin11 grown to stationary phase in TSAYE at 30°C. A low NaCl concentration was chosen to limit osmotic extraction of some meat components and interference with the inhibitory activity of the two bacteriocins. The contaminated cubes were then soaked for 20 min in a sterile 8.7-g/liter NaCl solution at 4°C either without bacteriocin (control) or with nisin A or pediocin AcH. The bacteriocin concentrations (up to 3,000 AU/ml for nisin A and 50,000 AU/ml for pediocin AcH) varied in different studies as noted in the result section. Meat cubes were removed from the decontamination bath and allowed to drain for 5 min at room temperature. They were then ground for 15 s using a household blender (Moulinette Coupe-Coupe, France) and stored aerobically at 5°C. More precisely, aliquots of about 50 g of ground meat was dispensed into 100 ml flasks plugged with plastic caps. At selected intervals up to 20 days a sample of 1 g of ground meat was withdrawn and mixed with 9 ml of cold TSAYE using a Stomacher Ultra Turrax T25 (Janke & Kunkel, Staufen, Germany). The suspensions were centrifuged for 10 min at 10,000 X g, and the supernatants were then assayed for bacteriocin activity. To estimate very low residual concentrations of bacteriocins in meat, the more sensitive agar diffusion method was preferred to the spot-on-lawn method. Residual nisin activity in meat was determined by the agar diffusion method, with Micrococcus luteus ATCC 10240 as indicator strain (38). For pediocin, the increase in sensitivity resulted not from use of a larger volume of the solution tested (50 μl extract in each well) but on the choice of L. ivanovichii L1PE as test organism, since a preliminary study had shown that this strain was more sensitive to pediocin AcH than L. innocua Lin11. Also, Tween 20 (1%) was added after sterilization to TSAYE containing agar (15 g/liter) to enhance the bactericidal activity of the bacteriocin. Solutions containing nisin A at 10^4 IU/ml and pediocin AcH at 5,000 AU/ml were used as reference solutions. To compensate for the presence of interfering substances extracted from meat, standard curves were obtained by assaying known amounts of nisin A and pediocin AcH added to bacteriocin-free meat extracts (38).

Stability of bacteriocins in ground meat

First, we determined whether there was a concentration (AU/g) of bacteriocin at which binding sites of meat may become saturated. On three occasions increasing amounts of nisin A and pediocin AcH were mixed with raw ground meat at 5°C and free
bacteriocin activity was determined about 5 min later on water extracts.

In one experiment ground meat was divided into two parts; one was immediately cooled at 5°C whereas the other was heated for 3 min at 100°C in a microwave oven to inactivate the proteases and then cooled at 5°C. Known amounts of nisin A and pediocin AcH were then added to unheated and heated ground meat and mixed properly for uniform distribution and stored aerobically at 5°C. At selected intervals a sample was taken, and the residual free and total bacteriocins were extracted and assayed by the aforementioned methods.

In another experiment raw and heated meat juice was used to tentatively separate the effect of adsorption of bacteriocins on solid meat components from enzyme degradation. One part ground meat and one part sterile deionized water (wt/wt) were homogenized for 30 s with an Ultra Turrax and the mixture was centrifuged for 10 min at 10,000 × g and 4°C. The supernatant was considered meat juice. Half of the volume was heated for 3 min at 100°C to inactivate proteases. Nisin A and pediocin AcH were prepared in sodium phosphate buffer (10 mM, pH 6.5) to final concentrations of 300 and 100 AU/ml, respectively. Then heated or raw meat juice was added at a concentration of 10% (vol/vol) to the bacteriocin solutions, which were then stored at 5°C. At intervals, bacteriocin activity of the mixture was determined by the agar diffusion technique, using appropriate test organisms.

RESULTS AND DISCUSSION

Preparation of partially purified pediocin AcH

The method of preparing pediocin AcH by desorption at low pH from the cell surface of the producing strain followed by ultrafiltration provided large amounts of pediocin AcH. One liter of culture broth (ca. 10^12 cells) allowed the production of about 10^7 AU of pediocin AcH, which represents at least 10 times more bacteriocin than from the original method described by Yang et al. (39). Both methods also are expected to eliminate any interference on bacteriocin activity by the components of the culture medium as in the case of precipitation of proteins from supernatant by ammonium sulfate (4, 5).

Relation of IU of nisin and AU

Commercial nisin is available with activity expressed as international units (IU), using Micrococcus luteus ATCC 10240 as indicator strain. As this strain is not sensitive to some other bacteriocins of lactic acid bacteria, these inhibitors are assayed on different sensitive bacterial strains and the activity is expressed as arbitrary or activity units per milliliter (AU/ml). To evaluate our results on a comparable basis, it was then important to determine the relation between IU and AU for nisin A and relate that to the AU of pediocin AcH. The activity titers of nisin and pediocin AcH stock solutions were determined using the spot-on-lawn method against L. innocua Lin11. From three separate determinations we found that one IU of nisin A corresponded to 0.3 AU. So, the factor 0.3 was used to convert IU of nisin A into AU. For pediocin AcH only AU values were used.

Effectiveness of bacteriocin-containing solutions in reducing listeria in ground meat

The effectiveness of decontaminating meat by soaking in saline solutions containing different concentrations of nisin A is illustrated in Fig. 1. Rapid reduction of L. innocua Lin11 occurred 20 min after addition followed by further reduction up to the second day of storage. The reduction increased as nisin concentration increased, but the Listeria cells surviving nisin treatment resumed growth after 2 days, at a rate similar to that of the control, and reached the same final population. Other researchers have also observed a similar reduction of listerial populations during soaking (9) and subsequent storage at 4°C (14).

The decontamination procedure was also efficient with pediocin AcH, but considerably more pediocin AcH (on a basis of activity units at 30°C) was necessary to obtain the same listerial reduction as nisin A (Fig. 2). In this experiment, growth of survivors to pediocin AcH was delayed for up to 8 days of storage, but this phenomenon was not confirmed in the other experiments carried out with this bacteriocin. Moreover, in contrast to that of nisin A, the bactericidal efficiency of pediocin AcH was found to depend on initial pH. While with nisin no difference in Listeria cell count reduction was observed between meat at relatively low pH (5.7 ± 0.1) and meat as a higher pH (6.1 ± 0.1), with pediocin AcH a difference at 0.5 log after grinding and a difference of 1 log after 2 days at 5°C were observed, which means about 3- and 10-fold differences in viable Listeria count in meat (Table 1). It is also worth noting that higher growth rates of survivors were observed in meat with the low than in meat with the high pH, regardless of the bacteriocin used. Growth rates in the absence of bacteriocins were not different from those in the presence of these inhibitors (data not presented).

Greater efficiency of nisin compared to pediocin AcH on some Listeria strains has been reported by other researchers (10, 15), but the influence of initial pH was not observed. Although low initial meat pH values could easily be
attributed to natural ante- and/or postmortem glycogen catabolism and not to bacterial spoilage, it is difficult to explain the differences in bacterial growth rates between low and high meat pH.

Evidence of resistance of survivors to bacteriocins under use

Results presented earlier showed that in meat treated with nisin and pediocin the loss of viability of listerial cells was rapid up to 2 days, at which point the growth of survivors began to appear (Figs. 1 and 2). Evidence of resistance of the survivors to nisin A and pediocin AcH was first demonstrated by addition of extra nisin A and pediocin AcH (300 and 1,000 AU/ml, respectively) in control and bacteriocin-treated meat samples after 12 days of storage (i.e., a long time after the surviving cells resumed growth). The addition of extra nisin induced a 2.4-log decrease in cell count in the control meat and only a 0.4-log reduction (i.e., 100 times less destruction) in nisin-treated meat. The corresponding results with pediocin AcH were reductions of 1.9 and 1.5 log, respectively (results not presented). This means that the Listeria populations in bacteriocin-treated meat have acquired some resistance to these compounds, with more resistance for nisin A than for pediocin AcH.

To confirm that the survivors in nisin-treated samples are resistant to this substance, and observe whether this resistance is stable during regrowth at 5°C, Listeria cells were also enumerated both on TSAYE and on TSAYE containing nisin A (15 AU/ml) and the results were compared with those obtained with meat not treated with nisin (control). The results are presented in Table 2. In control meat the population increased during storage due to growth (counts on TSAYE alone) whereas the level of nisin-resistant cells (counts on TSAYE plus nisin) remained almost constant. In contrast, enumeration on TSAYE alone and TSAYE plus nisin for nisin-treated meat samples gave similar counts after 2 days of storage. This means that most Listeria at 2 days were nisin-resistant, and that nisin resistance persisted during storage.

It has been shown that Listeria surviving nisin treatment have increased resistance to this bacteriocin (35, 36). This also has been observed with pediocin AcH in TSBYE (8, 36). It has been established that in a population of a sensitive strain there are some normal variant cells that are resistant to nisin (12, 19, 31, 36). In the presence of a bacteriocin the sensitive cells are killed, leaving the resistant variant alive. An increase in the bacteriocin concentration would result in lower numbers of survivors, but these bacteria are more resistant to the bacteriocin in use (36). Nisin-resistant Listeria were found to be resistant to pediocin AcH as well as to enterococcin EFS2, a newly discovered bacteriocin (27). Therefore, we can anticipate that nisin-resistant bacteria would be resistant to other bacteriocins. In the absence of the bacteriocin the resistant variant cells multiply and produce both sensitive and resistant cells, and the population soon reaches the original susceptible state, which is the true (genetically controlled) nature of the strain (31). However, in the presence of the bacteriocin the sensitive cells will die while the resistant surviving cells will multiply giving resistant populations. It is also expected that combined use of two appropriate bacteriocins would reduce the emergence of such resistant cells (18).

### TABLE 1. Effect of initial pH of fresh ground pork meat on the antilisterial activity of nisin A and pediocin AcH (average of three independent trials for nisin A and two for pediocin AcH, with the confidence interval at $P = 0.95$)$^a$

<table>
<thead>
<tr>
<th>Initial pH of meat</th>
<th>pH 5.7 $\pm$ 0.1</th>
<th>pH 6.1 $\pm$ 0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nisin A</td>
<td>Pediocin AcH</td>
</tr>
<tr>
<td>Reduction in viable cell count after grinding (log cycles)$^b$</td>
<td>1.9 $\pm$ 0.5</td>
<td>1.3 $\pm$ 0.7</td>
</tr>
<tr>
<td>Maximum reduction in viable cell count (log cycles)$^c$</td>
<td>2.4 $\pm$ 1.0</td>
<td>1.8 $\pm$ 1.0</td>
</tr>
<tr>
<td>Growth rate of survivors (log cycles/day)</td>
<td>0.18 $\pm$ 0.20</td>
<td>0.20 $\pm$ 0.10</td>
</tr>
</tbody>
</table>

$^a$ Before grinding, meat cubes contaminated with Listeria innocua Lin11 were soaked in a cold saline solution (4 to 6°C) containing nisin A (1,500 AU/ml) or pediocin AcH (50,000 AU/ml).

$^b$ The inoculation of pour plates took place approximately 20 min after addition of the bacteriocin to the meat.

$^c$ The inoculation of pour plates took place after 2 days of storage at 5°C (see Figs. 1 and 2).
TABLE 2. Enumeration (log CFU/g), on TSAYE and TSAYE containing nisin (15 AU/ml), of Listeria innocua during growth at 5°C in control or nisin-treated ground pork meat

<table>
<thead>
<tr>
<th>Enumeration medium</th>
<th>Listeria innocua Lin11 (log CFU/g)</th>
<th>Days of storage at 5°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control meat</td>
<td>TSAYE alone</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>TSAYE plus nisin</td>
<td>3.7</td>
</tr>
<tr>
<td>Nisin-treated meat</td>
<td>TSAYE alone</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>TSAYE plus nisin</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Effect of temperature on bactericidal effect of nisin A and pediocin AcH in a reference system

A faster decrease was observed in TSBYE at 30 than at 5°C, and total viability loss was significantly higher at 5 than at 30°C, but no difference between the two bacteriocins was observed (results not shown). This is somewhat surprising given the reported difference between the two bacteriocins in regard to energy requirements for bactericidal effectiveness (7). The reason is probably that Listeria spp. are psychrotrophic bacteria, and consequently they are able to produce energy from growth at low temperature. Moreover, it has been shown that growth at low temperature increases the susceptibility to nisin, presumably by modification of the cell membrane composition (1). Thus, the difference in inhibitory activity between nisin A pediocin AcH in ground meat at 5°C cannot be attributed to the low temperature. Since one AU of pediocin AcH has the same inhibitory activity as one AU of nisin A at 30°C on TSAYE, two hypotheses were investigated to explain why more pediocin AcH activity is needed to ensure the same viability loss in meat at 5°C than with nisin A (i) more rapid degradation of pediocin AcH by meat proteases and (ii) stronger adsorption of this bacteriocin to meat components, particularly fat (3).

Loss of activity of nisin and pediocin AcH in ground meat stored at 5°C

First, we investigated the efficiency of the cold water method of bacteriocin extraction from meat. Nisin A (300 AU/g) or pediocin AcH (1,000 AU/g) was added to fresh meat during grinding and subjected to cold water extraction immediately to determine the activity due to free bacteriocins. A second extraction using hot acid followed immediately to determine the activity due to free bacteriocins. An amount added to meat after cold water extraction (average of three independent trials) observed saturation levels in a slurry containing beef muscle at a concentration of 12.5% and for high pediocin AcH concentrations (above 100,000 AU/ml, though their AU is not necessarily equivalent to our AU, since both the indicator organism and the culture medium were different). Absence of saturation levels in meat could be the result of bacteriocin concentrations too low to counterbalance two factors acting concurrently, namely adsorption on meat components, particularly fat (3, 13), and enzymatic degradation. The observation that recovered activity remained essentially the same as the amounts of bacteriocins increased suggests that neither saturation of meat adsorption sites nor maximum enzymatic degradation rate (resulting from saturation of enzyme active sites by the bacteriocins as substrates) was attained.

In one experiment nisin A (600 AU/g) and pediocin AcH (2,600 AU/g) were added to fresh ground meat as well as ground meat that was heat treated (3 min at 100°C in microwave oven), and the bacteriocin activity of the extracts was monitored when the meat was stored for up to 9 days at 5°C (Fig. 3). The activity declined with storage time, with a more rapid loss in the raw meat than in the heat-treated meat, regardless of the bacteriocin or the mode of extraction used. Again, a more rapid activity loss was found with pediocin AcH than with nisin A. The results indicate that reduced recovery of activity of both bacteriocins in raw meat (Tables 3 and 4) could be due to protease action, as heated samples

TABLE 3. Percentage of nisin and pediocin AcH activity recovered from fresh ground pork meat after extraction with cold water and after a second extraction with hot acid

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Nisin A</th>
<th>Pediocin AcH</th>
</tr>
</thead>
<tbody>
<tr>
<td>First extraction: cold water</td>
<td>22.3</td>
<td>10.9</td>
</tr>
<tr>
<td>Second extraction: hot acid</td>
<td>48.2</td>
<td>13.6</td>
</tr>
<tr>
<td>Total</td>
<td>70.5</td>
<td>24.5</td>
</tr>
</tbody>
</table>

aNisin A (300 AU/g) and pediocin AcH (1,000 AU/g) were added to meat during grinding, and activity was determined immediately by the agar diffusion method using Listeria ivanovii L1PE as indicator organism.

TABLE 4. Effect of the concentration of added nisin A and pediocin AcH on the percentage of inhibitory activity recovered from ground meat after cold water extraction (average of three independent trials with nisin A and two with pediocin AcH, with the confidence interval at P = 0.95)

<table>
<thead>
<tr>
<th>Bacteriocin</th>
<th>Amount added (AU/g)</th>
<th>Activity recovered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin A</td>
<td>30</td>
<td>61.4 ± 12.4</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>65.1 ± 22.5</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>47.8 ± 23.1</td>
</tr>
<tr>
<td></td>
<td>1,500</td>
<td>40.8 ± 8.5</td>
</tr>
<tr>
<td>Pediocin AcH</td>
<td>500</td>
<td>9.3 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>10.3 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>10,000</td>
<td>14.5 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>15,000</td>
<td>12.5 ± 4.8</td>
</tr>
</tbody>
</table>
exhibited more activity. The results also suggest that pediocin AcH is more susceptible to protease activity than nisin.

Bacteriocin activity was also determined in phosphate buffer (10 mM, pH 6.5) containing nisin (100 AU/ml) or pediocin AcH (250 AU/ml) to which raw meat juice or meat juice that had been heated (3 min at 100°C in a boiling water bath) was added at a 10% concentration. Results showed that activity in presence of raw meat juice was less stable with pediocin AcH than nisin (data not presented).

Overall, the results of these experiments are in agreement with early studies showing that pediocin is susceptible to a higher number of proteases than nisin (5, 21). The reason why all bactericidal activity was not recovered from heated meat by hot acid extraction could be that microwave heating might not have completely inactivated the protease action.

Our work confirms that nisin activity in raw meat decreases during storage at low temperature (9, 16, 34), but it is much more stable than the activity of pediocin AcH. Other studies have reported that pediocin was stable on raw beef surfaces for 28 days at 4°C (28), in cooked vacuum-packaged wiener for 1 week at 25°C (14), or in cooked summer sausage for 60 days at 4°C (25). The rapid reduction of pediocin AcH activity in pork ground red meat could be due to high proteolytic activity in this kind of meat and proteolytic enzymes being made available in higher concentrations by the grinding. The efficiency of proteolytic systems is known to be higher in pork than in beef, and higher in white than red muscles (2, 23, 24, 32, 33). In sausages, proteases can be destroyed or their action reduced by processing (cooking) and by curing agents (37). The difference between nisin A and pediocin AcH stability in raw meat juice is consistent with in vitro studies which showed that several proteases can hydrolyze pediocin AcH whereas only one, namely α-chymotrypsin, degrades nisin (5, 21). The inactivation of bacteriocins by meat proteases could be expected, but this is the first study to show the rate of inactivation in ground pork.

In conclusion, our study demonstrates that decontamination of meat muscles by soaking in saline solutions containing an appropriate bacteriocin may, along with other methods (e.g., cold storage in modified atmosphere), extend the shelf life and safety of ground meat. Nisin A seems to be more effective than pediocin AcH in the type of meat we studied, probably because it is more resistant to meat proteases. However, routine use of nisin at concentrations insufficient to destroy all listeria could result in emergence of bacteriocin-resistant cells in the industrial environment and ultimately compromise the use of other efficient bacteriocins. Further investigations are needed to understand the mechanism(s) of bacterial destruction in situ and other factors which affect bacteriocin activity. In particular the inhibitory efficacy of a bacteriocin may depend on the type of meat examined as well as the pH of the meat itself.

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3. Bell, R. G., and K. M. De Lacy. 1986. Factors influencing the production of a bacteriocin, pediocin AcH, in in vitro and in vivo systems is known to be higher in pork than in beef, and

FIGURE 3. Loss of bacteriocin activity in ground meat stored at 5°C. (■) heat-treated meat and hot acid extraction, (●) raw meat and hot acid extraction, (△) raw meat and cold neutral extraction. Arrows indicate no activity detected (<1 AU/g).


