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Effects of Nisin on Growth of Bacteria Attached to Meat

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Nisin had an inhibitory effect on gram-positive bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*, and *Streptococcus lactis*) but did not have an inhibitory effect on gram-negative bacteria (*Serratia marcescens*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa*) attached to meat. Nisin delayed bacterial growth on meats which were artifically inoculated with *L. monocytogenes* or *Staphylococcus aureus* for at least 1 day at room temperature. If the incubation temperature was 5°C, growth of *L. monocytogenes* was delayed for more than 2 weeks, and growth of *Staphylococcus aureus* did not occur. We also found that the extractable activity of nisin decreased rapidly when the meats were incubated at ambient temperatures and that this decrease was inversely related to the observed inhibitory effect. These findings disclosed that nisin delays the growth of some gram-positive bacteria attached to meat. However, nisin alone may not be sufficient to prevent meat spoilage because of the presence of gram-negative and other nisin-resistant gram-positive bacteria.

It is estimated that there are between 24 million and 81 million cases of food-borne illness each year, and approximately 50% of these cases are associated with meat and poultry (6, 14, 23). Microbial contamination of raw meat and meat products has been the predominant cause of food-related illness. Outbreaks of food-borne diseases have led to considerable illness and even death (1, 21).

One efficient way to decrease the microbial contamination on meat is effective washing (11, 20). Although methods and devices have been developed to clean animal carcasses (2–4), complete sterilization has not been achieved.

Another way to prevent microbial contamination is to block the microbial attachment to meat. Although rates of attachment of bacteria to meat have been studied (22, 25), there is limited information on how to prevent this attachment. A logical approach is, therefore, to search for a good chemical or physical agent to block microbial attachment to meat.

Nisin, a small antimicrobial peptide produced by lactic acid bacteria (19), has been tested as a preservative for its antibotulinal effect on bacon (9, 30) and chicken frankfurter emulsion (31), although the conclusions of the previous studies (9, 30) were that nisin is not very effective. It can inhibit the outgrowth of *Bacillus licheniformis* (7), and *Clostridium sporogenes* PA3679 (27) spores and the growth of lactic acid bacteria from cured and fermented meat products (10) and in brewing (26). However, there is limited information on the effect of nisin on raw meat. The purpose of this study was to determine the effect of nisin on the attachment and growth of bacteria on meat and to assess its feasibility for use as a meat preservative.

MATERIALS AND METHODS

Nisin. Nisaplin, a commercial product of nisin that contains 10^6 IU/g, was provided by Aplin & Barret Ltd. (Trowbridge, England). One gram of Nisaplin was dissolved in 100 ml of 0.02 N HCl containing 0.75% NaCl, and the pH was brought to 3.0 with 1 N NaOH, giving 10^4 IU/ml. The solution was filter-sterilized, stored at 5°C, and used within 1 week (7).

Meat. Fresh lean beef muscle was obtained from the abattoir at the U.S. Meat Animal Research Center and stored at −15°C. Before the experiments were performed, samples were thawed at room temperature and cut with a sterile scalpel into pieces of 1.0 by 1.0 by 0.5 cm. These samples contained fewer than 100 CFU per sample.

Bacterial strains. *Serratia marcescens* ATCC 8100, *Staphylococcus aureus* ATCC 25923, *Streptococcus lactis* ATCC 11454, *Streptococcus faecalis* ATCC 27853, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 22853, and *Micrococcus flavus* ATCC 10240 were obtained from Difco Laboratories (Detroit, Mich.). *Listeria monocytogenes* Scott A was obtained from the Division of Microbiology, U.S. Food and Drug Administration, Cincinnati, Ohio. The bacteria were maintained on tryptic soy agar (Difco), but *Streptococcus lactis* was maintained on MRS agar (Difco) and *Micrococcus flavus* was maintained on brain heart infusion agar (Difco).

Organisms were grown in tryptic soy broth (Difco), but *Streptococcus lactis* was grown in MRS broth. They were incubated at 37°C, but *Listeria monocytogenes* was incubated at room temperature for 18 to 24 h. The cultures were centrifuged at 3,000 × *g* at 4°C for 10 min. Supernatants were then decanted, and the cell pellets were suspended in 10 ml of attachment medium (25). The cell suspensions were then diluted in the same medium to approximately 10^7/ml.

Effects during attachment. Cut meat pieces were soaked in the nisin solution (10^4 IU/ml) for 10 min at room temperature (ca. 23°C). Bacterial attachment to nisin-treated meat was compared with that to meat that was soaked in the control solution (0.02 N HCl with 0.75% NaCl [pH 3.0]).

The meat samples were transferred aseptically to a sterile beaker containing 20 ml of cell suspension at 10^7/ml and were incubated at room temperature for 0, 2, 5, and 10 min. The samples were gently rinsed aseptically with normal saline solution (0.87% NaCl) at the appropriate time interval. Rinsed samples were immediately transferred to a sterile bag

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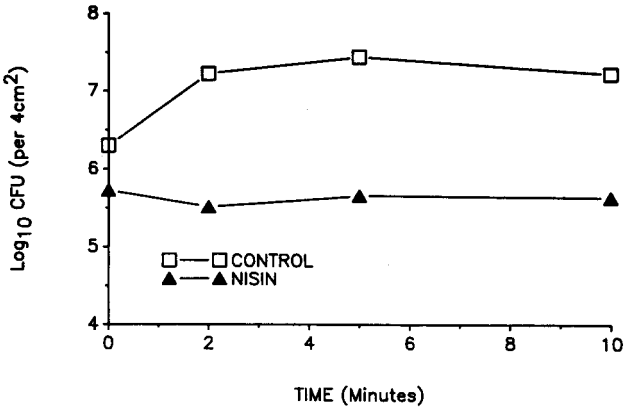

FIG. 1. Effect of nisin during the attachment of *L. monocytogenes* to meat at room temperature.

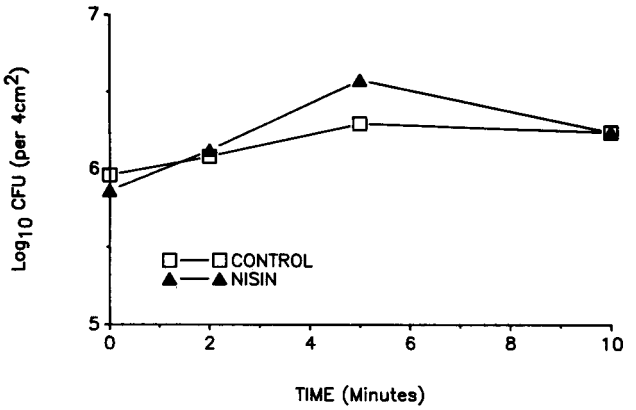

FIG. 2. Effect of nisin during the attachment of *Serratia marcescens* to meat at room temperature.

containing 99 ml of sterile Butterfields phosphate buffer (33) and stomached for 2 min in a stomacher (Stomacher 400; Tekmar Inc., Cincinnati, Ohio). Suspensions were then serially diluted in sterile buffer to the appropriate concentration and pour plated onto tryptic soy agar.

Effects on attached bacteria. The meat pieces were inoculated as indicated above and incubated for 10 min at room temperature. The inoculated samples were immersed in nisin solution (10^4 IU/ml); aseptically removed; and rinsed gently with saline solution at time intervals of 0, 2, 5, and 10 min. The number of bacteria remaining on the meat was determined by stomaching the meat samples and serially diluting and plating them as described above.

Bacterial growth on meat. The meat samples were first soaked in the nisin or control solution (0.02 N HCl with 0.75% NaCl [pH 3.0]) for 10 min and were immersed into the bacterial suspension (about 10^6/ml in the attachment medium) for 10 min. The inoculated meats were gently rinsed with saline solution, drained, and put into sterile petri dishes. The dishes were sealed with Parafilm (American Can Co., Greenwich, Conn.) to prevent dehydration and were incubated at either ambient temperature or 5°C. Culture counts were conducted at 0, 24, 48, and 72 h for those cultures that were incubated at ambient temperature and at 0, 1, 2, 3, and 4 weeks for those cultures that were incubated at 5°C.

TABLE 1. Effect of nisin on the attachment of bacteria to meat after 10 min

Organism	Treatment	No. of cells attached ($\log_{10}$ CFU/4 cm^2)[a]
Streptococcus lactis	Control	7.16[a]
	Nisin	5.27[b]
Staphylococcus aureus	Control	6.39[a]
	Nisin	5.39[b]
Listeria monocytogenes	Control	7.20[a]
	Nisin	5.62[b]
Salmonella typhimurium	Control	7.23[a]
	Nisin	6.63[a]
Serratia marcescens	Control	6.23[a]
	Nisin	6.23[a]
Pseudomonas aeruginosa	Control	7.64[a]
	Nisin	7.33[a]

[a] Means with different superscripts are significantly different ($P < 0.05$). Each bacterium was analyzed separately.

Decimal reduction time determination. Organisms were grown as described above and suspended in Butterfield phosphate buffer (33). The bacterial suspension (0.1 ml; or ca. 10^8/ml) was put into 10 ml of nisin solution (10^4 IU/ml); and culture counts were conducted at 30 s and 1, 2, 5, 10, and 30 min by the pouring plate method. The decimal reduction time (*D*) value was estimated for the time that was required to kill 1 log cycle of viable cells in the nisin solution at ambient temperature.

Nisin concentration determination. The plate diffusion assay method developed by Tramer and Fowler (32) was used to determine the nisin concentration. Each piece of nisin-treated meat was placed in 10 ml of control solution, macerated by hand, and stomached for 3 min. The suspension was then boiled for 5 min to release nisin and centrifuged. The supernatants were used for the assay. *Micrococcus flavus* was used as the test organism, and brain heart infusion agar was used as the assay medium.

Statistical analysis. Statistical analysis was performed by using the general linear models and mean least-squares procedures described by SAS (28). Unless otherwise noted, significance is expressed at the 5% level. Each experiment was performed at least in duplicate.

RESULTS

***D* value determination.** It was found that the control solution (0.02 N HCl with 0.75% NaCl [pH 3.0]) had no significant ($P > 0.05$) effect for up to 30 min on the viability of the gram-positive bacteria and for up to 10 min on the viability of the gram-negative bacteria that were tested. When the nisin activity was 10^4 IU/ml, *L. monocytogenes* decreased from 10^7/ml to less than 10/ml in 30 s; the *D* value for *Staphylococcus aureus* was 30 s and that for *Streptococcus lactis* was 5 min. *D* values for gram-negative bacteria were not determined.

Effects during attachment. The effect of nisin during the attachment of *L. monocytogenes* is shown in Fig. 1. There was a significant ($P < 0.05$) inhibitory effect of nisin on *L. monocytogenes* during attachment to meat. There was also a significant increase in the number of cells attached to the meat in the control solution between 0 and 2 min, whereas there was no significant increase in the numbers of cells on

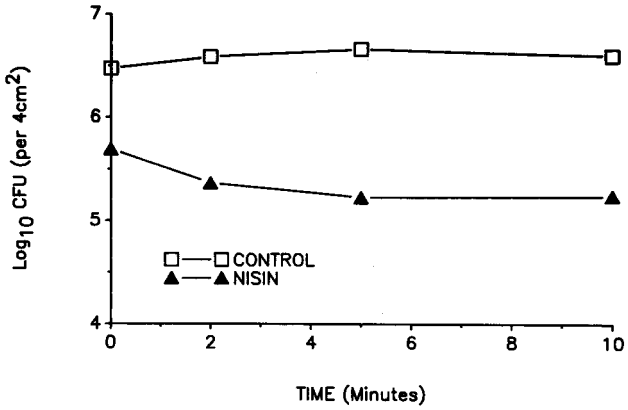

FIG. 3. Effect of nisin on attached *L. monocytogenes* on meat at room temperature.

TABLE 2. Effect of nisin on the growth of bacteria on meat incubated at room temperature

Organism inoculated	Treatment	Log CFU/4 cm² at[a]:			
		day 0	day 1	day 2	day 3
None	Control	2.00[a]	5.91[b]	8.82[d]	9.07[d]
	Nisin	2.00[a]	3.26[c]	7.07[e]	7.83[e]
Staphylococcus aureus	Control	5.26[a]	8.96[c]	10.23[d]	9.81[c]
	Nisin	3.28[b]	6.99[e]	9.20[c]	9.66[c]
Listeria monocytogenes	Control	5.77[a]	8.67[c]	8.86[c]	9.31[c]
	Nisin	3.17[b]	5.85[a]	8.27[e]	8.69[c]
Streptococcus lactis	Control	5.71[a]	8.89[c]	8.89[c]	8.96[c]
	Nisin	4.63[b]	8.84[c]	8.87[c]	8.76[c]

[a] Means with different superscripts within rows and columns for each organism are significantly different ($P < 0.05$).

the nisin-treated meat (zero time indicates that the determination was made as quickly as possible after the addition of nisin). Similar results were obtained with *Staphylococcus aureus* and *Streptococcus lactis*. Results with *Serratia marcescens* are shown in Fig. 2. There were no differences between the nisin and the control solutions with regard to the numbers of cells attached to the meat after 10 min of incubation. Similar results were also obtained with *Salmonella typhimurium* and *P. aeruginosa*. The effects of nisin during the attachment of bacteria to meat for up to 10 min are summarized in Table 1. Nisin had a significant effect on the attachment of gram-positive bacteria but not on that of gram-negative bacteria.

Effects on attached bacteria. The effect of nisin on attached *L. monocytogenes* is shown in Fig. 3. The effect of nisin on the cells attached to meat occurred within the first 2 min, but no additional effect was observed with an increased time of exposure. Similar results were also obtained with *Staphylococcus aureus* and *Streptococcus lactis*. When gram-negative bacteria were tested, no significant effect of nisin was observed. As an example, the results for *Serratia marcescens* are shown in Fig. 4.

Effect of nisin on bacterial growth on meat. The effect of nisin on the growth of bacteria on meat is shown in Tables 2 and 3. Table 2 shows the effect of nisin on the growth of bacteria on meat when they were incubated at ambient temperature. The number of bacteria on the nisin-treated uninoculated meat was approximately 1.2 log CFU less than that on the controls after 3 days. There was a significant ($P < 0.05$) difference between the control and treated samples for both *Staphylococcus aureus* and *L. monocytogenes* for the first 2 days, although there was not difference after 3 days. The population of *Streptococcus lactis* was significantly lower after the initial treatment, but there was no difference after 1 day of incubation.

Results of the effect of nisin on the growth of *Staphylococcus aureus* and *L. monocytogenes* on meat that was incubated at 5°C are shown in Table 3. There was a significant ($P < 0.05$) difference between the control and nisin-treated samples for both *Staphylococcus aureus* and *L. monocytogenes* for the test period of 4 weeks. *Staphylococcus aureus* did not grow at 5°C, whereas *L. monocytogenes* grew well at this temperature. Nisin treatment caused a significant reduction in the initial numbers of *L. monocytogenes* and a substantial delay in growth on meat.

Nisin concentrations on meat. The effects of incubation at both ambient temperature and 5°C on the concentration (activity) of nisin on meat are indicated in Table 4. We found that the activity of the nisin remaining on the meat decreased rapidly after incubation at ambient temperature. However, the activity of nisin did not decrease as rapidly when the meat was incubated at 5°C.

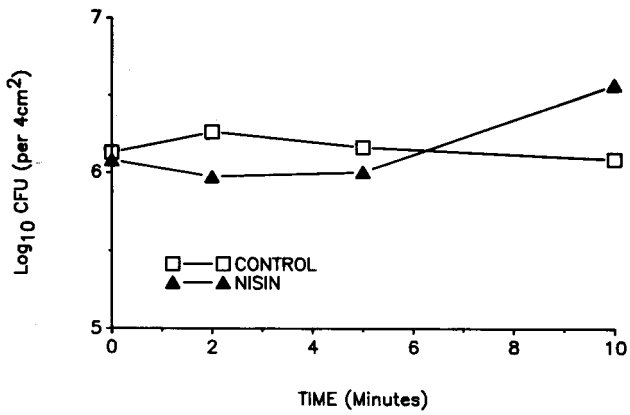

FIG. 4. Effect of nisin on attached *Serratia marcescens* on meat at room temperature.

DISCUSSION

Among the various kinds of antimicrobial agents, nisin has the unique function of being used as a food preservative. Nisin is a small polypeptide, and any residue remaining in food is digested; therefore, it is nontoxic (19). Results of this

TABLE 3. Effect of nisin on the growth of bacteria on meat at 5°C

Organism inoculated	Treatment	Log CFU/4 cm² at[a]:				
		0	1 wk	2 wk	3 wk	4 wk
Staphylococcus aureus	Control	5.30[a]	5.21[a]	NA	5.22[a]	4.88[b]
	Nisin	3.95[c]	4.27[d]	3.98[c]	4.04[c,d]	3.96[c]
Listeria monocytogenes	Control	4.45[a]	6.73[b]	7.99[c]	8.90[d]	8.45[c,d]
	Nisin	2.00[e]	2.00[e]	4.40[a]	7.32[f]	6.96[b,f]

[a] Means with different superscripts within rows and columns for each organism are significantly different ($P < 0.05$). NA, Not available.

TABLE 4. Effect of incubation temperature on the nisin activity on meat

Incubation condition	Remaining activity (IU/piece of meat) at:				
	0 day	1 day	2 days	3 days	4 days
Room temp	154.9	36.8	15.1	11.1	7.3
5°C	147.4	70.8	55.0	43.0	43.4

study indicate that nisin is not stable on meat. The activity of nisin decreased rapidly with time, especially at room temperature.

There may be many reasons for the loss of nisin activity on meat. It has been postulated that the binding of nisin to meat particles and surfaces might cause the loss of nisin activity (18). The decrease of nisin activity on meat was consistent with the antimicrobial activity (Tables 2 and 3). Nisin had a significant effect on meat spoilage by those gram-positive bacteria that were tested.

Recovery of nisin from meat and meat emulsions has been poor and variable (8). We experienced similar difficulties. The method of nisin determination on meat that we used in this study is merely an indication of the extractable nisin activity that is active against the gram-positive bacteria that were tested.

The inhibition of gram-positive bacteria by nisin during attachment to meat is probably due to the initial inhibitory effect. Nisin (16 IU/ml) had an initial effect on the growth of *L. monocytogenes*; however, after an initial 30-h lag period, the organism grew prolifically (24). Recent studies by Sommers et al. (29) and Doyle (12) also indicate that *L. monocytogenes* Scott A grows in the presence of 2,000 IU/ml in tryptic soy broth after an initial substantial reduction in number and a lag of a few days.

The antimicrobial effect of nisin is caused by its interaction with the phospholipid components of the cytoplasmic membrane and, thus, interference with membrane function (18). Nisin is more effective against spores than against vegetative cells (7, 27). The mode of action should help us to understand the applicability and limitation of its use as a food preservative.

Nisin has been used as an effective preservative in processed cheese. The treatment levels to meet storage temperature extremes and to satisfy the prolonged shelf-life requirement for processed cheese have been considered to be 500 IU/g (13). Nisin has also been used to extend the shelf-life of dairy desserts (5), as well as fresh and canned evaporated milk (15). Nisin has also been used in numerous canned vegetables such as potatoes, peas, and mushrooms (16), as well as in soups and cereal puddings. Recently, nisin has been used in the alcoholic beverage industry. Studies have indicated that the spoilage bacteria are principally *Lactobacillus* and *Pediococcus* species, which are sensitive to nisin (26). Use of nisin in fermentation mash inhibits *Lactobacillus* species, which increases the alcohol content in the distillate (17).

Use of nisin for meat preservation, however, has not had much success. There are problems of low solubility, uneven distribution, and lack of stability on the meat surface. Results of the present study indicate that nisin can delay the growth of those gram-positive bacteria that are attached to meat. However, nisin alone may not be sufficient to prevent spoilage, since gram-negative and nisin-resistant gram-positive bacteria such as lactic acid bacteria and are often associated with meat spoilage. Nisin with nitrite has been reported to be effective for the preservation of meat (27). Research in this direction, i.e., to illustrate the possible synergistic effect of nisin and other types of preservatives, should therefore be pursued.

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