Nutraceuticals & Food
Vol. 8, p. 196~199 (2003)

Nutraceuticals and Food

Inhibition of *Listeria monocytogenes* in Vacuum or Modified Atmosphere-Packed Ground Beef by Lactococcal Bacteriocins

– Research Note –

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Abstract

We investigated the antagonistic effects of two lactococcal bacteriocins, nisin or lacticin NK24, on the growth and the survival of *Listeria monocytogenes* in vacuum or modified atmosphere-packaged ground beef. Ground beef was inoculated with approximately 3 log colony-forming units (CFU) of *L. monocytogenes* ATCC 15313 culture per gram of ground beef. Inoculated samples were blended with/without 100 AU/g nisin or lacticin NK24, and subsequently vacuum or modified atmosphere packed at 4°C. Listeria in the bacteriocin-treated and control samples was subsequently isolated from both vacuum and modified atmosphere packs and enumerated as CFU on Listeria Isolation Agar medium. Microbial counts in ground beef treated with bacteriocin declined steadily, while those of non-treated beef samples increased steadily. The results obtained demonstrate that nisin inhibits the growth of *L. monocytogenes* more effectively than lacticin NK24 at 100 AU/g. The use of lactococcal bacteriocins, such as nisin or lacticin NK24, in vacuum or modified atmosphere packaging offers a promising approach for eliminating or reducing the risk of *L. monocytogenes* contamination in ground beef.

Key words: ground beef, bacteriocin, nisin, lacticin NK24, *Listeria monocytogenes*, modified atmosphere packaging, vacuum packaging

INTRODUCTION

The human and animal pathogen, *Listeria monocytogenes* is found in vegetables, dairy products, beef, pork, poultry and seafood (1,2). Its ability to withstand harsh environmental conditions, such as extreme pH, heat, and cold, dictates that special consideration be given to it when planning food storage and distribution. *L. monocytogenes* also poses a serious public health risk because it can cause morbidity and mortality among the very young, the old, and pregnant women with impaired immunity. The frequent incidence of *L. monocytogenes* infection emphasizes the need for improved food preservation or the elimination of the pathogen from meat and meat products in order to protect human health (3-5).

Recently, the consumer market has moved towards products with improved quality. Consumers are particularly demanding products that are preservative-free and safe, but which have also been mildly processed and have an extended shelf-life. To meet consumers' requirements, a new trend has emerged, which answers this need for increased shelf-life products using more natural approaches (6). Novel systems, such as biopreservation have generated much interest as a natural means of controlling the growth of pathogenic and spoilage organisms.

Some lactic acid bacteria (LAB) produce antimicrobial proteins such as bacteriocins, whch are bactericidal proteins with, generally speaking, a narrow spectrum of activity targeted specifically toward a species related to the producer culture. The search for LAB bacteriocins has, therefore, been directed towards substances, which target *Listeria* sp., and consequently this has led to the description of a large number of antilisterial bacteriocins. Nisin is probably the most studied antilisterial bacteriocin. Nisin is a class I bacteriocin, which is produced by strains of *Lactococcus lactis* and is used as a food preservative on a commercial scale in several countries (6,7). However, nisin is expensive, and the utility of this agent is hindered by its prohibitive cost in many industries, especially when high concentrations are needed to achieve satisfactory antimicrobial effects. In addition, the use of high concentrations of nisin may encourage the selective growth of nisin-resistant bacterial sub-populations. This particular problem could be overcome by combining nisin with other preservatives (i.e., NaCl or sorbic acid), other bacteriocin, and different packaging conditions such as vacuum packaging or modified atmosphere packaging, to utilize their synergistic effects. Moreover, this approach may be used to reduce the nisin concentration required for effective control of the relevant microorganisms (8,9). The use of

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bacteriocin coated packaging film combined with hydrostatic pressure has also been tested (10-12).

The objective of this study, therefore, was to evaluate whether the two bacteriocins, nisin or lacticin NK24, are capable of reducing the population of *L. monocytogenes* ATCC 15313 in vacuum or modified atmosphere-packaged ground beef.

MATERIALS AND METHODS

Bacterial strains, culture media, and growth conditions

Lactococcus lactis subsp. *lactis* ATCC 11454 was used as the nisin producer. *Lactococcus lactis* NK24, previously isolated from a Korean fermented fish food (Jeot-gal), was maintained at -70°C in lactobacilli MRS broth (Difco Laboratories, Detroit, USA) containing 20% (v/v) glycerol (13). Working cultures were propagated in MRS broth at 30°C for 12 hr before being used in experiments. *L. monocytogenes* ATCC 15313 was grown for 12 hr at 37°C in tryptic soy broth (Difco) containing 0.6% yeast extract. Prior to use, the strains from frozen stocks were subcultured twice in appropriate media.

Preparation of bacteriocin

The bacteriocins used in this study were nisin and lacticin Nk24. Lacticin NK24 is a bacteriocin produced by *L. lactis* NK24 and was isolated from Jeot-gal using a modified triple agar layer method (13). Nisin and lacticin NK24 were produced in a 5 L jar fermenter (3.0-liter working volume; Korea Fermenter Co., Inchon, Korea). *L. lactis* subsp. *lactis* ATCC 11454 or *L. lactis* NK24 was inoculated (1%, v/v) into 250 mL of sterile MRS broth and the seed culture (1%, v/v) transferred to the jar fermenter. The temperature was maintained at 30°C, and the pH at 6.0±0.1 by the addition of 3 N HCl and 3 N NaOH. The agitation speed in the jar fermenter was 200 rpm and no aeration was provided. The culture broth was centrifuged at 8,000×g for 20 min at 4°C and supernatants filter-sterilized through a 0.22 μm cellulose acetate filter. Partially purified nisin or lacticin NK24 were obtained by ethanol precipitation, which was performed by slowly adding ethanol to the culture supernatant to 40% at 4°C, with constant stirring. Slow stirring was continued for an additional 1 hr at 4°C. Precipitated proteins were centrifuged at 12,000×g for 20 min at 4°C, and the pellets resuspended in 100 mM phosphate buffer (pH 7.0). Ethanol was evaporated at 30°C over 2 h. The nisin or lacticin NK24 samples obtained were stored at -70°C.

Bacteriocin assay

Bacteriocin activity was measured using the spot-on-lawn assay (13).

Meat preparation and inoculation

Ground beef (boneless front leg meat) was purchased from a local food market and inoculated with 1×10^3 CFU/g of *L. monocytogenes* ATCC 15313, and subsequently treated with bacteriocins at a level of 100 AU/g. The inoculum was hand-kneaded into the meat for 2 min. Meat samples (two 8 g portions) were placed on polystyrene dishes and covered with an LLDPE wrap film. The control ground beef did not contain bacteriocin, but was also inoculated with 1×10^3 CFU/g of *L. monocytogenes* ATCC 15313. To mimic the effect of packaging, the inoculated beef was treated under vacuum or in a modified atmosphere at 4°C.

Enumeration of *L. monocytogenes*

L. monocytogenes ATCC 15313 cells were counted in various beef samples without special packaging following 0, 0.4, 1, 4, 7, 14, and 21 days of storage at 4°C after inoculation (Fig. 1); and following 0, 1, 4, 7, 14, 28, and 44 days of storage at 4°C after inoculation for vacuum packed (Fig. 2) and modified atmosphere packed samples (Fig. 3). After each storage period, 8 g ground beef portions were aseptically removed from each dish and placed in stomacher bags (Stomacher Lab Systems, Topley House, England) and mixed with distilled water; the final volume was adjusted to 80 mL by adding cell suspension media. Additional serial dilutions were subsequently made using sterile 0.1% peptone water. Cells were counted by spread plating 0.1 or 1 mL dilutions of the samples onto plates of Listeria Isolation Agar medium (LABM Limited, Bury, UK) and incubating the plates at 30°C for 48 hr. All experiments were performed in triplicate, the values shown represent the average of the three measurements.

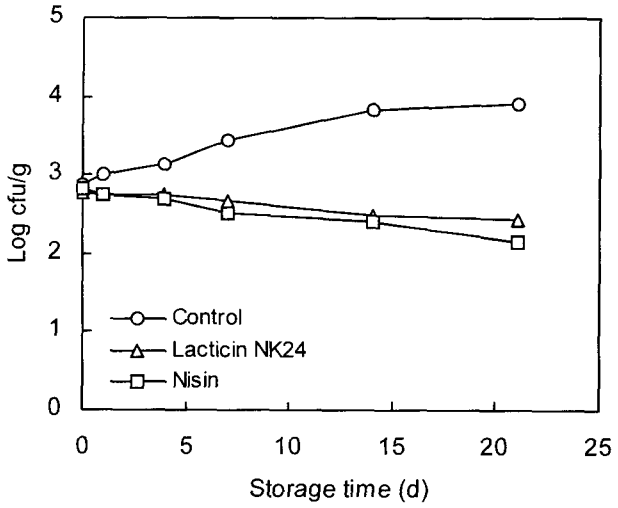

Fig. 1. Inhibitory effect of lactococcal bacteriocins on *Listeria monocytogenes* ATCC 15313 in ground beef during refrigerated storage (4°C).

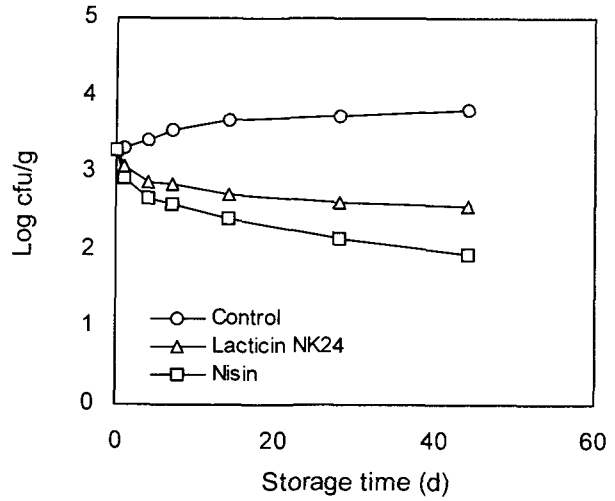

Fig. 2. Inhibitory effect of lactococcal bacteriocins on *Listeria monocytogenes* ATCC 15313 in vacuum packed ground beef.

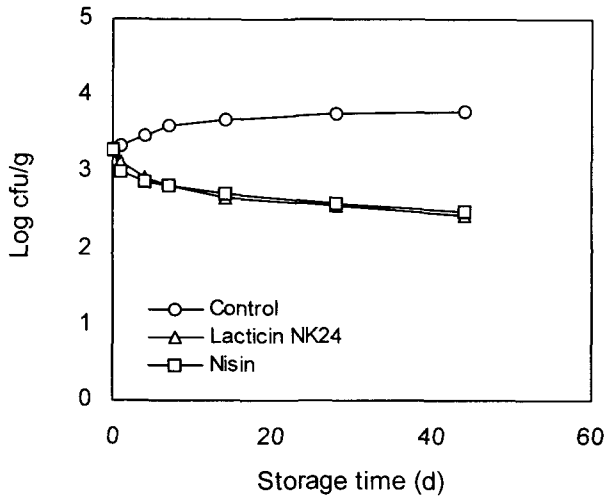

Fig. 3. Inhibitory effect of lactococcal bacteriocins on *Listeria monocytogenes* ATCC 15313 in modified atmosphere packed ground beef.

RESULTS AND DISCUSSION

The ability of *L. monocytogenes* to grow under a variety of conditions has been demonstrated (12,14). It is able to survive and grow on meat and meat products at temperatures commonly used for refrigeration (6,15). In this study, ground beef samples were inoculated with *L. monocytogenes* ATCC 15313, stored at 4°C, and the inhibitory effect of the two lactococcal bacteriocins on its growth evaluated. A decline in the cell count was observed during storage in ground beef treated with bacteriocins, whereas the *L. monocytogenes* propagated steadily in the bacteriocin-free control samples. Compared with the control, the bacteriocins reduced the number of bacteria by as much as 1 log cycle after 7 days, 1.4 log cycles after 14 days, and 1.7 log cycles after 21 days of storage at 4°C. However, *L. monocytogenes* ATCC 15313 was not completely inhibited. In raw meat, naturally occurring enzymes are known to degrade added bacteriocins, thereby impairing their effectiveness (16).

In the vacuum packed ground beef samples, compared with the control, the *L. monocytogenes* counts were reduced by as much as 1.0 or 0.7 log cycles after 7 days, 1.3 or 1.0 log cycles after 14 days, and 1.9 or 1.3 log cycles after 44 days, by the addition of nisin or lacticin NK24, respectively (Fig. 2).

In the samples using the modified atmosphere packaging, compared with the control, nisin or lacticin NK24 reduced the number of bacteria by as much as 1.3 or 1.2 log cycles after 1 day, 1.2 log cycles each after 7 days, and 1.3 log cycles after 44 days storage.

We did not observe a synergistic effect associated with the vacuum or modified atmosphere packaging. However, the synergistic effects of packaging systems with sakacin K have been reported (17). From the results obtained, it was apparent that the effects of packaging atmosphere on an indicator microorganism depend on the type meat; this could be due to the pH of the meat, its water activity level or the endogenous flora present. Increased effects of a combination of lacticin 3147 and hydrostatic pressure were reported by Morgan et al. (18). These results indicated that a combination of high pressure and lacticin 3147 may be used to improve the quality of minimally processed foods normally packed at lower hydrostatic pressure levels.

Although rapid progress is being made on the use of the bacteriocins from LAB as food biopreservatives, more research is needed.

ACKNOWLEDGEMENTS

This study was supported by the Good Health 21 program of the Korean Ministry of Health and Welfare (HMP-98-F-3-0010). This study was also supported by the Brain Korea 21 Project.

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(Received February 27, 2003; Accepted April 12, 2003)