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Inhibition of *Listeria monocytogenes* by *Lactobacillus bavaricus* MN in Beef Systems at Refrigeration Temperatures†

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The ability of *Lactobacillus bavaricus*, a meat isolate, to inhibit the growth of three *Listeria monocytogenes* strains was examined in three beef systems: beef cubes, beef cubes in gravy, and beef cubes in gravy containing glucose. The beef was minimally heat treated, inoculated with *L. bavaricus* at 10^5 or 10^3 CFU/g and *L. monocytogenes* at 10^2 CFU/g, vacuum sealed, and stored at 4 or 10°C. The meat samples were monitored for microbial growth, pH, and bacteriocin production. The pathogen was inhibited by *L. bavaricus* MN. At 4°C, *L. monocytogenes* was inhibited or killed depending on the initial inoculum level of *L. bavaricus*. At 10°C, at least a 10-fold reduction of the pathogen occurred, except in the beef without gravy. This system showed a transient inhibition of the pathogen during the first week of storage followed by growth to control levels by the end of the incubation period. Bacteriocin was detected in the samples, and inhibition could not be attributed to acidification. Low refrigeration temperatures significantly ($P \leq 0.05$) enhanced *L. monocytogenes* inhibition. Moreover, the addition of glucose-containing gravy and the higher inoculum level of *L. bavaricus* were significantly ($P \leq 0.05$) more effective in reducing *L. monocytogenes* populations in most of the systems studied.

Minimally processed, vacuum-packaged, refrigerated meat products have become increasingly popular. To fulfill consumer demands for "natural" foods, many of these products contain no preservatives (9). However, the microbial safety of these products is being questioned (33). They are susceptible to growth by psychrotrophic food-borne pathogens (37). Notably, *Listeria monocytogenes*, because of its ability to grow actively at refrigeration temperatures (7, 13, 42), poses a serious health threat to high-risk populations such as the unborn, newborn, or immunocompromised (28). Because of its ubiquitous distribution and its association with domestic livestock, *L. monocytogenes* is likely to occur in raw meats (4, 23). Furthermore, its prevalence both at the slaughterhouse (17) and in the processing environment (12) increases the potential for postprocessing contamination. The incidence and growth of *L. monocytogenes* in processed meat products are well documented (12, 19, 20, 23). Its prevalence ranges from 5 to 13% in ready-to-eat meat products, in which typical plate counts ranged from ≤10 to 1,000 CFU/g (23). Although most cases of human listeriosis associated with the consumption of ready-to-eat meat products appear to be sporadic (32, 38), the high mortality rate (30%) (27) has led the Food and Drug Administration to impose a zero-tolerance policy for *L. monocytogenes* in ready-to-eat meat products (14).

Novel strategies, such as biopreservation systems, have gained increasing attention as a means of "naturally" controlling the growth of pathogenic and spoilage organisms in ready-to-eat foods. Some lactic acid bacteria, such as those commonly associated with vacuum-packaged meats, produce antimicrobial proteins known as bacteriocins (24, 29). Thus, bacteriocins might be regarded as natural preservatives in refrigerated meat products (41). Several investigators have reported antilisterial activity of different bacteriocinogenic lactic acid bacteria in meat-related products (2, 3, 6, 8, 15, 30, 35, 40, 44). *Lactobacillus bavaricus* is commonly isolated from refrigerated vacuum-packaged meat (10). We have previously reported that *L. bavaricus* MN inhibits *L. monocytogenes* growth in a model meat gravy system (43). The antilisterial action was related to bacteriocin production and enhanced by lower temperatures and acid production. The purpose of this study was to validate the use of *L. bavaricus* MN to inhibit *L. monocytogenes* growth in minimally heat-treated, vacuum-packaged beef. We report here the inhibition of *L. monocytogenes* in all of the beef systems studied, although to different extents.

MATERIALS AND METHODS

Bacterial cultures and media. *L. monocytogenes* Scott A, F5069, and 19115, all carrying the plasmid pGK12 coding for chloramphenicol and erythromycin resistance (15), were the gift of P. M. Foegeding (North Carolina State University, Raleigh). They were grown at 30°C in Trypticase soy broth without glucose (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 0.6% yeast extract (Difco Laboratories, Detroit, Mich.), 0.5% glucose (Fisher Scientific Company, Pittsburgh, Pa.), 3.5 μg of chloramphenicol per ml (Sigma Chemical Co., St. Louis, Mo.), and 3.5 μg of erythromycin per ml (Sigma). The culture was maintained as a stab by adding 1.5% Bacto-Agar (Difco) to the broth. *L. bavaricus* MN was grown in Lactobacilli MRS broth (Difco) at 30°C and was maintained as stabs by adding 1.5% Bacto-Agar (Difco) to the broth. All stab cultures were kept at 4°C and transferred monthly.

L. monocytogenes was enumerated on tryptose phosphate broth (Difco) supplemented with 3.5 μg of chloramphenicol and erythromycin per ml and containing 1.5% Bacto-Agar (TPA). *L. bavaricus* MN was enumerated on Lactobacilli MRS broth with 1.5% Bacto-Agar. Total aerobic plate count was enumerated with plate count agar (Difco) (PCA).

Inoculum preparation. All three strains of *L. monocytogenes* were used as a pool. Cells of each strain were

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propagated overnight at 30°C in Trypticase soy broth containing yeast extract and glucose (10 ml) supplemented with chloramphenicol and erythromycin. Cells were pelleted by centrifugation (5,000 × *g* for 20 min, 4°C), washed twice in 10 ml of 0.1% peptone water, and resuspended in 5 ml of 0.1% peptone water. The A_{660} was measured, and each suspension was diluted as necessary to obtain approximately equal cell densities of each isolate. Equal volumes of the three suspensions were combined and diluted in 0.1% peptone to obtain the desired inoculum level. The cell suspension of *L. bavaricus* was prepared separately in a similar fashion and was resuspended in 0.1% peptone water to obtain the desired inoculum level.

Meat preparation and inoculation. Frozen beef (85% lean) diced cubes (0.75 in. [ca. 1.9 cm]) were obtained through the dining services of Rutgers, The State University of New Jersey. Before use, the meat was thawed at 4°C for 1 day. One hundred grams of meat was placed into individual gas-impermeable bags, and bags were steamed for 10 min at 100°C. Heat-treated bags were taped shut and held at 4°C until inoculation.

The inoculum was added to the meat at 1% (vol/wt) (100 g of meat received 1 ml of peptone water containing the inoculum preparation). To inoculate the beef in gravy, the inocula were added to 15 ml of gravy containing either 0 or 0.5% glucose. The 15 ml of inoculated gravy was then added to 100 g of beef. The gravy was formulated to contain 1.8% Trypticase peptone (BBL), 1.2% beef extract (Difco), 0.6% yeast extract (Difco), 0.2% carrageenan type II (Sigma), and 2% starch (National Starch & Chemical Corporation, Bridgewater, N.J.). The gravy was sterilized by autoclaving for 15 min at 15 lb/in^2. In each of the three meat experiments, the peptone water or gravies were prepared so that when added into the meat the final inoculum levels were 10^5 CFU of *L. bavaricus* per g and 10^2 CFU of *L. monocytogenes* per g or 10^3 CFU of *L. bavaricus* per g and 10^2 CFU of *L. monocytogenes* per g. In each experiment, *L. bavaricus* was inoculated alone at 10^5 or 10^3 CFU/g, *L. monocytogenes* was inoculated alone at 10^2 CFU/g, and uninoculated meat samples were used as controls.

After the inoculum was spread throughout the meat sample by massaging, the bags were vacuum sealed and stored at 4 or 10°C for up to 6 or 4 weeks, respectively.

Sampling and analysis. Three bags of meat from each treatment were sampled at selected times to determine *L. monocytogenes* and *L. bavaricus* populations and the pH values. Individual gas-impermeable bags were introduced into stomacher bags, opened, and stomached for 1 min with 100 ml of peptone water. *L. monocytogenes* was enumerated on TPA (supplemented with chloramphenicol and erythromycin) pour plates after 48 h at 30°C. Total aerobic plate counts were determined with PCA plates. Since PCA plates allow the growth of *L. monocytogenes* and the indigenous microflora, the difference in counts between PCA and TPA was used to determine the level of indigenous microflora. *L. bavaricus* MN was surface plated on MRS. The MRS and PCA surface platings were done with a spiral plater (Spiral System Instruments, Inc.), and colonies were enumerated after incubation for 48 h at 30°C with a bacterial colony counter (model 500A; Spiral System Instruments, Inc.). The pH of each bag sample was determined by inserting a flat-surface pH electrode (standardized against buffer at pH 4.0 and 7.0 and connected to a Markson pH meter) into the interior of the meat bag and obtaining readings from three different locations within the sample; the average value was reported.

Agar diffusion assay for bacteriocin production. Bacteriocin production was determined essentially as described by Rogers and Montville (34). Briefly, *Lactobacillus sake* ATCC 15521, the bacteriocin-sensitive organism, was grown in Lactobacilli MRS broth at 30°C overnight and diluted to 10^5 to 10^6 CFU/ml in tempered MRS broth containing 1.5% Noble agar (Difco). The inoculated agar (20 ml) was pipetted into sterile petri dishes, and 6.8-mm wells were cut after the agar solidified. At the end of each experiment described above, 1 ml of the bag contents was microcentrifuged for 10 min. The cell-free supernatant was removed and neutralized with 1 N NaOH, and 50 μl was pipetted into each well. The plates were preincubated at 4°C overnight and incubated at 30°C for 24 h. The clearing zones formed around the well were measured as the distance from the edge of the well to the edge of the inhibition zone of the sensitive organism.

The proteinaceous nature of the bacteriocin was confirmed by adding 10 μl of α-chymotrypsin (bovine pancrease, type II) (Sigma), 10 mg/ml in 10 mM sodium phosphate buffer (pH 7.5), into a smaller well, 3.8 mm in diameter, cut adjacent to the 6.8-mm one (25). A negation of the inhibition zone in the region of the protease indicated inhibitor sensitivity to the protease and thus confirmed the proteinaceous nature of the inhibitory substance.

In order to compare the amount of bavaricin MN with equivalent nisin units, 1 g of Nisaplin (Aplin & Barret Ltd., Trowbridge, Wiltshire, England) (1,000,000 IU/g) was serially diluted in 0.02 N HCl–0.75% NaCl (pH 5.2). Fifty microliters was added to the wells, and standard curves of zone sizes versus concentrations were generated.

Statistical analysis. An analysis of the variance (*F* distribution and individual or multiple *t*-confidence intervals) of the meat treatments was performed to determine the significance of any differences. Data from final pH values were analyzed by the Student *t* test. Significance is expressed at the 5% level in all analysis.

RESULTS AND DISCUSSION

Growth and survival of *L. monocytogenes* in minimally heat-treated, vacuum-packaged meat cubes inoculated with high (10^5 CFU/g) or low (10^3 CFU/g) levels of *L. bavaricus* MN at 4 or 10°C are shown in Fig. 1 and 2, respectively. In the absence of *L. bavaricus*, the *L. monocytogenes* population grew to 10^7 CFU/g at 4°C or to 10^8 CFU/g at 10°C by the end of a 6- or 4-week incubation period, respectively, as observed with TPA plate counts. The presence or absence of gravy in the meat did not influence final *L. monocytogenes* levels. By using a composite of three *L. monocytogenes* strains, we decreased the chances of selecting a strain that was very sensitive or resistant to the bacteriocin. These strains, transformed with the pGK12 plasmid (15), which conferred resistance to chloramphenicol and erythromycin, enabled us to quantitatively monitor the listerial population from a mixed background microflora. Foegeding et al. (15) have shown that both wild-type and transformed strains were similarly sensitive to bacteriocin activity.

The minimal heat treatment applied to the meat samples (steaming for 10 min at 100°C) did not render a product sterile. Low levels of indigenous microflora ($\leq 10^2$ CFU/g) were observed at time zero in the uninoculated bags. This native flora grew under vacuum conditions at refrigeration temperatures, reaching levels of 10^6 to 10^8 CFU/g at the end of the storage period, depending on the initial microflora load (data not shown). However, this native microflora did not inhibit *L. monocytogenes* growth. The 10- to 100-fold differ-

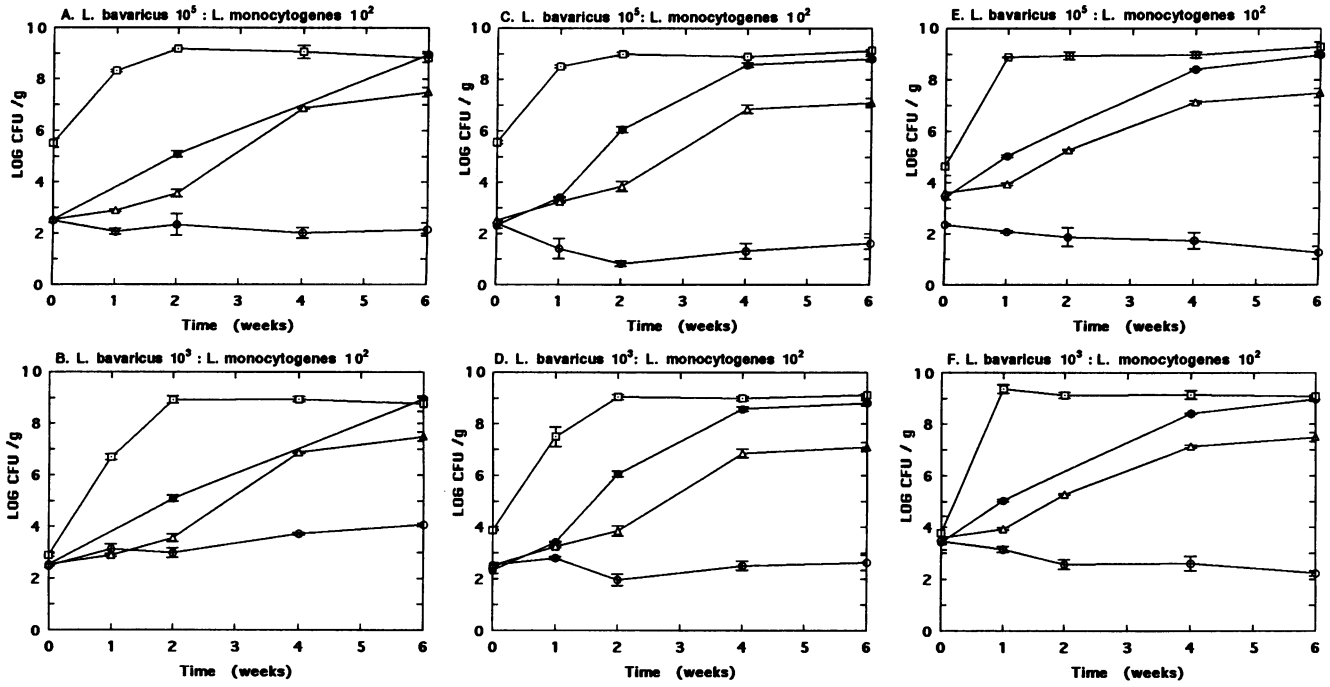

FIG. 1. Growth of *L. monocytogenes* and *L. bavaricus* MN in minimally heat-treated, vacuum-packaged meat at 4°C. Panels A and B represent beef cubes packaged without gravy. Panels C and D represent beef cubes packaged in gravy, and panels E and F represent beef cubes packaged in gravy containing 0.5% glucose. Symbols: ⊡, *L. bavaricus* coinoculated with *L. monocytogenes* enumerated on MRS; ○, *L. monocytogenes* coinoculated with *L. bavaricus* enumerated on TPA; △, *L. monocytogenes* alone enumerated on TPA; ●, *L. monocytogenes* alone enumerated by total plate counts. Vertical bars represent the standard deviation of the mean.

ence between the PCA and TPA population counts from samples inoculated only with *L. monocytogenes* can be explained by the growth of the indigenous background microflora and gives evidence of the noninhibitory action of the microflora against the pathogen.

L. bavaricus MN, an isolate from a species usually associated with red meats stored chilled in vacuum packs (10), grew faster than *L. monocytogenes*, reaching levels of at least 10^8 CFU/g during the first 2 weeks of incubation. *L. bavaricus* growth patterns were similar, independent of the initial inoculum level, the temperature of incubation, or the presence or absence of gravy. Moreover, the presence of *L. monocytogenes* had no apparent effect on the growth of *L. bavaricus* MN (data not shown). In all three meat systems incubated at 4°C, *L. bavaricus* inhibited *L. monocytogenes* growth (Fig. 1). Independent of the meat system (gravy containing 0 or 0.5% glucose or no gravy added), all samples inoculated with *L. bavaricus* at 10^5 CFU/g showed antagonistic action against *L. monocytogenes*, but no significant difference among the meat systems was observed (Fig. 1A, C, and E). Those systems in which a low inoculum level of *L. bavaricus* was added (10^3 CFU/g) were either bacteriostatic (Fig. 1F) or strongly inhibitory towards *L. monocytogenes*. There was a 10,000-fold suppression of listerial growth in glucoseless gravy (Fig. 1D) or a 1,000-fold reduction compared with the control in beef cubes without gravy (Fig. 1B). In these samples (low initial inoculum level of *L. bavaricus*), the addition of glucose-containing gravy significantly enhanced the pathogen inhibition, as compared with that for treatment in which no gravy was added. At 4°C, the antilisteric action was significantly enhanced by the higher inoculum levels of the lactic acid bacteria.

In those meat systems incubated at 10°C, *L. monocytogenes* was inhibited only when the gravy was present (Fig. 2C to F). For the meat containing no gravy, a transient inhibition of the pathogen during the first week of storage occurred but was followed by growth to control levels by the end of the incubation period (Fig. 2A and B). The *L. monocytogenes* population was significantly decreased (with both high and low inoculum levels of the lactic acid bacteria) when glucose-containing gravy was added to the meat. Since it was reported that *L. monocytogenes* may become resistant to bacteriocins (15, 21), *L. monocytogenes* isolates from coinoculated samples were tested for sensitivity to *L. bavaricus* MN at the end of each experiment by the agar diffusion assay. No resistance was acquired towards bavaricin MN during this period of time (data not shown).

The pH was monitored over the course of the experiment for each meat system. Initial pH values of the various meat systems were 6.13 ± 0.07 (data not shown) and decreased in the inoculated samples to levels ranging from 5.09 to 5.72 (Table 1). The final pH values in uninoculated samples were ≥5.73. In most cases, no significant difference between the final pH values of the coinoculated combination and that of the corresponding control coinoculated with *L. monocytogenes* alone was observed, regardless of the presence of glucose or gravy or the temperature of incubation. The only situation in which a significant difference was observed (in comparison with the control) was the combination of *L. bavaricus* at 10^5 and *L. monocytogenes* at 10^2 CFU/g (pH of 5.09 ± 0.23) in the beef in gravy containing glucose incubated at 10°C. Growth of *L. monocytogenes* at pH values less than 5.0 has been reported (18). The inhibition of *L. monocytogenes* in this study cannot be explained by a

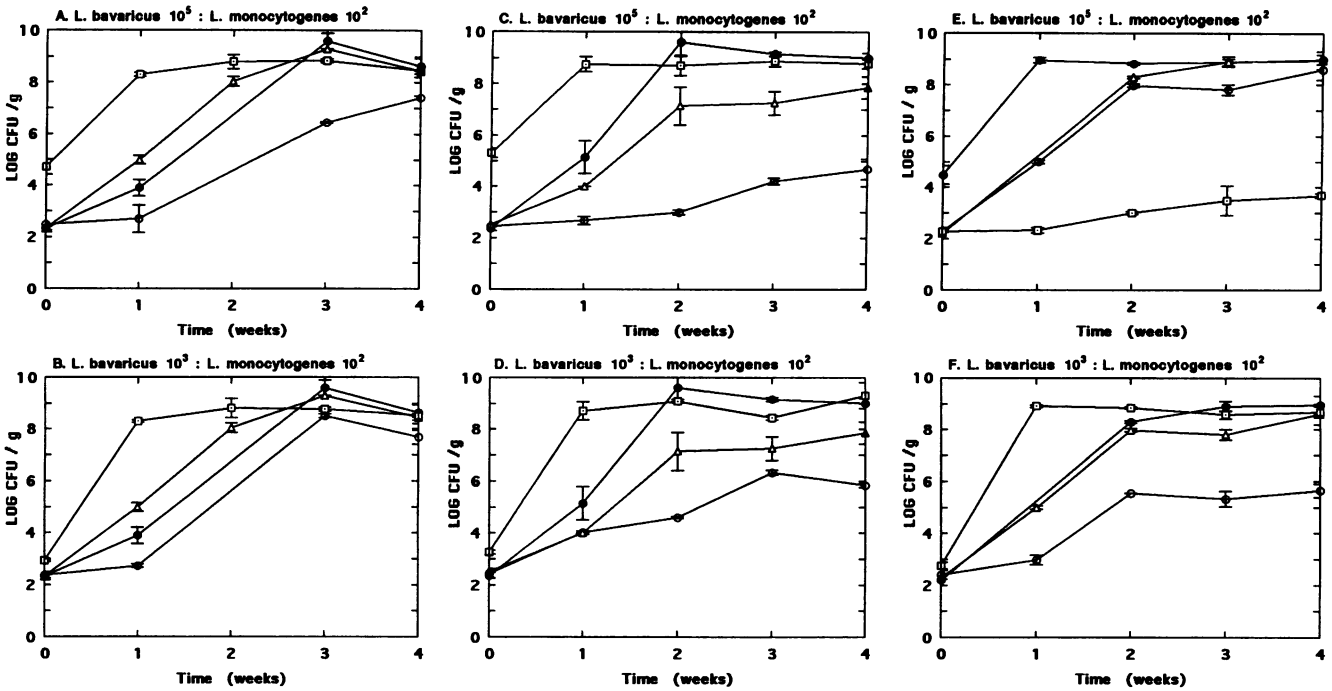

FIG. 2. Growth of *L. monocytogenes* and *L. bavaricus* MN in minimally heat-treated, vacuum-packaged meat at 10°C. Panel and symbol definitions are as described for Fig. 1.

decrease in pH. Furthermore, inhibition of the pathogen was observed when pH values were still close to 6.1.

Previous studies in our laboratory have demonstrated bacteriocin production by *L. bavaricus* MN in medium (25), as well as in a model gravy system (43). Furthermore, bavaricin MN has been characterized (26). In the studies reported here, we demonstrated bacteriocin production in meat samples by using the well diffusion assay (data not shown). In this regard, cell-free neutralized supernatants from all meat systems inoculated with *L. bavaricus* MN produced a clearing zone on lawns containing a sensitive indicator organism. Furthermore, their proteinaceous nature was confirmed by the negation of the zone of inhibition in the presence of a protease (data not shown). Bacteriocin production was independent of the presence of glucose in the meat. In a few instances, smaller zones of inhibition were observed with uninoculated samples or those samples inoculated only with *L. monocytogenes*. This may be the result of bacteriocin production by indigenous microflora. Yet, it failed to inhibit *L. monocytogenes* growth as discussed above. Schillinger et al. (35) reported that sakacin A, produced by *L. sake* Lb 706, was inactivated after 1 week in minced meat stored at 8°C. In these studies, bacteriocin production was observed after 6 or 4 weeks of storage at 4 and 10°C, respectively, suggesting that bavaricin MN is stable in minimally processed meat, as reported for pediocin PA1 (31). When the zones of inhibition obtained from meat supernatants in these experiments were compared with zones of inhibition of known concentrations of Nisaplin, values ranging from 0.4 to 1.2 equivalent nisin units were observed (data not shown). In this regard, 100 IU of pure nisin was shown to decrease the number of *L. monocytogenes* cells by 100,000-fold when added directly into Trypticase soy broth with yeast extract and glucose (5).

This study shows the antagonistic action of *L. bavaricus* MN against *L. monocytogenes* in minimally heat-treated, vacuum-packaged meat stored at refrigeration temperatures. Inhibition was not due to acidification, since the antagonistic effect was observed at noninhibitory pH levels. Similar experiments using an isogenic Bac⁻ *L. bavaricus* MN would be required to definitively prove the bacteriocin's role. Unfortunately, exhaustive efforts at generating such a Bac⁻ strain were fruitless. Yet, even though the pH was not greatly reduced in those meats in gravy with glucose, addition of the sugar enhanced the antagonistic action towards

TABLE 1. Final pH values of beef systems inoculated with *L. bavaricus* and/or *L. monocytogenes*[a]

Temp (°C)	Inoculum (CFU/g)[b]		Final pH value for beef cubes (±SD):		
	MN	SA	Without gravy	In gravy without glucose	In gravy with glucose
4	0	10^2	5.67 ± 0.12	5.64 ± 0.08	5.59 ± 0.02
	10^5	0	5.54 ± 0.06	5.44 ± 0.42	5.11 ± 0.24
	10^3	0	5.50 ± 0.06	5.56 ± 0.23	5.23 ± 0.19
	10^5	10^2	5.55 ± 0.05	5.46 ± 0.11	5.59 ± 0.07
	10^3	10^2	5.58 ± 0.05	5.61 ± 0.11	5.54 ± 0.05
	0	0	5.85 ± 0.29	5.96 ± 0.18	6.24 ± 0.01
10	0	10^2	5.72 ± 0.15	5.62 ± 0.23	5.60 ± 0.11
	10^5	0	5.56 ± 0.04	5.29 ± 0.05	5.51 ± 0.19
	10^3	0	5.62 ± 0.09	5.38 ± 0.18	5.42 ± 0.08
	10^5	10^2	5.51 ± 0.22	5.64 ± 0.04	5.09 ± 0.23
	10^3	10^2	5.72 ± 0.12	5.48 ± 0.23	5.58 ± 0.03
	0	0	5.73 ± 0.08	5.96 ± 0.39	6.15 ± 0.03

[a] pH values measured after 6 weeks at 4°C or 4 weeks at 10°C. The initial pH value for the three systems was 6.13 ± 0.07.
[b] MN, *L. bavaricus* MN; SA, *L. monocytogenes* SA.

the pathogen. Previous reports support the fact that inhibition of *L. monocytogenes* by bacteriocinogenic lactic acid bacteria may be independent of acid production (3, 6, 35, 40). These observations lead us to speculate that bavaricin MN may be acting in an energy-dependent manner, as is the case for other bacteriocins (16, 39). Lower refrigeration temperatures enhanced the antagonistic action. *L. monocytogenes* is also more sensitive to the bacteriocin produced by *Carnobacterium piscicola* LK5 at 5°C than at 19°C (6). This evidence suggests that a multiple-barrier system best inhibits pathogen growth. That the antilisterial activity was greater in most of those products containing gravy with glucose suggests that the addition of gravy to the meat served as a matrix to improve bacteriocin diffusion and thus facilitated access to the pathogen target organism.

Recent reports suggest that a high initial inoculum level of the starter culture is needed to inhibit the target organism (2, 40). High cell densities (10^8 to 10^9 CFU) may account for either depletion of essential nutrients (29, 40) or the generation of a bacteriocin MIC (36). However, we report that successful inhibition of the pathogen occurred even with a low initial inoculum level of *L. bavaricus*. As we have previously reported (43), the fact that inhibition of *L. monocytogenes* is evident after the onset of *L. bavaricus* exponential growth may suggest that bavaricin production occurs during early growth stages, as is reported for other meat lactic acid bacteria (1, 11, 22).

This study confirms the antagonistic action of *L. bavaricus* MN towards *L. monocytogenes* in minimally heat-treated, vacuum-packaged meat. The use of bacteriocinogenic lactic acid bacteria as potential biopreservatives may be appealing to the food industry. Not only does *L. bavaricus* MN grow and produce bacteriocin at refrigeration temperatures, but also it is effective at nonacidic pH values. Thus, meeting consumers' demands for natural preservatives, it also serves as an additional safety barrier to ensure product quality at refrigeration temperatures.

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REFERENCES

1. **Anh, C., and M. E. Stiles.** 1990. Antibacterial activity of lactic acid bacteria isolated from vacuum-packaged meats. J. Appl. Bacteriol. **69**:302–310.
2. **Berry, E. D., R. W. Hutkins, and R. W. Mandigo.** 1991. The use of bacteriocin-producing *Pediococcus acidilactici* to control post-processing *Listeria monocytogenes* contamination of frankfurters. J. Food Prot. **54**:681–686.
3. **Berry, E. D., M. B. Liewen, R. Mandigo, and R. W. Hutkins.** 1990. Inhibition of *Listeria monocytogenes* by bacteriocin producing *Pediococcus* during the manufacture of fermented semi-dry sausage. J. Food Prot. **53**:194–197.
4. **Brackett, R. E.** 1988. Presence and persistence of *Listeria monocytogenes* in food and water. Food Technol. **42**(4):162–164.
5. **Bruno, M. E. C., A. Kaiser, and T. J. Montville.** 1992. Depletion of proton motive force by nisin in *Listeria monocytogenes* cells. Appl. Environ. Microbiol. **58**:2255–2259.
6. **Buchanan, R. L., and L. A. Klawitter.** 1992. Effectiveness of *Carnobacterium piscicola* LK5 for controlling the growth of *Listeria monocytogenes* Scott A in refrigerated foods. J. Food Saf. **12**:217–234.
7. **Buchanan, R. L., H. G. Stahl, and R. C. Whiting.** 1989. Effects and interactions of temperature, pH, atmosphere, sodium chloride, and sodium nitrite on the growth of *Listeria monocytogenes*. J. Food Prot. **52**:844–851.
8. **Chung, K. T., J. S. Dickson, and J. D. Crouse.** 1989. Effects of nisin on growth of bacteria attached to meat. Appl. Environ. Microbiol. **55**:1329–1333.
9. **Corlett, D. A., Jr.** 1989. Refrigerated foods and use of hazard analysis and critical control point principles. Food Technol. **43**(2):91–94.
10. **Dainty, R. H., and B. M. Mackey.** 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage process. J. Appl. Bacteriol. **73**:103S–114S.
11. **Degan, A. J., A. E. Yousef, and J. B. Luchansky.** 1992. Use of *Pediococcus acidilactici* to control *Listeria monocytogenes* in temperature-abused vacuum-packaged wieners. J. Food Prot. **55**:98–103.
12. **Doyle, M. P.** 1988. Effect of environmental and processing conditions on *Listeria monocytogenes*. Food Technol. **42**(4):169–171.
13. **Farber, J. M., and P. I. Peterkin.** 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. **55**:476–511.
14. **Federal Register.** 1989. Revised policy for controlling *Listeria monocytogenes*. Fed. Regist. **54**:22345–22346.
15. **Foegeding, P. M., A. B. Thomas, D. H. Pilkington, and T. R. Klaenhammer.** 1992. Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. Appl. Environ. Microbiol. **58**:884–890.
16. **Gao, F. H., T. Abee, and W. N. Konings.** 1991. Mechanism of action of the peptide antibiotic nisin in liposomes and cytochrome *c* oxidase-containing proteoliposomes. Appl. Environ. Microbiol. **57**:2164–2170.
17. **Genigeorgis, C. A., D. Dutulescu, and J. F. Garayzabal.** 1989. Prevalence of Listeria spp. in poultry meat at the supermarket and slaughterhouse level. J. Food Prot. **52**:618–624.
18. **George, S. M., B. M. Lund, and T. F. Brocklehurst.** 1988. The effect of pH and temperature on initiation of growth of *Listeria monocytogenes*. Lett. Appl. Microbiol. **6**:153–156.
19. **Glass, K. A., and M. P. Doyle.** 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. Appl. Environ. Microbiol. **55**:1565–1569.
20. **Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4–7.
21. **Harris, L. J., H. P. Fleming, and T. R. Klaenhammer.** 1991. Sensitivity and resistance of *Listeria monocytogenes* ATCC 19115, Scott A and UAL 500 to nisin. J. Food Prot. **54**:836–840.
22. **Hastings, J. W., and M. E. Stiles.** 1991. Antibiosis of *Leuconostoc gelidum* isolated from meat. J. Appl. Bacteriol. **70**:127–134.
23. **Johnson, J. F., M. P. Doyle, and R. G. Cassens.** 1990. *Listeria monocytogenes* and other *Listeria* spp. in meat and meat products: a review. J. Food Prot. **53**:81–91.
24. **Klaenhammer, T. R.** 1988. Bacteriocins of lactic acid bacteria. Biochimie **70**:337–349.
25. **Lewus, C. B., A. Kaiser, and T. J. Montville.** 1991. Inhibition of food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl. Environ. Microbiol. **57**:1683–1688.
26. **Lewus, C. B., and T. J. Montville.** 1992. Further characterization of bacteriocins Plantaricin BN, Bavaricin MN and Pediocin A. Food Biotechnol. **6**:153–174.
27. **Lovett, J.** 1989. *Listeria monocytogenes*, p. 283–310. *In* M. P. Doyle (ed.), Foodborne bacterial pathogens. Marcel Dekker, Inc., New York.
28. **Marth, E. H.** 1988. Disease characteristics of *Listeria monocytogenes*. Food Technol. **42**(4):165–168.
29. **Marugg, J. D.** 1991. Bacteriocins, their role in developing natural products. Food Biotechnol. **5**:305–312.
30. **Mattila-Sandholm, T., A. Haikra, and E. Skyttä.** 1991. The effect of *Pediococcus damnosus* and *Pediococcus pentosaceus* on the growth of pathogens in minced meat. Int. J. Food Microbiol. **13**:87–94.
31. **Nielsen, J. W., J. S. Dickson, and J. D. Crouse.** 1990. Use of a bacteriocin produced by *Pediococcus acidilactici* to inhibit *Listeria monocytogenes* associated with fresh meat. Appl. En-

viron. Microbiol. **56**:2142–2145.
32. **Pinner, R. W., A. Schuchat, B. Swaminathan, P. S. Hayes, K. A. Deaver, R. E. Weaver, B. D. Plikaytis, M. Reeves, C. V. Broome, J. D. Wenger, and the Listeria Study Group.** 1992. Role of foods in sporadic listeriosis. JAMA **267**:2046–2050.
33. **Rhodehamel, E. J.** 1992. FDA's concerns with *sous vide* processing. Food Technol. **46**(12):73–76.
34. **Rogers, A. M., and T. J. Montville.** 1991. Improved agar diffusion assay for nisin quantification. Food Biotechnol. **5**:161–168.
35. **Schillinger, U., M. Kaya, and F. K. Lüke.** 1991. Behavior of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*. J. Appl. Bacteriol. **70**:473–478.
36. **Schillinger, U., and F. K. Lüke.** 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Microbiol. **55**:1901–1906.
37. **Schofield, G. M.** 1992. Emerging food-borne pathogens and their significance in chilled foods. J. Appl. Bacteriol. **72**:267–273.
38. **Schuchat, A., K. A. Deaver, J. D. Wenger, B. D. Plikaytis, L. Mascola, R. W. Pinner, A. L. Reingold, C. V. Broome, and the Listeria Study Group.** 1992. Role of foods in sporadic listeriosis. JAMA **267**:2041–2045.
39. **Schuller, F., R. Benz, and H. G. Sahl.** 1989. The peptide antibiotic subtilin acts by formation of voltage-dependent multistage pores in bacterial and artificial membranes. Eur. J. Biochem. **182**:181–186.
40. **Skyttä, E., W. Hereijgers, and T. Mattila-Sandholm.** 1991. Broad spectrum antibacterial activity of *Pediococcus damnosus* and *Pediococcus pentosaceus* in minced meat. Food Microbiol. **8**:231–237.
41. **Stiles, M. E., and J. W. Hastings.** 1991. Bacteriocin production by lactic acid bacteria: potential for use in meat preservation. Trends Food Sci. Technol. **2**:247–251.
42. **Walkers, S. J., P. Archer, and J. G. Banks.** 1990. Growth of *Listeria monocytogenes* at refrigeration temperatures. J. Appl. Bacteriol. **68**:157–162.
43. **Winkowski, K., and T. J. Montville.** 1992. Use of meat isolate *Lactobacillus bavaricus* MN to inhibit *Listeria monocytogenes* growth in a model meat gravy system. J. Food Saf. **13**:19–31.
44. **Yousef, A. E., J. B. Luchansky, A. J. Degnan, and M. P. Doyle.** 1991. Behavior of *Listeria monocytogenes* in wiener exudates in the presence of *Pediococcus acidilactici* H or pediocin AcH during storage at 4 or 25°C. Appl. Environ. Microbiol. **57**:1461–1467.