ELSEVIER

International Journal of Food Microbiology 70 (2001) 111–119

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Evaluation of the ability of lysozyme and nisin to control meat spoilage bacteria

F.M. Nattress*, C.K. Yost, L.P. Baker

Agriculture and Agri-Food Canada, Lacombe Research Centre, 6000 C&E Trail, Lacombe, Alberta, Canada AB T4L 1W1

Received 7 November 2000; received in revised form 8 March 2001; accepted 17 April 2001

Abstract

The antimicrobials lysozyme, nisin, and mixtures of the two were studied to ascertain their abilities to control the growth of the meat-borne spoilage bacteria, *Brochothrix thermosphacta* B2 and *Carnobacterium* sp. 845. The goal was to optimize an antimicrobial for potential use in preservation of fresh meats. Their efficacies were evaluated in APT broth, in a meat juice extract and on cores of lean and fat pork tissue. Both lysozyme and nisin alone as well as mixtures of the two effectively inhibited *B. thermosphacta* B2 at 250 μg/ml in APT broth, the lowest concentration evaluated, for 10 days at 2°C. In the presence of 500 μg/ml lysozyme, *B. thermosphacta* B2 grew after 12 days incubation. Only 125 μg of antimicrobial/ml was required to inhibit *B. thermosphacta* B2 for 27 days at 2°C in pork juice. An estimated surface concentration of 130 $\mu g/cm^2$ of each of the antimicrobials effectively inhibited *B. thermosphacta* B2 on inoculated cores of fat and lean pork tissue when the cores were incubated in vacuum packages for 6 weeks at 2°C. In APT broth and in pork juice, lysozyme showed no antimicrobial activity against *Carnobacterium* sp. 845 at concentrations of 500 and 1000 μg/ml, respectively. Nisin and mixtures of the two antimicrobials inhibited *Carnobacterium* sp. 845 so that its numbers were at least 3 log units lower than untreated samples after 26 and 27 days incubation for APT and pork juice, respectively. The antimicrobial effect was concentration dependent. On lean pork tissue, numbers of *Carnobacterium* sp. 845 were significantly lower than untreated samples or samples treated with 195 $\mu g/cm^2$ lysozyme when 260 $\mu g/cm^2$ of a 1:3 (w/w) ratio of nisin to lysozyme was introduced to the cores. The inhibitory effect lasted for 14 of 42 days incubation in vacuum at 2°C. On fat tissue, both lysozyme alone and the 1:3 nisin/lysozyme mixture inhibited *Carnobacterium* sp. 845 for 21 days storage in vacuum at 2°C. On fat and lean tissue, mixtures of nisin and lysozyme would be more effective antimicrobials than either nisin or lysozyme alone. Crown Copyright © 2001 Published by Elsevier Science B.V. All rights reserved.

Keywords: Antimicrobial; Lysozyme; Nisin; Meat spoilage

1. Introduction

Spoilage of raw meat accounts for major annual losses to processors and retailers. Globally, the red meat industry has increased its use of anoxic preservative packaging systems to satisfy the demand for extended product storage life and reduction of spoilage losses. During extended anoxic storage, growth of putrefactive bacteria is limited and lactic acid bacteria (LAB) becomes the dominant bacteria. Eventually, their numbers attain maximum levels of 10^6–10^7 cfu/cm^2 and spoilage by LAB occurs (Egan, 1983). One approach to extending the storage and shelf life of fresh meats is to introduce antimi-

* Corresponding author. Tel.: +1-403-782-8140; fax: +1-403-782-6120.

E-mail address: nattressf@em.agr.ca (F.M. Nattress).

0168-1605/01/$ - see front matter. Crown Copyright © 2001 Published by Elsevier Science B.V. All rights reserved.
PII: S0168-1605(01)00531-1

crobials, preferably naturally occurring antimicrobials, to the surface of the meat (Chung et al., 1989; Cutter and Siragusa, 1996a,b, 1997; Cutter, 1999, 2000; Gill and Holley, 2000a,b). Lysozyme, a lytic enzyme found in foods such as milk and eggs, is a muraminidase that hydrolyses β1–4 linkages between *N*-acetylmuramic acid and *N*-acetylglucosamine (Cunningham et al., 1991). It is known to inhibit some gram-positive bacteria, but alone it is ineffective against gram-negative bacteria. Commercially, lysozyme has been used primarily to prevent late blowing in semi-hard cheeses, which is caused by the fermentation of lactate by butyric acid bacteria, primarily *Clostridium tyrobutyricum* (Bester and Lombard, 1990; Cunningham et al., 1991). The potential use of lysozyme as a food preservative has invoked considerable interest, particularly in Japan (Cunningham et al., 1991). Nisin, produced by *Lactococcus lactis* subsp. *lactis*, is a small, heat-stable protein, classified as a lantibiotic (Holzapfel et al., 1995) and is the best known of the bacteriocins. Its spectrum of activity is limited and includes many gram-positive bacteria and their spores but does not include gram-negative bacteria or fungi (Delves-Broughton et al., 1996). Nisin is generally recognized as safe (GRAS) for use as a food additive in many countries.

Evaluation of the antimicrobial activity of nisin in raw beef using selected organisms (Cutter and Siragusa, 1994, 1996a,b, 1997) has shown that nisin activity is lost over time. Although there were initial reductions in numbers of gram-positive organisms inoculated onto surfaces, after extended storage, numbers increased to the levels on untreated samples. When nisin was embedded in a calcium alginate gel, the antimicrobial activity of the nisin was increased. Numbers of *B. thermosphacta*, a potent meat spoilage organism, remained low for up to 7 days (Cutter and Siragusa, 1996b). Synergy between nisin and lysozyme has been shown in vitro (Chung and Hancock, 2000).

This work was initiated to evaluate the synergistic effect of lysozyme and nisin on two bacteria isolated from meat and associated with spoilage, *B. thermosphacta* B2 and *Carnobacterium* sp. 845. Studies were done in vitro and in meat systems. Often antimicrobials are evaluated in an in vitro system only (Szabo and Cahill, 1998a). These types of studies give some insight into what may happen in a food system, but the natural system is very different. When food systems are evaluated, the efficacies of antimicrobial treatments are frequently much reduced (Scannell et al., 1997; Szabo and Cahill 1998b; Chumchalova et al., 1998; Greer et al., 2000).

2. Materials and methods

2.1. Growth media

A preliminary trial was done in APT broth (Difco Laboratories, Detroit, MI, USA). Subsequent trials were done in a pork juice medium (Greer et al., 2000), which was supplemented with 0.4% dextrose (Sigma, St. Louis, MO, USA) by the addition of 20 ml/l from a 20% (w/v) sterile stock solution prior to use in trials.

2.2. Pork cores

Sterile cores of fat and lean tissue with a surface area of 25 cm^2 were excised from pork loins that had generous fat coverings, using the procedure described by Greer et al. (2000).

2.3. Antimicrobials

Nisin (Chrisin (2.5% w/w nisin), 1000 IU/mg, Christian Hansen, Denmark), lysozyme (> 22,800 Shugar units/mg, Canadian Inovatech, Abbotsford, BC, Canada) and co-dried mixtures of the two at ratios of 1:3 nisin/lysozyme and 1:1 nisin/lysozyme and 3:1 nisin/lysozyme (Canadian Inovatech) were used at concentrations of 250, 500 and 1000 μg/ml in APT broth and at concentrations of 125, 250, 500 and 1000 μg/ml in pork juice medium. The antimicrobials were dissolved either in APT broth or in pork juice medium to attain the appropriate final concentrations. Growth media containing antimicrobials were filter sterilized through 0.2-μm Nalgene® Membrane Filter (Nalgene, Rochester, NY, USA) prior to dispensing into sterile flasks for use in the experiment. Nisin, lysozyme and mixtures of the two at weight to weight ratios of 1:1 nisin/lysozyme and 1:3 nisin/lysozyme were used at concentrations of 2500, 5000 and 10,000 μg/ml to

attain estimated concentrations of antimicrobial on cores of pork fat and lean tissue of 65, 130 and 260 μg/cm^2, respectively, depending on the experiment. The volume of antimicrobial adhering to the meat surface was estimated by dipping 25 cores of lean and 25 cores of fat tissue into a known volume of antimicrobial. The reduction in volume of antimicrobial after the treatment of the cores was measured and the volume of antimicrobial adhering to each core was calculated. Antimicrobials were dissolved in sterile water and were used immediately. They were not filter sterilized before use.

2.4. Bacterial cultures

The psychrotrophic meat-borne bacteria isolated from spoiled beef, *B. thermosphacta* B2 (G.G. Greer, Agriculture and Agri-Food Canada) and *Carnobacterium* sp. 845 were used in this study. *Carnobacterium* sp. 845 was isolated from carbon dioxide packaged beef at the time of spoilage (F.M. Nattress) and was tentatively identified as *Lactobacillus* sp. using CH50 strips (Biomérieux, St. Laurent, QC, Canada). Genotypic analysis using the methods of Nissen et al. (1994) and Yost and Nattress (2000) showed it to be a *Carnobacterium* sp. It is the same organism as the *Lactobacillus* sp. 845 described in the work of Chung and Hancock (2000). The organisms were maintained in Cooked Meat Medium (Difco). Bacteria were subcultured twice in Tryptic Soy Broth (TSB) (Difco) using a 1% inoculum, and they were incubated aerobically without shaking at 25°C for 48 h. For studies in broth and pork juice, 4 log cfu/ml inocula from these starter cultures were used. When the organisms were used to inoculate pork cores, cells from 25 ml of starter culture were pelleted (15,000 rpm for 3 min), resuspended in 50 ml of 0.1% peptone (Difco) and washed twice to remove growth medium. Pellets were resuspended in 0.1% peptone to a final concentration of log 7 cfu/ml of cells.

2.5. Sample preparation and sampling plan

Growth medium supplemented with antimicrobial was inoculated to give a final concentration of bacterial cells of log 4 cfu/ml. Samples were incubated aerobically at 2°C with no shaking. Two to three times per week, samples were removed and bacteria were enumerated. Samples of 0.1 ml were removed and 10-fold dilutions prepared in 0.1% peptone diluent. Samples of 0.1 ml were spread onto the surface of plates of Tryptic Soy Agar (TSA, Difco). Inoculated plates were incubated aerobically at 25°C for 48 h and bacterial numbers/ml of sample were calculated. Two replications of the trial in pork juice medium were done and triplicate samples were examined in each replication.

Cores of lean and fat pork tissue were dipped in the suspensions of *B. thermosphacta* B2 or *Carnobacterium* sp. 845 to introduce approximately 3.5 log cfu/cm^2 bacteria to the lean cores and 4 log cfu/cm^2 to the fat cores. The procedure has been described by Greer et al. (2000). Cores were packaged in vacuum pouches (Winpak, Winnipeg, MB, Canada) with an oxygen transmission rate of 40–50 cm^3/m^2 in 24 h at 23°C. The pouches were vacuum packaged (Multivac, Kansas City, MO, USA) and were incubated at 2°C. At 0 time, and weekly thereafter, for 6 weeks, cores were removed and sampled. For enumeration of bacteria, 1:1 dilutions of the cores were prepared by homogenizing the cores for 2 min in 25 ml of 0.1% peptone diluent using a Stomacher® Lab-Blender Model '400' (Seward Laboratory, London, England). Further dilutions were 10-fold and 0.1 ml samples were plated on TSA. Inoculated plates were incubated aerobically at 25°C for 48 h. Bacterial numbers were calculated as log cfu/cm^2.

The Commission Internationale de l'Eclairage (CIE, 1978) values (L^*, a^* and b^*) were measured objectively using a Minolta chroma meter II (Minolta Camera, Japan) and the pH was measured using an Oktron Digital pH meter (Model Wo-0060500-000, Anachemia Scientific, Calgary, AB, Canada) and a flat surface polymer body combination electrode (Fisher Scientific, Nepean, ON, Canada).

2.6. Statistical analysis

Data were analyzed using the General Linear Models Analysis of Variance Procedure of the Statistical Analysis System (SAS Institute, 1995). Means were compared by the Student's *t*-test and differences were declared significant when $P < 0.05$. The models included treatment or time of incubation and

treatment and their interaction. Data were sorted by organism and incubation temperature and time where applicable.

3. Results

3.1. APT broth

The effect on growth of *B. thermosphacta* B2 incubated at 2°C in APT broth supplemented with nisin, lysozyme, 1:1 nisin/lysozyme, 1:3 nisin/lysozyme or 3:1 nisin/lysozyme at a concentration of 250, 500 and 1000 μg/ml was examined. All treatments inhibited the growth of *B. thermosphacta* B2 during 10 days incubation at 2°C (data not shown). The trials using 500 μg/ml of antimicrobial were continued for 26 days and were repeated twice. *B. thermosphacta* B2 started to grow after 10 days incubation (Fig. 1A) in the flasks treated with lysozyme alone. Nisin and all the mixtures inhibited *B. thermosphacta* B2 throughout the trial period.

Lysozyme alone had no effect on *Carnobacterium* sp. 845 in APT broth and the growth of the organism was the same as in the sample with no antimicrobial (Fig. 1B). Mixtures of 3:1 nisin/lysozyme, 1:1 nisin/lysozyme and 1:3 nisin/lysozyme at a concentration of 500 μg/ml inhibited bacteria growth for 10–12 days. When bacteria did grow, the cultures reached stationary phase approximately 5 log units lower than the samples with no antimicrobial.

3.2. Pork juice

When *B. thermosphacta* B2 was grown in pork juice medium at 2°C, its numbers increased from the initial inoculation level of 4 to 8 log cfu/ml after 27 days. When the medium was supplemented with nisin, lysozyme, 1:1 nisin/lysozyme, 1:3 nisin/lysozyme or 3:1 nisin/lysozyme at concentrations of 125, 250 and 500 μg/ml and inoculated with 4 log cfu/ml *B. thermosphacta* B2, the bacterial numbers were immediately reduced to below the detectable limit of 1 log cfu/ml. No bacteria were recovered during the entire 27-day incubation period.

The growth of *Carnobacterium* sp. 845 at 2°C in pork juice amended with 250, 500 and 1000 μg/ml of 3:1 nisin/lysozyme1:1 nisin/lysozyme and 1:3 nisin/lysozyme was monitored (Fig. 2A,B,C). As in APT broth, lysozyme alone failed to inhibit growth of the bacterium, even at a concentration of 1000 μg/ml, and bacterial numbers were the same as in untreated samples ($P > 0.05$). Bacterial numbers in all the other treatments were significantly lower than in untreated and lysozyme treated samples ($P < 0.05$).

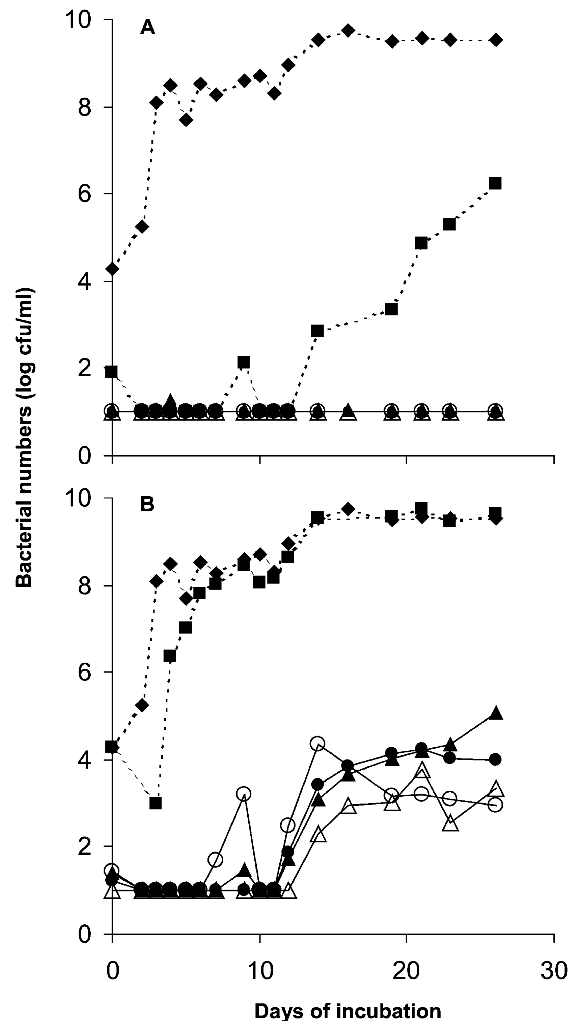

Fig. 1. Growth of *B. thermosphacta* B2 (A) and *Carnobacterium* sp. 845 (B) at 2°C in APT broth (◆) or APT broth supplemented with 500 μg/ml nisin (▲), lysozyme (■), 1:1 nisin/lysozyme (●), 1:3 nisin/lysozyme (△), 3:1 nisin/lysozyme (○). Least square means are shown. The standard error for A is 0.3 and for B is 0.7.

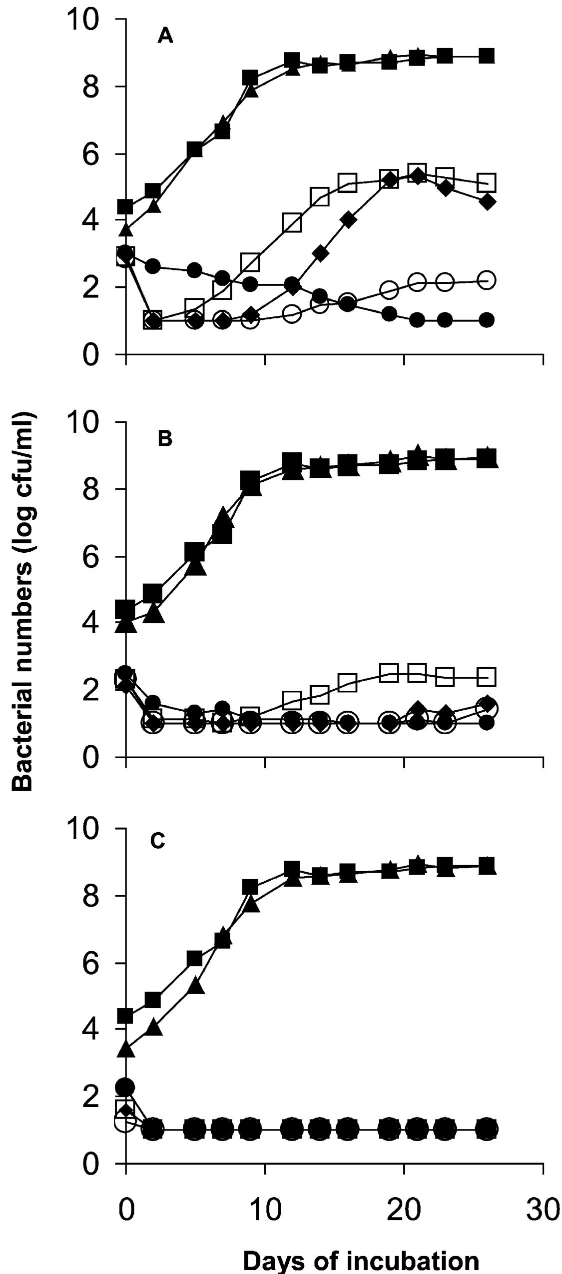

Fig. 2. Growth of *Carnobacterium* sp. 845 at 2°C in a pork juice medium (■) or a pork juice medium supplemented with nisin (●), lysozyme (▲), 1:1 nisin/lysozyme (◆) or 1:3 nisin/lysozyme (□) or 3:1 nisin/lysozyme (○). The concentrations used were 250 μg/ml (A, standard error = 0.3), 500 μg/ml (B, standard error = 0.4) and 1000 μg/ml (C, standard error = 0.1). Least square means are shown.

3.3. Pork cores

B. thermosphacta B2 did not grow on lean tissue, but the original inoculum level of 4 log cfu/cm^2 was maintained. Lysozyme, nisin, 1:1, 1:3 and 3:1 nisin/lysozyme mixtures reduced *B. thermosphacta* B2 numbers to below the detectable limit of 0 log cfu/cm^2 and no bacteria were detected throughout 42 days of vacuum storage at 2°C (Fig. 3A). How-

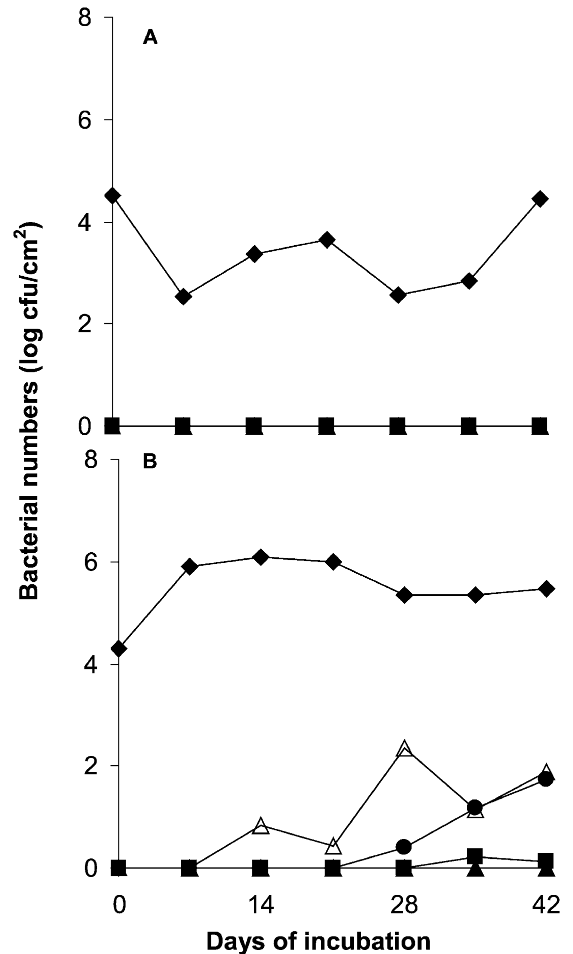

Fig. 3. Growth of *B. thermosphacta* B2 when it was inoculated onto sterile cores of pork lean (A) or fat (B) tissue. The tissue was subsequently dipped in antimicrobial solutions supplemented with 5000 μg/ml of antimicrobial so that approximately 130 μg/cm^2 adhered to the surface of each core (■, nisin; △, lysozyme; ●, 1:1 nisin/lysozyme; ▲, 3:1 nisin/lysozyme) or they were dipped in sterile water, ◆. Cores were vacuum packaged and stored at 2°C for up to 6 weeks. Least square means are shown. The standard error for A is 0.2 and for B is 0.3.

ever, the organism did grow on fat tissue from 4 to 6 log cfu/cm^2 (Fig. 3B). Bacterial numbers in the treated samples were significantly lower than in untreated samples ($P < 0.05$) and were at least 3.5 log units less than untreated samples after 42 days of storage in vacuum at 2°C.

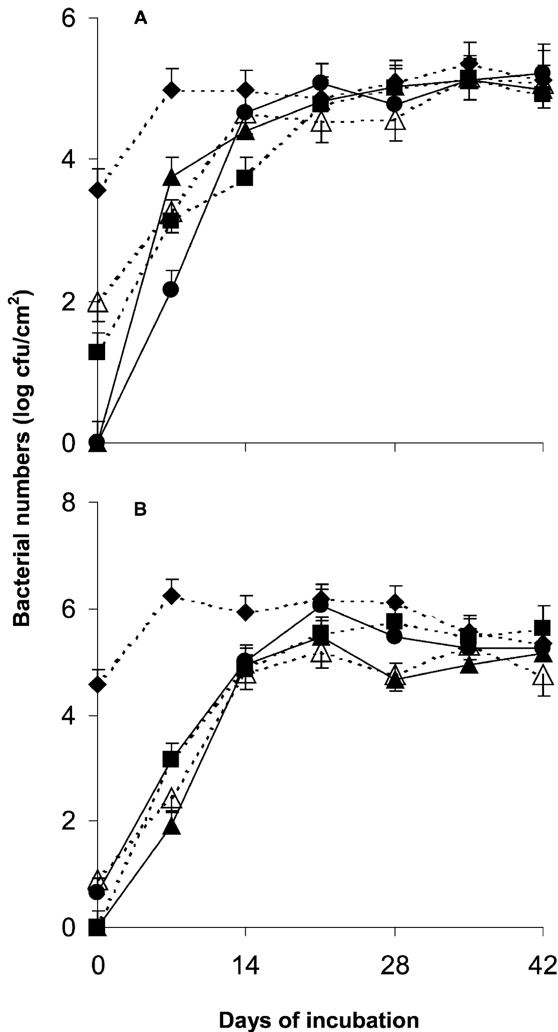

Fig. 4. Growth of *Carnobacterium* sp. 845 when it was inoculated onto sterile cores of pork lean (A) or fat (B) tissue. The tissue was subsequently dipped in antimicrobial solutions supplemented with 5000 μg/ml of antimicrobial so that approximately 130 μg/cm^2 adhered to the surface of each core (■, nisin; △, lysozyme; ●, 1:1 nisin/lysozyme; ▲, 3:1 nisin/lysozyme) or they were dipped in sterile water, ◆. Cores were vacuum packaged and stored at 2°C for up to 6 weeks. Least square means and standard errors are shown.

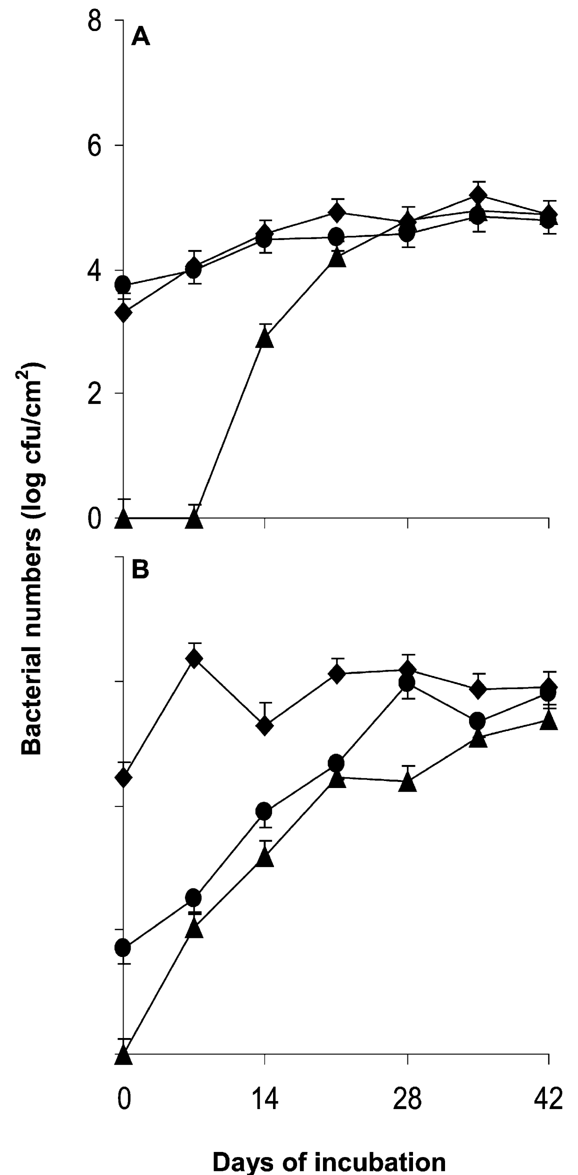

Fig. 5. Growth of *Carnobacterium* sp. 845 when it was inoculated onto sterile cores of pork lean (A) and fat (B) tissue. The tissue was subsequently dipped in water supplemented with lysozyme (●) at a concentration of 7500 μg/ml so that approximately 195 μg/cm^2 lysozyme adhered to each core or with 1:3 nisin/lysozyme (▲) at a concentration of 10,000 μg/ml so that approximately 260 μg/cm^2 adhered to each core. Cores for the positive control were dipped in sterile water (◆). Cores were vacuum packaged and stored at 2°C for up to 6 weeks. Least square means and standard errors are shown.

When *Carnobacterium* sp. 845 was introduced to cores which were subsequently treated with antimicrobial, bacterial numbers on both fat and lean tissue were reduced by all the treatments. They were reduced to below the detectable limit of 0 log cfu/cm^2 by 1:1 (lean tissue) and 3:1 (lean and fat tissue) mixtures of nisin/lysozyme at a concentration of 130 μg/cm^2 (Fig. 4). On lean tissue (Fig. 4A), the reduction in numbers was not significant ($P > 0.05$) after 14 days incubation. On fat tissue (Fig. 4B), treatments with a higher lysozyme concentration were significantly lower ($P < 0.05$) at 28 days. Considering these results, a ratio of 1:3 at a concentration of 260 μg/cm^2 of core surface was used for a further trial using *Carnobacterium* sp. 845. Bacteria were undetected for 7 days on lean tissue, but after that time bacteria grew quickly to reach levels of untreated samples after 21 days incubation (Fig. 5A). Neither nisin nor lysozyme alone at concentrations of 65 and 195 μg/cm^2 were as effective as the mixture of the two antimicrobials. On fat tissue, bacteria were undetectable at time = 0 in the 1:3 nisin/lysozyme treated samples (Fig. 5B). Only after 35 days of vacuum storage were the differences between the samples treated with the antimicrobial mixture and the controls no longer significant.

CIE L^* and a^* were not affected by any of the treatments. CIE b^* was significantly higher in treated samples (data not shown). The pH of the lean and fat tissue were 5.5 and 6.2, respectively and pH was not affected by the treatments.

4. Discussion

Lysozyme, nisin, and mixtures of the two, were shown to be effective at inhibiting the growth of the meat spoilage organism, *B. thermosphacta* B2, in APT broth at a concentration of 250 μg/ml, the lowest concentration evaluated, during a 12-day incubation period at 2°C. In a pork juice medium, 125 μg/ml effectively inhibited *B. thermosphacta* B2 during 27 days of incubation. A surface concentration of approximately 130 μg/cm^2 of antimicrobial also inhibited the bacterium's growth on cores of pork lean and fat tissue when the cores were incubated in vacuum at 2°C for 6 weeks. When beef surfaces were treated with nisin and stored in vacuum for up to 28 days at 4°C, *B. thermosphacta* B2 numbers were less than in untreated samples (Cutter and Siragusa, 1996a). Antimicrobial activity was evident for 21 days on both fat and lean tissue, indicating that nisin was active over the storage time (Cutter and Siragusa, 1996a). These results and those of the present study suggest the antimicrobial activity of nisin may not be completely inactivated on raw meat by an enzymatic reaction with glutathione as postulated by Rose et al. (1999).

Lysozyme alone was ineffective at inhibiting the growth of *Carnobacterium* sp. 845 at concentrations of 1000 μg/ml in APT broth and a pork juice medium. On pork cores of fat and lean tissue, however, numbers of *Carnobacterium* were lower than on untreated samples and its effectiveness was similar to that of the nisin treatment. A similar result was shown by Cutter and Siragusa (1994), where their *Carnobacterium divergens* culture exhibited nisin resistance in well-diffusion assays, but when applied to the surface of beef, nisin effectively inhibited the organism. Lysozyme has some properties that can make evaluation of its performance from one substrate and test system to another difficult to interpret. It has been shown to adhere to glass and to lose its activity quickly when studied in Pyrex, polypropylene or polyethylene containers (Cunningham et al., 1991). It can be inactivated by the presence of peptone, beef liver extract and boiled soybean, ingredients found in bacteriological media (Cunningham et al., 1991).

Our data suggested that nisin had the effect of reducing numbers at time = 0, an observation also made by Cutter and Siragusa (1997). Also, the more lysozyme there was in the antimicrobial mixture, the longer the antimicrobial was efficacious. Therefore, it was hypothesized that lysozyme, in combination with nisin, at a higher concentration than 65 μg/cm^2 used in the initial trials, might improve the antimicrobial activity of the mixture, as well as extend the time during which it would be effective. Since it is likely that nisin may only have significant antimicrobial activity at early times during refrigerated storage (Rose et al., 1999), its concentration in an antimicrobial mixture should only be high enough to quickly reduce numbers of sensitive bacteria. Considering the high cost of nisin and the regulatory implications of using antimicrobials, a mixture of 1:3 nisin/

lysozyme at an estimated surface concentration of 260 μg/cm^2 was used to further evaluate the efficacy of the antimicrobials on pork cores. On lean tissue, bacterial counts were significantly lower with the combination treatment, as compared to samples treated with lysozyme or nisin alone, or untreated for up to 14 days. On fat, the reduction in bacterial numbers persisted for 28 days.

Chung and Hancock (2000) observed synergy between nisin and lysozyme, and also demonstrated that a 1:3 combination of nisin/lysozyme was more active than either individual molecule in an in vitro system. The results of this study also demonstrate synergy between lysozyme and nisin. The mixtures of the antimicrobials were more effective than either parent molecule in the same concentration at reducing numbers of *Carnobacterium* sp. 845 at 0 and 3 days in pork juice, and at 0 time on cores of lean pork tissue. Chung and Hancock (2000) demonstrated by electron micrographs a dramatic morphological effect of mixtures of 1:3 and 3:1 nisin/lysozyme that are not apparent when the antimicrobials are used alone.

Since the antimicrobial spectra of nisin and lysozyme in the absence of chelators include only gram-positive bacteria (Bester and Lombard, 1990; Davidson et al., 1993; Delves-Broughton et al., 1996), their value, on their own, for use on fresh meats is limited. If they can be shown to inhibit lactic acid bacteria associated with spoilage of anoxically packaged meats, they may have some practical value, particularly if an antimicrobial or a packaging system can be found that will inhibit gram-negative bacteria. Packaging in carbon dioxide, for instance, has been shown to inhibit the growth of enterics and pseudodomonads, but lactic acid bacteria are not inhibited (Greer et al., 1993). Incorporation of nisin and lysozyme into such a system could have value. With the introduction of new products such as moisture enhanced pork, the possibility of the introduction of bacteria to normally sterile interior muscle tissue is increased, and antimicrobials could have a role in controlling the growth of these organisms.

The effectiveness of mixtures of nisin and lysozyme on meat surfaces against a lactic acid bacterium that is resistant to inhibition in bacteriological media and in pork juice has been demonstrated. Although many antimicrobials have been shown to be effective in culture media, few have shown efficacy when used on a natural product and few studies have demonstrated activity over extended storage periods. Exporters of Canadian pork require a storage life of 42 days in vacuum at low temperature. The data from this study show that mixtures of nisin and lysozyme maintain their activity on fresh pork and that they may have potential to extend the storage life of naturally contaminated fresh pork, thus improving industry's ability to attain the required storage life of their product.

Acknowledgements

The authors wish to acknowledge Meghan Morris and Sheri Perepelitza for their excellent technical assistance. This work was supported by Canadian Inovatech, Abbotsford, BC, Canada and the Matching Investment Initiative of Agriculture and Agri-Food Canada.

References

Bester, B.H., Lombard, S.H., 1990. Influence of lysozyme on selected bacteria associated with Gouda cheese. J. Food Prot. 53, 306–311.

Chumchalova, J., Josephsen, J., Plockova, M., 1998. The antimicrobial activity of acidocin CH5 in MRS broth and milk with added NaCl, $NaNO_3$ and lysozyme. Int. J. Food Microbiol. 43, 33–38.

Chung, W., Hancock, R.E., 2000. Action of lysozyme and nisin mixtures against lactic acid bacteria. Int. J. Food Microbiol. 60, 25–32.

Chung, K.-T., Dickson, J.S., Crouse, J.D., 1989. Effects of nisin on growth of bacteria attached to meat. Appl. Environ. Microbiol. 55, 1329–1333.

Commission Internationale de l'Eclairage (CIE), 1978. Recommendations on uniform color spaces, color difference equation, psychometric color terms, pp. 8–12. CIE Publication No. 15 (E-1.3.1) 1891/(TC-1.3), Supplement No. 2, Paris, France.

Cunningham, F.E., Proctor, V.A., Goetsch, S.J., 1991. Egg-white lysozyme as a food preservative: an overview. World's Poult. Sci. J. 47, 141–163.

Cutter, C.N., 1999. The effectiveness of triclosan-incorporated plastic against bacteria on beef surfaces. J. Food Prot. 62, 474–479.

Cutter, C.N., 2000. Antimicrobial effect of herb extracts against *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium* associated with beef. J. Food Prot. 63, 601–607.

Cutter, C.N., Siragusa, G.R., 1994. Decontamination of beef carcass tissue with nisin using a pilot scale model carcass washer. Food Microbiol. 11, 481–489.

Cutter, C.N., Siragusa, G.R., 1996a. Reductions of *Listeria innocua* and *Brochothrix thermosphacta* on beef following nisin spray treatments and vacuum packaging. Food Microbiol. 13, 23–33.

Cutter, C.N., Siragusa, G.R., 1996b. Reduction of *Brochothrix thermosphacta* on beef surfaces following immobilization of nisin in calcium alginate gels. Lett. Appl. Microbiol. 23, 9–12.

Cutter, C.N., Siragusa, G.R., 1997. Growth of *Brochothrix thermosphacta* in ground beef following treatments with nisin in calcium alginate gels. Food Microbiol. 14, 425–430.

Davidson, P.M., Post, L.S., Branen, A.L., McCurdy, A.R., 1993. Naturally occurring and miscellaneous food antimicrobials. In: Branen, A.L., Davidson, P.M. (Eds.), Antimicrobials in Foods. Marcel Dekker, New York, pp. 371–419.

Delves-Broughton, J., Blackburn, P., Evans, R.J., Hugenholtz, J., 1996. Applications of the bacteriocin nisin. Antonie van Leeuwenhoek 69, 193–202.

Egan, A.F., 1983. Lactic acid bacteria of meat and meat products. Antonie van Leeuwenhoek 49, 327–336.

Gill, A.O., Holley, R.A., 2000a. Inhibition of bacterial growth on ham and bologna by lysozyme, nisin and EDTA. Food Res. Int. 33, 83–91.

Gill, A.O., Holley, R.A., 2000b. Surface application of lysozyme, nisin, and EDTA to inhibit spoilage and pathogenic bacteria on ham and bologna. J. Food Prot. 63, 1338–1346.

Greer, G.G., Dilts, B.D., Jeremiah, L.E., 1993. Bacteriology and retail case life of pork after storage in carbon dioxide. J. Food Prot. 56, 689–693.

Greer, G.G., Paquet, A., Dilts, B.D., 2000. Inhibition of *Brochothrix thermosphacta* in broths and pork by myristoyl-L-methionine. Food Microbiol. 17, 177–183.

Holzapfel, W.H., Geisen, R., Schillinger, U., 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int. J. Food Microbiol. 24, 343–362.

Nissen, H., Holck, A., Dainty, R.H., 1994. Identification of *Carnobacterium* spp. and *Leuconostoc* spp. in meat by genus-specific 16S rRNA probes. Lett. Appl. Microbiol. 19, 165–168.

Rose, N.L., Sporns, P., Stiles, M.E., McMullen, L.M., 1999. Inactivation of nisin by glutathione in fresh meat. J. Food Sci. 64, 759–762.

SAS Institute, 1995. SAS/STAT User's Guide. SAS Institute, Cary, NC.

Scannell, A.G.M., Hill, C., Buckely, D.J., Arendt, E.K., 1997. Determination of the influence or organic acids and nisin on shelf-life and microbiological safety aspects of fresh pork sausage. J. Appl. Microbiol. 83, 407–412.

Szabo, E.A., Cahill, M.E., 1998a. Nisin and ALTA™ 2341 inhibit the growth of *Listeria monocytogenes* on smoked salmon packaged under vacuum or 100% CO_2. Lett. Appl. Microbiol. 28, 373–377.

Szabo, E.A., Cahill, M.E., 1998b. The combined effects of modified atmosphere, temperature, nisin and ALTA™ 2341 on the growth of *Listeria monocytogenes*. Int. J. Food Microbiol. 43, 21–31.

Yost, C.K., Nattress, F.M., 2000. The use of multiplex PCR reactions to characterize populations of lactic acid bacteria associated with meat spoilage. Lett. Appl. Microbiol. 31, 129–133.