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A Research Note

Treatments with Nisin and Chelators to Reduce *Salmonella* and *Escherichia coli* on Beef†

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ABSTRACT

Salmonella typhimurium ATCC 14028 or *Escherichia coli* O157:H7 attached to lean beef tissue were treated with citrate, lactate, sodium hexametaphosphate, or EDTA, alone or in combination with nisin in simple buffers, and incubated at 4°C for up to 3 days. Lactate with nisin reduced *S. typhimurium* attached to beef by 0 40 $\log_{10}$ CFU/cm², while EDTA and nisin reduced *E. coli* O157:H7 by 0.42 $\log_{10}$ CFU/cm². Unlike earlier in vitro studies in which treatments with nisin and chelating agents resulted in reductions of > 4 $\log_{10}$ CFU/cm², such reductions were not observed in situ.

Key words: *Escherichia coli* O157:H7, *Salmonella typhimurium*, nisin, chelators, beef

Various food-grade chelators (citrate, phosphate, EDTA, EGTA) have been examined for extending the antimicrobial activity of nisin to gram-negative bacterial cells *(1, 8, 9, 10)*. In vitro treatments of various gram-negative bacteria with nisin and chelators resulted in log reductions as high as 6 $\log_{10}$ CFU/ml *(1, 8, 10)*. In our laboratories, inclusion of nisin in different buffers reduced populations of *S. typhimurium* ATCC 14028 up to 3.8 $\log_{10}$ CFU/ml and *E. coli* O157:H7 up to 5.6 $\log_{10}$ CFU/ml *(3)*. Compounds such as 1% sodium hexametaphosphate or 500 mM lactate, as well as EDTA (50 mM), or citrate (100 mM) enhanced nisin activity against gram-negative pathogens in vitro *(3)*. In the present study, we examined the effects of nisin and chelators against *E. coli* O157:H7 and *S. typhimurium* ATCC 14028 attached to lean beef tissue under refrigerated conditions.

†Names are necessary to report factually on available data: however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable

MATERIALS AND METHODS

Bacterial strains

Salmonella cholerasuis subsp. *cholerasuis* serotype *typhimurium* ATCC 14028 (*S. typhimurium* ATCC 14028) was obtained from the American Type Culture Collection (ATCC Rockville, MD). *Escherichia coli* O157:H7 was obtained from the Food Research Institute (Madison, WI). Cultures were maintained in 75% glycerol at −20°C and propagated in trypticase soy broth (BBL, Cockeysville, MD) at 37°C. From an 18-h culture, *E. coli* O157:H7 or *S. typhimurium* 14028 cells were inoculated into fresh medium, grown to approximately 7 $\log_{10}$ CFU/ml (OD_{600}, ca 0.1), centrifuged (15 min, 2800 × g, 5°C), and resuspended in the original volume of the respective buffer (see below).

Nisin, buffers, and chelators

Purified nisin (Ambicin™) was obtained from Applied Microbiology (New York, NY) and stored at 4°C. Buffers used in this study were 1 mM 3-[N-morpholino] propanesulfonic acid (MOPS;) (Sigma Chemical Co. St. Louis, MO) and 1 mM potassium phosphate-buffered saline (PBS). Test solutions were prepared by adding disodium EDTA (Fisher Scientific Co., Pittsburgh, PA), citric acid monohydrate (Sigma), DL-lactate (85%, wt/vol) (Sigma), and sodium hexametaphosphate (HMP) (FMC, Norwalk, CT) to the respective buffer containing 50 μg/ml of nisin, for final concentrations of 50 mM, 100 mM, 500 mM, and 1% wt/vol, respectively. Solutions were adjusted to pH 7.0 with HCl or NaOH.

Experimental design

Internal pieces of postrigor lean beef muscle tissue (24-h postslaughter; pH 5.8) were obtained from beef carcasses at the abattoir of the Roman L. Hruska U.S. Meat Animal Research Center, cut to 2.5 by 2.5 cm pieces, sterilized by U.V. light (60-watt germicidal bulbs; 51-cm distance from tissue, 20 min), stored aseptically at −20°C, and thawed to 25°C prior to use. Individual pieces of tissue were attached to sterile alligator jaw clips and inoculated with bacteria by submerging each piece into 10 ml of a cell suspension containing approxi-

mately 9 $\log_{10}$ CFU/ml for 15 min at 25°C. Each piece of inoculated tissue was submerged into 10 ml of sterile test solution (15 min, 25°C), hung in a covered, sterile beaker and incubated at 4°C until bacterial populations were enumerated. Pieces of inoculated lean beef tissue, submerged only in sterile buffer (MOPS or PBS) and subjected to similar incubation conditions were used as untreated controls throughout this experiment. Three individual replicates of the experiment were performed.

Bacterial enumeration

After treatments at day 0, and following incubation at days 1 and 3, randomly selected pieces of beef tissue were pummeled for 2 min (Stomacher 400, Tekmar, Inc., Cincinnati, OH) in 90 ml of 0.1% buffered peptone water (BPW) (Difco Laboratories, Detroit, MI). Serial dilutions were made in 0.1% BPW and samples were plated in duplicate on trypticase soy agar (BBL) using a spiral plater (Model D, Spiral Biosystems, Bethesda, MD). Plates were enumerated after incubation for 36 h at 37°C.

Calculations and statistical analyses

Bacterial populations were converted to $\log_{10}$ CFU/cm^2 values. Statistical analysis of data was performed using the General Linear Models procedure of SAS *(7)*. The probability level was $P \leq 0.05$, unless otherwise noted. The log reduction factor (LRF) was calculated as the difference between least square means (LSM) of populations of inoculated beef tissue, submerged in buffer, and LSM of populations of inoculated beef submerged in test solution (LRF: LSM $\log_{10}$ CFU/ml untreated –LSM $\log_{10}$ CFU/cm^2 treated).

RESULTS

Examining the effects of nisin alone or in combination with chelators in PBS or MOPS buffers against *E. coli* O157:H7 attached to lean beef tissue over the 3 days indicated that there were no significant 2-way interactions of treatment by buffer, treatment by day, buffer by day, or a 3-way interaction of treatment by buffer by day; however, treatment, buffer, and day were significant factors. The effect of treatment against bacterial populations of *E. coli* O157:H7 attached to beef tissue was not significantly different between test solutions (Fig. 1); however, treatments with nisin and EDTA or lactate were statistically different ($P < 0.004$) from the untreated control. Analysis of the buffer effect on bacterial populations indicated that treatments of *E. coli* O157:H7 with test solutions in MOPS buffer ($\log_{10}$ 6.96) were statistically different ($P \leq 0.004$) from test solutions in PBS buffer ($\log_{10}$ 6.71). With regard to the day effect, populations of *E. coli* O157:H7 were statistically different from each other at days 0 ($\log_{10}$ 7.23), day 1 ($\log_{10}$ 6.84), and day 3 ($\log_{10}$ 6.44).

In the case of *S. typhimurium* attached to lean beef tissue, only treatment was a statistically significant ($P \leq 0.002$) factor. Data indicated that of the treatments against *S. typhimurium*, only nisin and lactate or EDTA combinations with nisin resulted in populations that were statistically different from untreated controls (Fig. 2). No significant population differences for *S. typhimurium* were detected over the 3-day incubation, nor were differences between buffer types observed.

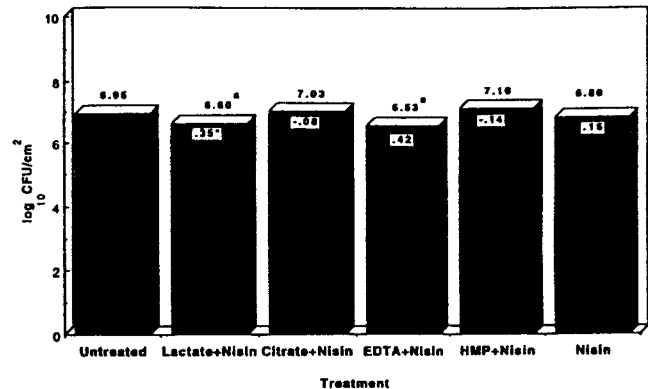

Figure 1. *Populations of* E. coli O157:H7 *attached to lean beef tissue following treatments with nisin or nisin and chelator combinations in either buffer. *Denotes log reduction factor. [a]Denotes a statistically significant different* ($P \leq 0.04$) *population from that on untreated beef tissue.*

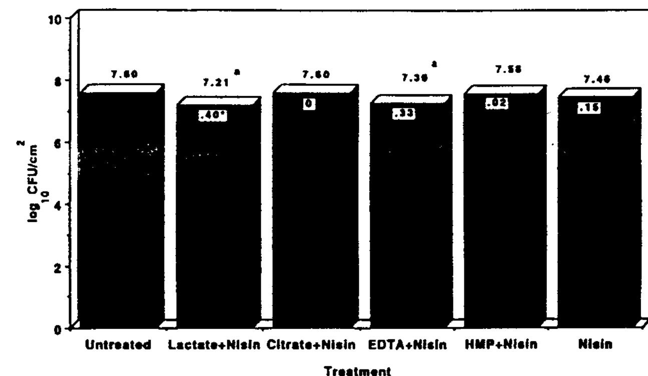

Figure 2. *Populations of* S. typhimurium *attached to lean beef tissue following treatments with nisin or nisin and chelator combinations in either buffer. *Denotes log reduction factor. [a]Denotes a statistically significant different* ($P \leq 0.002$) *population from that on untreated beef tissue.*

DISCUSSION

The effects of nisin against gram-positive pathogens attached to red meat have been examined previously *(2)*. In these studies, nisin delayed the growth of *Staphylococcus aureus* and *Listeria monocytogenes* at least 1 day at room temperature, and also delayed growth for up to 2 weeks at 5°C *(2)*. While nisin activity remained stable on red meat for up to 2 weeks under refrigerated conditions, the bacteriocin did not affect gram-negative bacteria attached to meat *(2)*. Synergistic treatments with chelators are known to enhance nisin activity against gram-negative bacteria cells in vitro *(1, 8, 9, 10)*. Given that nisin and chelator combinations in two buffer types were effective against *Salmonella* and *E. coli* under refrigerated conditions *(3)*, this study was performed to determine if the same combinations would reduce bacterial populations attached to postrigor red meat held at 5°C for up to 3 days. From the present study, it can be concluded that combinations of nisin and chelators resulted in very slight reductions of *E. coli* O157:H7 and *S. typhimurium*

ATCC 14028 attached to meat. These reductions were not greater than 0.42 log_{10} CFU/cm^2.

There may be many reasons for the inability of these treatments to reduce populations of gram negative bacteria attached to meat. Bacteriocins may be degraded by endogenous proteases associated with meat or bind preferentially to adipose tissues *(6)*. No attempt was made in this study to determine the amount of nisin present after treatment or incubation for 3 days.

Buffers with mild chelating capacities (MOPS, PBS, Tris) appeared to bind calcium and magnesium from the surrounding solution, but apparently not from the outer membrane of the gram-negative cells *(3)*. If magnesium was not chelated from the lipopolysaccharide layer of the cells, it was possible that the outer membrane remained impermeable to nisin, and populations were not drastically reduced. While excessive concentrations of chelators as well as buffers with chelating properties were used in the present study, the abundance of other ions associated with the surface of red meat *(5, 11)* may have interfered with the ability of the chelators to bind magnesium from the LPS of the bacteria. Additionally, attached bacteria were not as readily accessible to the treatments compared to organisms in suspension *(3)*. Increasing the concentration of chelating compounds to compensate for additional cations on the meat surface may increase the magnitude of inactivation of the gram-negative bacteria. Other methods for delivering these compounds to the carcass surface *(4)* and reducing populations of pathogens are also worthy of investigation.

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