LACTIC ACID AND NISIN EFFECT ON BEEF SPOILAGE BACTERIA ATTACHMENT

H. W. OCKERMAN, An. Sci. Dept., OSU, 2029 Fyffe Road, Columbus, OH 43210-1095, USA.
H. R. RODRIGUEZ, and N. A. PENSEL, Instituto de Tecnología de Carnes CICV-INTA, C.C. 77, 1708 Morón, Buenos Aires, ARGENTINA.

Summary

Lactic acid proved to have a strong inhibitory effect on the attachment of Pseudomonas fluorescens but did not exert a marked reduction of the adherence of Escherichia coli; nonetheless, it was the most effective treatment for E. coli. Lactic acid did not show any effect against Lactobacillus casei. Nisin proved to be effective in preventing the attachment of L. casei but did not have an effect on P. fluorescens or E. coli. The combination of lactic acid-nisin reduced the attachment of P. fluorescens; however, its effect was not as marked as 2% lactic acid.

Introduction

It has been estimated that microbial spoilage accounted for up to 20 million pounds of meat losses in the USA. Ninety five percent of these losses would correspond to the packaging industry and retailers (Breidenstein, 1986). Microbial contamination of raw meat and meat products is the main cause of spoilage. Fresh meat will spoil due to microbial action unless some action is taken to prevent such a process. Anderson and Marshall (1990) reported that a one log reduction in bacterial count can be obtained through a good commercial beef carcass washing. Although, further methods to decontaminate animal carcasses have been utilized (Smulders, et al 1985), complete sterilization has not been accomplished. Yet under the current slaughtering practices some bacterial contamination might be expected.

One way of preventing or retarding bacterial spoilage could be to block or prevent the bacterial attachment to meat surfaces. Attachment to meat surfaces has been studied (Benedict, 1988); still, there is a lack of information on how to prevent microbial meat adherence particularly regarding spoilage organisms. A sensible approach would be to seek for chemical agents to block this attachment.

Lactic acid is an organic acid that has been used primarily to decrease surface microbial contamination and to increase shelf-life on carcasses and retail cuts (Smulders et al. 1986). Nisin is a polypeptidic antibiotic produced by Streptococcus lactic used as a food preservative. Nisin has been used in preservation of meat (Rayman et al 1981) in an attempt to lower nitrite contents and to take advantage of its antibotulinal effect. Information of nisin effect on raw meat is, however, scarce (Chung, et al 1989). The objective of this study was to evaluate the effectiveness of lactic acid and nisin, alone or in combination, during the attachment of spoilage bacteria to sterile lean beef muscle tissue.

Materials and methods

Muscle tissue: Samples from beef longissimus dorsi (LD) muscle were obtained by using the Hone et al. (1997) procedure to collect aseptic tissue. Sterile was defined as having <1 CFU/g. Lactic acid: A lactic acid Baker Analyzed Reagent (J. T. Baker Chemical Co.) ACS grade containing 89.9% DL-lactic acid was used. Nisin: Nisin from Sigma Chemical Co., with an activity not less than 1 million IU per gam was used. Bacterial strains: Pseudomonas fluorescens (Pf), Lactobacillus casei (Lc) and Escherichia coli (Ec) were obtained from the OSU Department of Microbiology. Brochothrix thermosphacta (Bt) ATCC 11509 was obtained from the American Type Culture Collection. Organisms were grown following standards procedures (Rodríguez, 1990). Cultures were centrifuged (at 3,000 g at 4° C for 10 min) and washed with saline solution (0.85% aqueous solution of Na Cl) twice. Cell suspensions were diluted in the same medium to have approximately $1x10^7$/ml of target bacterium (attachment medium). To enumerate Pf and Ec Tryptic Soy Agar (Difco Lab.) (25°C-24hs) was used; MRS Agar (Beckton

Dickinson, Mic. Sys.) (35°C-48hs) was used for Lc and Brain Heart Infusion Agar (Difco Lab.) (25°C-48hs) was utilized for Bt. Detachment experiment: Meat pieces were soaked in the 2% lactic solution, 10^3IU/ml nisin or 1% lactic acid solution plus 500 IU/ml nisin for 1 minute at room temperature (ca 22°C). Bacterial attachment to antimicrobial agent-treated meat was compared with that to LD muscle soaked into distilled water. LD samples were transferred aseptically to a sterile beaker containing 20 ml of attachment medium and were incubated at room temperature for 0, 30, 60, 90 and 120 min. The samples were thoroughly rinsed with saline solution at each appropriate attachment time. Rinsed samples were immediately transferred to a sterile bag containing 50 ml of 0.1% peptone water and stomached for 2 min in a Stomacher Lab-Blender (Tekmar, Co.). Suspensions were then decimally diluted in 0.1% peptone water, and appropriate dilutions were enumerated by surface spread plate method. Decimal reduction determination: The decimal reduction time (D value) was estimated as the time that was required to decrease 1 log cycle of viable cells in the antimicrobial agent solutions at room temperature. Organisms were grown as described above and suspended in saline solution. The bacterial suspension (0.1 ml, or 10^8 CFU/ml) was placed in contact with 100 ml of the antimicrobial agent and at appropriate time intervals counts were performed. Data analysis: A three-way analysis of variance on the decimal log transformed counts were performed by using the SAS GLM procedure. Each experiment was replicated 6 times.

Results and discussion

Effectiveness of an antimicrobial treatment can be measured as the differences between the average count of the samples exposed to the control and the bacterial count of each particular sample subjected to a given antimicrobial agent treatment. The larger the difference the better the effect of the treatment in retarding or blocking the bacterial attachment. No treatments were applied on Bt since this bacterium did not show attachment. There were no differences ($P>0.05$) in the action of lactic acid and lactic-nisin during the attachment of Pf (Fig. 1A). There were differences ($p<0.05$), however, between actions of lactic acid and lactic acid-nisin combination during the adherence of Pf except at 0 min. Lactic acid was the most effective agent in preventing attachment of Pf and nisin the least effective. Pf decreased from 10^6/ml to less than 10/ml in 30 sec, when exposed to 2% lactic acid. Results of Attachment experiments, however, suggest no effect on cell viability that should have affected microbial adherence. Lactic acid action against Pf is particularly relevant since this bacterium is the most important spoilage organism in aerobically stored meat and it become attached almost instantly. When comparing the differences within each treatment (Fig. 1B), there were no differences ($p>0.05$) for nisin among the various attachment times. Nisin (10^3/ml) showed no effect up to 30 min on Pf viability. Antimicrobial action of lactic acid became stronger after 0 and 60 min (Fig. 1B).

The combination lactic acid-nisin proved to be effective against Lc attachment suggesting some synergistic effect. (Fig. 2A). Lc decreased from 10^6 to less than 10/ml in 60 sec when exposed to 10^3/ml nisin. Lactic acid appeared to be the least effective against Lc attachment (Fig 2A). Whereas, 2% lactic acid had no effect up to 30 min on the viability of Lc. There were a significant increase in nisin action between 0 min and the rest of the attachment times (Fig. 2B). Nisin is effective against Gram-positive bacteria and its effectiveness on Listeria monocytogenes attachment has been reported (Chung, et al 1989). Use of nisin on preserved meats has not had much success so far. However, lactic acid have proved to be effective particularly against aerobes organisms (Anderson and Marshall, 1990). Results of our study suggest that nisin might prevent adherence of Gram-positive spoilage organism. Particularly, promising are the results of the combination lactic acid-nisin that might prevent adherence of Gram-negatives as well. Recently (Harris, et al 1991) have reported that lactic acid enhanced nisin effect against Listeria monocytogenes.

There were no differences ($p>0.05$) among treatment against Ec attachment at any attachment time (Fig. 3A). Nevertheless, data showed that lactic acid might be effective in

retarding the adherence of Ec. D-value for Ec was 13 min, while exposed to 1% lactic acid plus 500 IU/ml nisin was 17 min. Lactic acid was effective at 120 min rather than at 0 or 30 min (Fig. 3B).

Conclusions

Lactic acid proved to be very effective in preventing attachment of Gram-negative spoilage organisms particularly P. fluorescens. Nisin showed action on Gram-positive spoilage bacteria. Employment of synergistic antimicrobial agents, to prevent bacterial adherence, on naturally contaminated meat should be pursued.

REFERENCES

Anderson, M.E. and Marshall, R.T. 1990. Reducing microbial population on beef tissues: concentration and temperature of lactic acid. J. Food Safety 10,181-190.

Benedict, R.C. 1988. Microbial attachment to meat surfaces. In: "Recip. Meat Conf. Proc." Vol. 41,1-6pp.

Breidenstein, B. 1986. Economic impact of meat spoilage organisms. In "Recip. Meat Conf. Proc." Vol. 39,1-4pp.

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

Harris, L.J., Fleming, H.P., Klaennhammer, T.R. 1991.Sensitivity and resistance of Listeria monocytogenes ATCC 19115, Scott A, and UAL500 to nisin. J. Food Prot. 54,836-840.

Rayman, M.K., Aris, B. and Hurst, A. 1981. Nisin: a possible alternative or adjunct to nitrite in the preservation of meats. App. and Env. Microbiol.41,375-380.

Rodríguez, H.R. 1990. Effect of lactic acid and nisin during the attachment of spoilage bacteria to sterile beef muscle tissue. M Sc Thesis The Ohio State University, Columbus, OH.

Smulders, F.J.M. and Woolthuis, C.H. 1985. Immediate and delayed microbiological effects of lactic acid decontamination of calf carcasses. Influence on conventionally boned versus hot-boned and vacuum-packaged cuts. J. Food Prot. 48,838-847.

Figure 1

Differences between control and treatments for Pseudomonas fluorescens

Graph A: Comparisons among differences at each attachment time
Points among differences at the same attachment time bearing the same letter showed no significant difference (P>0.05).

Graph B: Comparisons among attachment times within each difference
Points within individual differences bearing the same letter showed no significant difference (P>0.05).

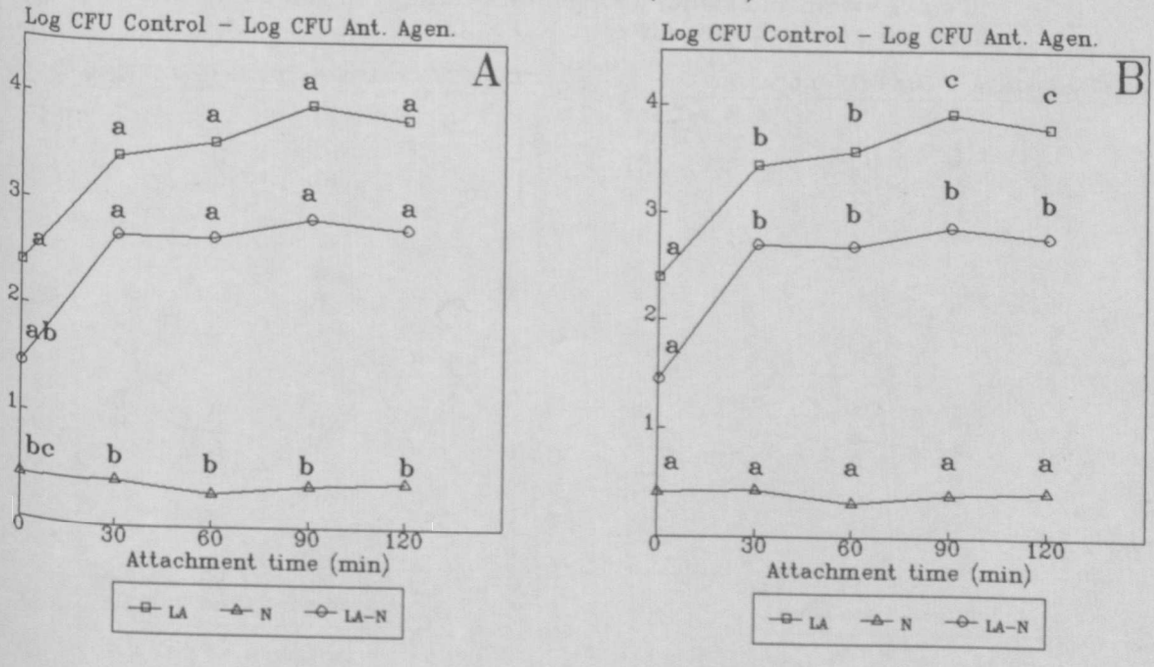

Figure 2

Differences between control and treatments for Lactobacillus casei

Graph A: Comparisons among differences at each attachment time
Points among differences at the same attachment time bearing the same letter showed no significant difference (P>0.05).

Graph B: Comparisons among attachment times within each difference
Points within individual differences bearing the same letter showed no significant difference (P>0.05).

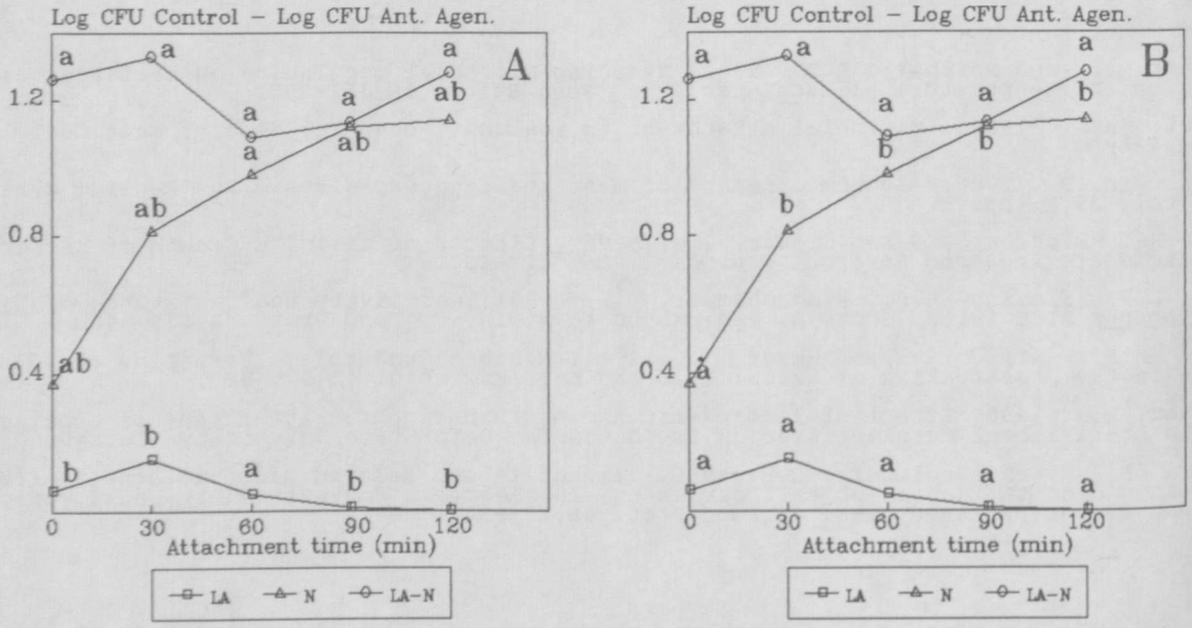

Figure 3

Differences between control and treatments for Escherichia coli

Graph A: Comparisons among differences at each attachment time
Points among differences at the same attachment time bearing the same letter showed no significant difference (P>0.05).

Graph B: Comparisons among attachment times within each difference
Points within individual differences bearing the same letter showed no significant difference (P>0.05).