BEHAVIOR OF ACID-ADAPTED *LISTERIA MONOCYTOGENES* IN MEAT DECONTAMINATION WASHINGS

John Samelis, John N. Sofos, Patricia A. Kendall, and Gary C. Smith

Center for Red Meat Safety, Department of Animal Sciences, Colorado State University, Fort Collins, CO 80523-1171, USA

Background

Animal carcass decontamination technologies are of increasing commercial application in slaughterhouses of North America to reduce microbiological contamination of meat, while their use on primal cuts and trimmings of meat is under consideration (5, 6). These technologies are of two major types, organic acid (5) and nonacid (6); both can reduce meat surface contaminants by 1 to 3 logs. Concerns, however, arise as to whether meat decontamination, especially with organic acids, may alter the microbial ecology of meat by developing stressed bacterial pathogens and reducing the numbers of their natural competitors on the treated meat or in the plant (5). Pathogens may also survive in meat decontamination waste fluids and develop resistance to food preservation processes, colonize plants and increase safety risks by cross-contaminating meat (6). Indeed, we have recently shown that *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Typhimurium DT104 may survive, in decreasing order, in undiluted 2% lactic (pH 2.3 to 2.5) or 2% acetic (pH 3.0 to 3.2) acid washings from meat for 2 to 7 d at 4°C or 10°C (4). Survival may be further enhanced if the pathogens are previously acid-adapted (1) and the acidic meat washings are accidentally mixed with water meat washings to result in decontamination waste fluids of sublethally low pH in plants.

Objectives

This study evaluated survival/growth of *L. monocytogenes* in plain water or organic (2% lactate or 2% acetate) acid spray-washings from fresh beef, or in their mixtures, stored at 4 or 10°C. *Listeria monocytogenes* was selected because of its high incidence on meat and prevalence in meat plants (2), its potential to survive meat decontamination when present as a natural contaminant (3), and its resistance to multiple stresses and superior ability to form biofilms (1,2).

Materials and Methods

Washings of meat were prepared by spraying top round cuts of beef (approximately 2 kg) with water (10 or 85°C) or 2% lactic or acetic acid (55°C) solutions in a simulated spray-washing apparatus (CHAD Company, Olathe, KS), as described previously (4). Water (10 or 85°C) washings were initially mixed at a ratio 1:1 to obtain a composite plain water washing mixture, followed by mixing of either 2% lactate or 2% acetate washings with this composite at ratios 1:1, 1:9 and 1:99 to obtain mixtures of acid-containing washings of variable pH. The original washings or their mixtures were inoculated (10^5 CFU/ml) with an either acid-adapted (previously grown in glucose-free tryptic soy broth plus 0.6% yeast extract, TSBYE-G, with 1% added glucose, TSBYE+G, at 30°C for 24 h) or nonadapted (previously grown in TSBYE-G) inoculum of *L. monocytogenes* strain N-7144Sm+. This streptomycin-resistant (Sm+, 800 mg/l) derivative was selected because of this property and its acid tolerance that was the highest among other strains of *L. monocytogenes* tested, but similar to its parental strain N-7144. Inoculated washings were incubated statically at 4 or 10°C for up to 14 d; water, 2% lactic acid or 2% acetic acid washings with no inoculation served as controls. Microbial survival/growth was monitored at 0, 2, 4, 7, and 14 d of storage at 4 or 10°C by plating on tryptic soy agar plus 0.6% yeast extract (TSAYE), TSAYE with 800 mg/l of streptomycin (TSAYE+Sm) and PALCAM agar. Colonies on agar plates were counted after incubation at 30°C for 48 h. Changes in pH of the washings were also measured during storage. In addition, portions (1 ml) of bacterial suspensions surviving in meat washings were re-exposed to 9 ml of TSBYE acidified to pH 3.5 with lactic acid at 2 and 7 d of storage at 10°C. This was done to determine whether pre-exposure to washings of different pH altered the acid tolerance of surviving *L. monocytogenes*, wherever survivors occurred, compared to the inoculum and/or with time of storage. Acid survivors were determined at 0, 60 and 120 min by plating, as above. All microbiological counts were converted to log CFU/ml (<1 log CFU/ml was the lowest detection limit).

Results and Discussion

After 2 d at 10°C, low surviving numbers (1-1.8 log CFU/ml) of inoculated (10^5 CFU/ml) acid-adapted *L. monocytogenes* were detected in 2% lactate washings or their 1:1 and 1:9 mixtures with water (i.e., pH < 3.2), while the nonadapted inocula became undetectable (<1.0 log CFU/ml). *Listeria monocytogenes* survived better in undiluted 2% acetate washings and their mixtures (1:1 and 1:9) with water (i.e., pH < 3.8), since acid-adapted survivors could be detected in those washings after 4 d at 4°C (data not shown). The pH of all the above acid washings (pH 2.2 to 3.8) did not change during storage (14 d) at either temperature. These results are in general agreement with our previous studies on the ability of partially acid-adapted (TSBYE with 0.25% glucose) *L. monocytogenes* to survive for 2-4 d in 2% lactate or acetate washings (4). Thus, acid-adapted *L. monocytogenes* may survive in meat washings of pH < 4.0 for times that may allow cross-contamination of meat, but survival would be minimal, and lower than that of *E. coli* O157:H7 (4).

Unlike in acidic washings of pH < 4.0, however, *L. monocytogenes* survived throughout storage (14 d) in mixtures (1:99) of 2% lactate or acetate washings with water washings (pH 4.2-4.5) at 4 (data not shown) or 10°C (Fig 1). Interestingly, at 10°C, the initial declines in pathogen populations were reversed to growth after 4 to 7 d (Fig. 1A). Growth of *L. monocytogenes* was greater in acetic/water than in lactic/water washings and for the nonadapted compared to acid-adapted populations (Fig. 1A), while no such increases were observed at 4°C (data not shown). This behavior of *L. monocytogenes* at 10°C was associated with the ability of the natural flora to overcome the inhibitory effect of low acid concentrations in diluted (1:99) acidic washings and, eventually, to grow (Fig. 1B). Growth of natural flora was faster and greater in washings stored at 10°C (Fig. 1B) than at 4°C (data not shown), and in

lactate- compared to acetate-containing washings (Fig. 1B). Interestingly, while in acetic/water washings the natural flora (TSAYE) was mainly composed of yeasts, in lactic/water washings the flora that eventually reached 10^8 CFU/ml was of the same type with that developed in plain water washings (Fig. 1B). In those washings, *L. monocytogenes* increased by approximately 2.5 and 1.5 logs at 10°C (Fig. 1A) and 4°C (data not shown), respectively, while it was rapidly (2 to 4 d) outgrown by putrefactive meat spoilage bacteria (i.e., 100% gram-negative, >85% oxidase positive) (Fig. 1B). The pH of water washings increased during storage due to the proteolytic activity of the natural flora, especially at 10°C (Fig. 1C). Growth on PALCAM (Fig. 1A) was very similar to growth on TSAYE+Sm (data not shown). TSAYE+Sm was unsuitable for the selective enumeration of *L. monocytogenes* in acid-containing washings carrying high or predominant underlying populations of yeasts, since these organisms were not inhibited by streptomycin.

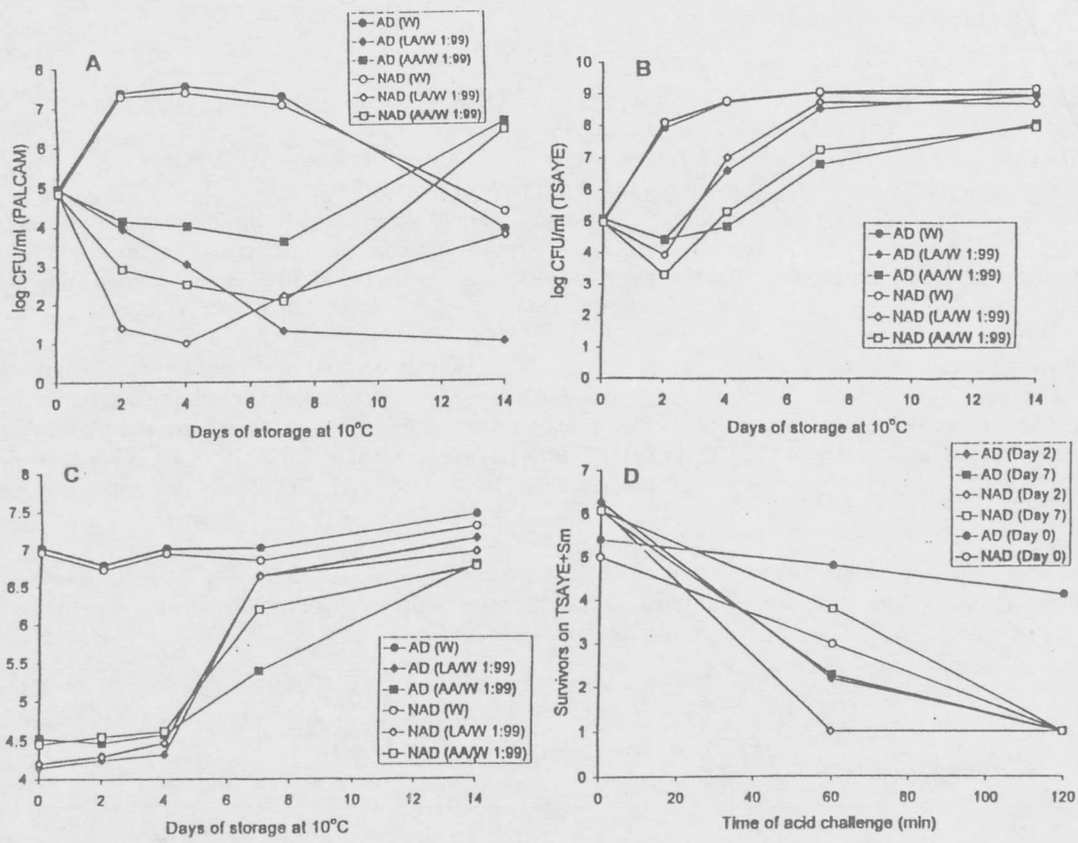
When *L. monocytogenes* populations grown in plain water washings for 2 or 7 d at 10°C were subsequently exposed to lactic acid (pH 3.5), their survival was much lower than that of the inoculum, while the initially nonadapted became more acid tolerant than the acid-adapted populations by day-7 (Fig. 1D). The very low numbers of survivors in all acidic (pH < 4.0) washings did not allow us to perform challenge tests. Also, at day 7 the fairly low (approx. 2-3 logs) populations of *L. monocytogenes* from acid/water (1:99) washings exposed to lactic acid pH (3.5) became undetectable (<1.0 log CFU/ml) at 60 min (data not shown).

Conclusions

Limited survival of *L. monocytogenes* may be expected in undiluted acidic (lactate or acetate) meat decontamination washings, or mixed with water washings, when the resulting pH is less than 4.0. However, acid-containing washings with a pH above 4.0 may support survival and potential growth for extended times, while in nonacid (water) washings the pathogen may increase to high numbers. However, the parallel increases of natural flora (i.e., gram-negative bacteria and yeasts) in such moderate to neutral pH washings may contribute to an acid sensitization of *L. monocytogenes*. Interestingly, acid sensitization seems to become greater in previously acid-adapted than nonadapted cells with prolonged storage of plain water washings at 10°C. The potential contribution of these effects on meat cross-contamination needs to be considered.

References

1. Lou, Y., and A.E. Yousef. 1997. Appl. Environ. Microbiol. 63:1252-1255.
2. Lou, Y., and A. E. Yousef. 1999. p. 131-224. In *Listeria*, Listeriosis and Food Safety. E.T. Ryser and E.H. Marth (ed.).
3. Prasai, R.K., C.L. Kastner, P.B. Kenney, D.H. Kropf, D.Y.C. Fung, L.E. Mease, et. al. 1997. J. Food Prot. 60:795-798.
4. Samelis J., J.N. Sofos, P.A. Kendall, and G.C. Smith. 2001. J. Food Prot., in press.
5. Smulders, F.J.M., and G.G. Greer. 1998. Int. J. Food Microbiol. 44:149-169.
6. Sofos, J.N., and G.C. Smith. 1998. Int. J. Food Microbiol. 44:171-188.



igure 1. Behavior of inoculated (10^5 CFU/ml), acid-adapted (AD) or nonadapted (NAD), *Listeria monocytogenes* (A) in co-culture ith the natural flora (B) in plain water (W) meat washings, or mixed (1:99) with 2% lactic (LA) or 2% acetic (AA) acid washings, nd associated pH changes (C), during storage at 10°C. The acid tolerance of the inoculated pathogen was assessed at 0, 2 and 7 days f exposure to plain water washings by subsequent exposure to pH 3.5 with lactic acid, and survivors (log CFU/ml) were determined ter 0, 60 and 120 min (D).