Effect of Acid Adaptation on Survival of *Escherichia coli* O157:H7 in Meat Decontamination Washing Fluids and Potential Effects of Organic Acid Interventions on the Microbial Ecology of the Meat Plant Environment

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ABSTRACT

The acid tolerance of *Escherichia coli* O157:H7 may be pH inducible. Correspondingly, organic acid meat decontamination washing fluids may enhance the establishment of acid-adapted *E. coli* O157:H7 strains in packing plants, especially in mixtures with water washings from meat that may be of sublethal pH. Acid-adapted and nonadapted cultures of a rifampin-resistant derivative of the acid-resistant *E. coli* O157:H7 strain ATCC 43895 were tested to evaluate their survival in meat-washing fluids over a wide pH range. The cultures were exposed (10^5 CFU/ml) to acidic (2% lactic acid, 2% acetic acid, or a mixture of the two with water washings at ratios of 1/1, 1/9, or 1/99 [vol/vol]) or nonacid (water) meat washings for up to 14 days at 4 or 10°C storage. *E. coli* O157:H7 survived in water washings, but the low storage temperatures and predominant natural microbiota synergistically inhibited its growth. Compared with acid-adapted populations, nonadapted populations displayed greater potential for survival and a tendency to initiate growth in water meat washings at 10°C. The pathogen survived in most of the acid washings throughout storage (14 days), sometimes with minimal population reductions. Overall, nonadapted populations declined faster than acid-adapted populations, while the declines increased as the acid concentration and temperature of storage increased and were more dramatic in lactate, compared to acetate, washings. Acid-containing washings were selective for growth of lactic acid bacteria and yeasts, indicating that organic acid treatments may alter the microbial ecology of meat plant environments and potentially that of the meat. These results should be considered when selecting decontamination technologies for meat.

Recent outbreaks of illness due to the consumption of various foods contaminated with *Escherichia coli* O157:H7 (2, 5, 9) have emphasized the need for control of this pathogen throughout the entire food chain (26). Cattle are a main reservoir of *E. coli* O157:H7, while the most likely mode of transmission to fresh meat and other foods is through bovine fecal contamination (13, 26). *E. coli* O157, which has been reported to be a *Shigella* with a little cloak of *E. coli* antigens (20), may have evolved in response to severe stress (26, 31). Evidently, *E. coli* O157:H7 has become naturally acid resistant (3, 10), particularly in the stationary phase (7, 22) and under starvation conditions (1). In addition, it may attain a pH-inducible acid tolerance (6, 27) that could enhance survival in acidic foods (21) or in the stomach to ultimately cause disease. The adaptation to physical stresses and evolution of *E. coli* O157:H7 that resulted in acid resistance may have increased its virulence (8, 31), as is indicated by its seemingly low oral infectious dose (i.e., 100 to 200 or even <10 cells in susceptible groups of consumers (2, 9, 26)).

Controlling the transmission of *E. coli* O157:H7 to fresh meat and its products by bovine fecal contamination (13) requires effective measures at both the farm and the slaughterhouse level (26, 35). In response, the U.S. Department of Agriculture Food Safety and Inspection Service established the requirement for *E. coli* biotype 1 enumeration as a means of verifying that the slaughter process is under control (14). This has renewed interest in meat decontamination technologies, which may include the spraying of carcasses with either organic acid solutions (32) or hot water, steam, or nonacid chemical solutions (35) to reduce microbial contamination and assist in passing the regulatory criteria (14, 34). Concerns, however, have been expressed about whether acidic decontamination technologies for meat may induce acid adaptation and eventually lead to the development of acid-resistant strains of *E. coli* O157:H7 and other pathogens following its potential survival on the meat or in the plant environment or during a posttreatment recontamination of meat (32, 35). In addition, organic acid interventions may reduce the numbers or alter the composition of the background flora on fresh meat or other foods, thereby leading to potentially compromised safety of decontaminated food products by enhancing the opportunity for surviving pathogens to proliferate in the absence of natural competitors (18).

These safety concerns have intensified research to address the potential implications of decontamination on...
pathogen behavior as well as on the microbial ecology of meat overall (4, 12, 24, 29, 36). Vold et al. (36) showed that high numbers of bacteria in the natural flora inhibited E. coli O157:H7 proliferation in ground beef stored aerobically or anaerobically at 12°C, with inhibition being more pronounced under anaerobic conditions. Conversely, on beef that had been decontaminated with lactic acid, the pathogen increased by nearly 3 logs within 5 days of storage at 10°C under air or vacuum, while the increases were only 1 log and virtually nonexistent on untreated beef (24). Berry and Cutter (4) demonstrated that previous acid adaptation of two strains of E. coli O157:H7 (ATCC 43895 categorized as acid resistant and ATCC 43889 demonstrating an inducible acid tolerance) resulted in higher numbers of survivors on beef carcass tissue during 14 days of 4°C storage, following spraying with 2% acetic acid, compared to nonadapted cultures. Interestingly, for both strains, numbers of acid-adapted populations remaining on the tissue following 2% acetic acid treatments were similar to numbers of both acid-adapted and nonadapted populations remaining on the tissue following spraying with water (4). Thus, the adaptation of E. coli O157:H7 to acid may negatively influence the effectiveness of 2% acetic acid spray washings in reducing pathogen numbers on carcasses (4).

Consistent with the findings of Berry and Cutter (4), we recently showed that the acid-resistant strain ATCC 43895 could survive at 4 or 10°C for 2 to 7 days in undiluted 2% lactate (pH 2.3 to 2.5) or 2% acetate (pH 3.0 to 3.2) spray washings on beef (29). The survival rate of E. coli O157:H7 at 4°C was greater and longer lasting in 2% acetic acid washings, and it was higher than that of Listeria monocytogenes and Salmonella Typhimurium DT104 under all conditions tested (29). Notably, the inoculated pathogen cells that survived under those pH conditions were not fully adapted to acid, because the cultures were prepared in broth with 0.25% instead of 1% glucose, which results in full adaptation (6). In that previous study (29), we did not evaluate the potential for survival of E. coli O157:H7 in acidic rinses that had been mixed with additional water from washes, which inadvertently happens in meat plants and may result in decontamination waste fluids that are sublethal to the pathogen. As a result, the aim of this study was to evaluate (i) the potential survival of E. coli O157:H7 at low temperatures in meat decontamination washings over a wide pH range, which could be the result of mixtures of water with either lactic or acetic acid solution washings under packing plant conditions, and (ii) the potential differences in survival between previously acid-adapted or nonadapted E. coli O157:H7.

MATERIALS AND METHODS

Bacterial strains and culture conditions. A rifampin-resistant (100 µg/ml) derivative of E. coli O157:H7 ATCC 43895 prepared in our laboratory (29) was used throughout this study. This rif+ derivative was selected because its parental strain is a raw meat isolate implicated in a hemorrhagic colitis outbreak from hamburgers (2) and because it is acid resistant (3, 4, 10). The ability of strain ATCC 43895rif+ to retain a high acid tolerance response similar to that of its parental strain was confirmed by challenge tests in trypticase soy broth (BBL, Becton Dickinson, Cockeysville, Md.) with 0.6% yeast extract (TSBYE; Difco, Sparks, Md.) acidified to pH 3.5 or 3.7 with lactic or acetic acid (28). Acid challenge treatments used suspensions (105 CFU/ml) of both the parental and rif+ derivative strains grown (30°C, 24 h) in glucose-free TSBYE (TSBYE-G; BBL) or with 1% added glucose (TSBYE+G; Sigma Chemical Co., St. Louis, Mo.) to prevent or induce acid adaptation (6). Acid-adapted or nonadapted cultures, prepared as described above, were used to inoculate meat washings. The pH values of cultures used as inocula were measured with a digital pH meter (Accumet 50, Fisher Scientific, Houston, Tex.) equipped with a glass electrode (Hanna Instruments, Ann Arbor, Mich.). Inoculation cultures were prepared from working cultures of strain ATCC 43895rif+, kept at 4°C on slants of trypticase soy agar (BBL) with 0.6% yeast extract (TSAYE), and by two subcultures in TSBYE at 30°C for 24 h before use in the experiments.

Preparation of fresh beef decontamination washings. Fresh (≤72 h postmortem), nondecontaminated top rounds of beef were obtained from a commercial plant or from the Meat Science Laboratory of Colorado State University, Fort Collins. Each top round was cut into four portions weighing approximately 2 kg each. Portions were individually spray washed with 2 liters of either cold (10°C) or hot (85°C) tap water or with warm (55°C) solutions of 2% lactic acid (10-lactic acid, 85% [wt/wt] syrup, Sigma) or 2% acetic acid (100% glacial, Mallinkrodt Baker, Inc., Paris, Ky.). Spraying was accomplished in a simulation model spray washer (Chad Co., Olathe, Kans.) as described previously (29). Separate washings were collected, distributed in 500-ml sterile bottles (Nalgene, Nalge Co., Rochester, N.Y.), stored at −70°C, and used in the experiments within 30 days.

Preparation of washing mixtures and inoculation with E. coli O157:H7. Frozen meat washings were thawed at 4°C overnight to minimize microbial and chemical changes. Initially, spray washings of meat with cold (10°C) water were mixed at a ratio of 1/1 with hot (85°C) water washings to obtain a mixture of nonacid meat-washing fluids. This was performed after earlier studies in our laboratory (29, 30) showed no differences in the behavior of the natural biota present compared to that of the pathogens inoculated in washings resulting from the spraying of meat with water at 10 or 85°C. Then, 2% lactic or 2% acetic acid solution washings were mixed with the composite water washings at ratios of 1/1, 1/9, and 1/99 to obtain mixtures of acid-containing washings with progressively higher pH values. Plain water washings, 2% acid solution washings, and the mixtures described earlier were individually transferred (100 ml) into presterilized 250-ml culture bottles (Nalgene). Sets of four bottles were prepared; two bottles were inoculated (106 CFU/ml) with the previously acid-adapted culture (TSBYE+G), and the other two bottles were inoculated with the nonadapted culture (TSBYE-G) of E. coli O157:H7 strain ATCC 43895rif+, as described previously (29). Then, the bottles of inoculated washings were transferred into two cooled incubators, one at 4 ± 0.5°C and the other at 10 ± 0.5°C, together with bottles of uninoculated water washings, 2% lactic acid washings, or 2% acetic acid washings, to serve as controls. Samples were incubated statically at the appropriate temperature for up to 14 days.

Microbiological and pH analyses. Changes in the populations of natural microbiota and those of inoculated (106 CFU/ml) E. coli O157:H7, as well as changes in the pH of these cultures, were monitored at 0, 2, 4, 7, and 14 days of storage at 4 or 10°C. On each sampling day, 1 ml of each culture was serially diluted
in 0.1% buffered peptone water (Difco). Then, the appropriate dilutions were spread (0.1 ml) in duplicate on TSAYE or TSAYE agar plates with 100 mg/liter rifampin (TSAYE-rif) to determine the populations of natural microbiota and strain ATCC 43895rif+.

Sorbitol MacConkey agar was not used in this study because it has been shown to be inhibitory to acid-stressed cells (28) as well as of low selectivity (29). In contrast, TSAYE-rif supported the growth and recovery of acid-stressed cells to levels similar to those on TSAYE but inhibited growth of indigenous bacteria (29).

Colonies on agar plates were counted after incubation at 30°C for 48 h. The pH of the washings was also measured. Preliminary biochemical characterization of colonies grown on countable TSAYE plates was performed by testing for colony morphology, microscopic appearance, gram reaction, catalase, and oxidase, as described previously (30). When the first two tests indicated the presence of yeasts, a tentative characterization was based on morphological criteria (colony characteristics, cell shape, cell budding or fusion ascii formation, and numbers of ascospores per ascus) and simplified taxonomic keys (17), as described previously (23). All experiments were performed in triplicate with different batches of meat washings. Microbiological counts were converted to log CFU/ml, and the means and standard deviations were calculated.

### RESULTS AND DISCUSSION

**Initial pH of undiluted or mixed washings from meat.** To follow the behavior of *E. coli* O157:H7 in acidic or plain water washings or in their mixtures (1/1, 1/9, or 1/99), the pH of the washings (Table 1) at inoculation is important. The average initial (day 0) pH values of all samples inoculated either with acid-adapted or nonadapted *E. coli* O157:H7 (e.g., \( n = 6 \)) and receiving either 2% lactate or 2% acetate washings were 2.34 ± 0.02 and 3.00 ± 0.03, respectively, while the average initial pH of water washings was 6.97 ± 0.12. Mixtures of 2% lactate solution washings with water washings at ratios of 1/1, 1/9, and 1/99 had average pH values of 2.64 ± 0.07, 3.21 ± 0.07, and 4.56 ± 0.15, respectively. The corresponding pH values of acetate-containing washing mixtures (i.e., 1/1, 1/9, or 1/99) were 3.21 ± 0.06, 3.76 ± 0.02, and 4.68 ± 0.07, respectively. The pH values of washings during storage (Table 1) are discussed in the following paragraphs.

**Acid tolerance of the inoculum.** The average pH values of acid-adapted and nonadapted cultures of strain ATCC 43895rif+ were 5.08 ± 0.04 and 6.71 ± 0.15, respectively. After a 120-min exposure to TSBYE acidified to pH 3.5 with lactic acid, the average reductions in the initial populations of acid-adapted and nonadapted strain ATCC 43895rif+ were 0.2 ± 0.1 and 0.8 ± 0.3 log CFU/ml, respectively. Thus, *E. coli* O157:H7 was acid resistant at inoculation (day 0), especially when previously acid adapted.

**Survival of *E. coli* O157:H7 in meat washings.** Inoculated (4.8 to 5.1 log CFU/ml) *E. coli* O157:H7 survived for at least 7 days at 4 or 10°C in most of the treatments with meat washings, regardless of its previous adaptation to acid (Table 2). Specifically, in acidic treatments, surviving populations of *E. coli* O157:H7 were constantly higher and present in the washings for longer times when stored at 4°C rather than at 10°C and when the initial inoculum was acid adapted rather than nonadapted. At 4°C, in particular, considerably high (3.7 to 4.7 logs) acid-adapted populations survived throughout storage (14 days) in all treatments, except for undiluted 2% lactate washings or 2% lactate washings diluted (1/1) with water washings, when the pathogen became undetectable at 7 to 14 days. Overall, reductions in *E. coli* O157:H7 populations during storage

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**Table 1.** Mean (\( n = 3 \)) pH values of water, acidic, or mixed fresh beef decontamination spray washings inoculated with acid-adapted or nonadapted *Escherichia coli* O157:H7 strain ATCC 43895rif+ and stored at 4 or 10°C

<table>
<thead>
<tr>
<th>Type of meat washings</th>
<th>Storage temperature</th>
<th>Acid-adapted culture</th>
<th>Nonadapted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 2 4 7 14</td>
<td>0 2 4 7 14</td>
</tr>
<tr>
<td>Lactic acid (2%)</td>
<td></td>
<td>2.35 2.34 2.55 2.36 2.45</td>
<td>2.33 2.36 2.55 2.34 2.46</td>
</tr>
<tr>
<td>Lactic acid/water (1/1)</td>
<td></td>
<td>2.63 2.56 2.85 2.54 2.75</td>
<td>2.65 2.61 2.71 2.55 2.72</td>
</tr>
<tr>
<td>Lactic acid/water (1/9)</td>
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<td>3.20 3.11 3.24 3.02 3.18</td>
<td>3.22 3.15 3.30 3.03 3.16</td>
</tr>
<tr>
<td>Lactic acid/water (1/99)</td>
<td></td>
<td>4.56 4.33 4.54 4.33 5.68</td>
<td>4.55 4.49 4.31 4.38 5.91</td>
</tr>
<tr>
<td>Acetic acid (2%)</td>
<td></td>
<td>2.99 2.91 3.09 2.80 3.14</td>
<td>3.01 2.91 3.10 2.83 3.00</td>
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<tr>
<td>Acetic acid/water (1/1)</td>
<td></td>
<td>3.20 3.09 3.30 3.03 3.30</td>
<td>3.21 3.15 3.30 3.03 3.20</td>
</tr>
<tr>
<td>Acetic acid/water (1/9)</td>
<td></td>
<td>3.76 3.66 3.84 3.46 3.73</td>
<td>3.76 3.68 3.80 3.47 3.73</td>
</tr>
<tr>
<td>Acetic acid/water (1/99)</td>
<td></td>
<td>4.66 4.52 4.70 4.39 4.70</td>
<td>4.70 4.53 4.71 4.38 4.72</td>
</tr>
<tr>
<td>Water</td>
<td>10°C</td>
<td>6.98 6.82 7.04 7.33 7.43</td>
<td>6.95 6.80 6.86 7.07 7.34</td>
</tr>
<tr>
<td>Lactic acid (2%)</td>
<td></td>
<td>2.35 2.37 2.51 2.40 2.52</td>
<td>2.33 2.38 2.45 2.34 2.42</td>
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<td></td>
<td>2.63 2.59 2.70 2.62 2.71</td>
<td>2.65 2.58 2.69 2.64 2.63</td>
</tr>
<tr>
<td>Lactic acid/water (1/9)</td>
<td></td>
<td>3.20 3.13 3.26 3.09 3.28</td>
<td>3.22 3.17 3.28 3.18 3.20</td>
</tr>
<tr>
<td>Lactic acid/water (1/99)</td>
<td></td>
<td>4.56 4.42 4.71 6.58 6.98</td>
<td>4.55 4.61 4.74 6.49 7.14</td>
</tr>
<tr>
<td>Acetic acid (2%)</td>
<td></td>
<td>2.99 3.00 3.03 2.88 3.03</td>
<td>3.01 3.00 3.03 2.84 3.00</td>
</tr>
<tr>
<td>Acetic acid/water (1/1)</td>
<td></td>
<td>3.20 3.20 3.30 3.21 3.27</td>
<td>3.21 3.15 3.23 3.17 3.27</td>
</tr>
<tr>
<td>Acetic acid/water (1/9)</td>
<td></td>
<td>3.76 3.74 3.78 3.57 3.65</td>
<td>3.76 3.72 3.76 3.66 3.70</td>
</tr>
<tr>
<td>Acetic acid/water (1/99)</td>
<td></td>
<td>4.66 4.54 4.69 4.62 4.68</td>
<td>4.70 4.71 4.68 4.71 4.67</td>
</tr>
</tbody>
</table>

* SD range, 0.01 to 0.51, except for 1/99 lactate/water samples at 14 days, which displayed an SD range of 0.93 to 1.10 for reasons discussed in “Results.”
became smaller as the proportion of water washings in the acidic mixtures increased. However, for acid-adapted cells, a 10-fold increase (from 1/99 to 1/9) in acetic acid concentration in the washing mixtures did not result in any increase in lethal effects during 14 days' storage at either temperature. This was because acetic-containing washings were less lethal to the pathogen than lactate-containing washings when undiluted or diluted at the same proportion with water washings. It seems alarming that acid-adapted \textit{E. coli} O157:H7 populations as high as 3.7 log CFU/ml survived in undiluted 2\% acetic acid washings for as long as 14 days at 4°C. Moreover, both the 1/9 dilution of 2\% acetic acid washings with water washings and the 1/99 dilution of 2\% solution washings of either acid with water washings allowed survival (2.9 to 4.7 logs) of \textit{E. coli} O157:H7 for at least 14 days, regardless of temperature (4 or 10°C) or previous adaptation of the pathogen to acid (Table 2).

These results clearly indicate the ability of \textit{E. coli} O157:H7 to survive for extended periods of time in acid-containing waste fluids from meat decontamination. As mentioned, potential safety risks associated with survival (up to 7 days) of partially acid-adapted \textit{E. coli} O157:H7 populations in meat washings and, thus, in the surrounding plant environment were first indicated in a previous study (29). The present study further suggests that the survival of the pathogen may become higher in number or that it may last longer upon previous acid adaptation of the pathogen or dilution of the original acid solution washings with water washings or rines (Table 2). Survival may increase in meat plants that use acetic rather than lactic acid for carcass decontamination. That acetic acid is lower in effectiveness than lactic acid against \textit{E. coli} O157:H7 has previously been reported by others (4, 16, 32, 35). Also, acetic acid may dissipate more rapidly than lactic acid in decontamination waste fluids, thereby permitting enhanced survival of the pathogen regardless of the ultimate pH value.

Moreover, \textit{E. coli} O157:H7 survived in plain water washings but did not multiply at either temperature (4 or 10°C) (Table 2). Its populations remained constant or tended to decline in water washings from days 7 to 14; notably, declines were greater for acid-adapted than for nonadapted populations at 10°C. Similar declines, which were higher at 10°C (i.e., approximately 1.5 logs) than at 4°C (i.e., approximately 0.5 log), were observed previously when, as indicated, partially acid-adapted cultures of \textit{E. coli} O157:H7 were used as inocula (29). These results suggest that \textit{E. coli} O157:H7 may have a greater potential to adapt to neutral pH but otherwise stressful conditions prevailing in water meat decontamination washings if previously grown without glucose. Particularly at 10°C, which is within the growth temperature range of the pathogen in culture broth (25), nonadapted \textit{E. coli} O157:H7 was likely to initiate growth (i.e., slight increases of 0.3 to 0.5 log CFU/ml were observed in some of the experiments) at 2 to 4 days of storage in water washings (Table 2). This is supported by more recent studies in our laboratory indicating the ability of \textit{E. coli} O157:H7 to multiply by 1 to 2 logs in water washings from meat stored at 15°C (unpublished data). At 10°C, however, the growth tendencies of \textit{E. coli} in water washings were suppressed (Table 2) soon after the competitive natural microbiota increased to high levels (>8 log CFU/ml) (Table 3). Thus, the growth potential of \textit{E. coli} O157:H7 in fresh meat environments under microbial competition, such as in water meat decontamination washings, may be crucially affected by the temperature and degree of oligotrophy in the natural substrate (19, 25, 29) as well as, most importantly, the history of the inoculum (6).

### TABLE 2. Populations (mean log CFU/ml [SD], n = 3) of inoculated, acid-adapted, or nonadapted \textit{Escherichia coli} O157:H7 strain ATCC 43895rif+ (TSAYE +rif) in water, acidic, or mixed fresh beef decontamination spray washings stored at 4 or 10°C

<table>
<thead>
<tr>
<th>Type of meat washings</th>
<th>Storage temperature</th>
<th>Acid-adapted culture</th>
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<tbody>
<tr>
<td></td>
<td>0 2 4 7 14</td>
<td>0 2 4 7 14</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid (2%)</td>
<td>4.9 (0.1) 4.7 (0.1) 4.5 (0.2) 4.5 (0.2) 4.4 (0.2)</td>
<td>5.1 (0.1) 5.0 (0.0) 4.8 (0.0) 4.8 (0.1) 4.7 (0.10)</td>
<td></td>
</tr>
<tr>
<td>Lactic acid/water (1/1)</td>
<td>4.8 (0.0) 4.4 (0.2) 2.6 (1.5) 1.8 (0.8)</td>
<td>5.0 (0.1) 1.5 (0.9) 1.0 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Lactic acid/water (1/9)</td>
<td>4.9 (0.1) 4.7 (0.1) 4.7 (0.1) 4.3 (0.0) 3.7 (0.3)</td>
<td>5.0 (0.1) 4.3 (0.2) 3.3 (0.4) 2.1 (0.5)</td>
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<tr>
<td>Lactic acid/water (1/99)</td>
<td>4.9 (0.1) 4.9 (0.0) 4.8 (0.0) 4.8 (0.1) 4.7 (0.1)</td>
<td>5.0 (0.0) 5.0 (0.0) 4.9 (0.1) 4.7 (0.2) 4.5 (0.1)</td>
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</tr>
<tr>
<td>Acetic acid (2%)</td>
<td>4.9 (0.1) 4.7 (0.7) 4.5 (0.1) 4.4 (0.1) 3.7 (0.9)</td>
<td>5.0 (0.1) 4.5 (0.2) 3.9 (0.4) 2.8 (0.2) 1.0 (0.0)</td>
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</tr>
<tr>
<td>Acetic acid/water (1/1)</td>
<td>4.9 (0.1) 4.7 (0.1) 4.7 (0.1) 4.5 (0.1) 4.2 (0.1)</td>
<td>5.0 (0.1) 4.4 (0.0) 3.8 (0.1) 3.3 (0.6)</td>
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</tr>
<tr>
<td>Acetic acid/water (1/9)</td>
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<td>5.0 (0.1) 4.8 (0.0) 4.5 (0.1) 4.1 (0.2) 3.4 (0.4)</td>
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</tr>
<tr>
<td>Acetic acid/water (1/99)</td>
<td>4.9 (0.1) 4.9 (0.1) 4.8 (0.1) 4.8 (0.1) 4.7 (0.0)</td>
<td>5.0 (0.1) 5.0 (0.1) 4.9 (0.1) 4.8 (0.1) 4.5 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>10°C</td>
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<tr>
<td>Lactic acid (2%)</td>
<td>4.9 (0.1) 4.2 (0.4) 4.3 (0.4) 4.2 (0.4) 3.8 (0.3)</td>
<td>5.1 (0.1) 5.2 (0.2) 5.2 (0.4) 5.2 (0.3)</td>
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<tr>
<td>Lactic acid/water (1/1)</td>
<td>4.8 (0.0) 2.5 (1.5) &lt;1.0 1.0 &lt;1.0</td>
<td>5.0 (0.1) 5.0 (0.1) 5.0 (0.1) 1.0 (1.0)</td>
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<tr>
<td>Lactic acid/water (1/9)</td>
<td>4.9 (0.1) 4.7 (0.1) 4.3 (0.1) 3.0 (0.3)</td>
<td>5.0 (0.1) 4.0 (0.1) 2.7 (0.5) 1.3 (0.5)</td>
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<tr>
<td>Lactic acid/water (1/99)</td>
<td>4.9 (0.1) 4.8 (0.1) 4.5 (0.0) 4.2 (0.0) 3.9 (0.3)</td>
<td>5.0 (0.0) 4.7 (0.1) 4.6 (0.1) 4.3 (0.1) 3.9 (0.0)</td>
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<tr>
<td>Acetic acid (2%)</td>
<td>4.9 (0.1) 4.8 (0.1) 4.3 (0.4) 2.9 (1.5)</td>
<td>5.0 (0.1) 4.1 (0.4) 2.3 (1.3) 1.1 (0.2) 1.0 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Acetic acid/water (1/1)</td>
<td>4.8 (0.1) 4.8 (0.1) 4.7 (0.1) 4.1 (0.1) 1.7 (0.9)</td>
<td>5.0 (0.1) 4.2 (0.1) 3.1 (0.2) 1.8 (0.2) 1.0 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Acetic acid/water (1/9)</td>
<td>4.8 (0.0) 4.9 (0.1) 4.8 (0.1) 4.8 (0.1) 4.6 (0.1)</td>
<td>5.0 (0.1) 4.8 (0.2) 4.5 (0.1) 4.1 (0.3) 2.9 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Acetic acid/water (1/99)</td>
<td>4.9 (0.1) 4.8 (0.1) 4.8 (0.1) 4.8 (0.1) 4.6 (0.2)</td>
<td>5.0 (0.1) 5.0 (0.2) 4.9 (0.2) 4.7 (0.3) 4.5 (0.6)</td>
<td></td>
</tr>
</tbody>
</table>
Another factor that could have negatively influenced the behavior of acid-adapted cells was the major pH shift, as indicated, from an average value of 5.08 (day 0) to an average value of 6.97 (Table 2). The corresponding declines of acid-adapted populations at 10°C may have contributed to their subsequent decreased survival, compared to nonadapted cells, in water washings during storage at 10°C (Table 2). The corresponding declines of acid-adapted populations at 4°C were approximately 0.5 log lower (Table 2), because the low storage temperature would have suppressed any tendency of those lactate/water washings containing acid-adapted populations to a low pH after 24 h at 30°C.

Behavior of natural microbiota in meat decontamination washings. As indicated, plain water washings supported the growth of high numbers (>8 log CFU/ml) of natural microbiota, and increases were faster in samples stored at 10°C than at 4°C (Table 3). This microbiota was predominantly composed of gram-negative (100% of colonies on countable TSAYE plates), oxidase-positive (>90% of colonies) Pseudomonas-like bacteria acquired from the meat. The inoculation in the washings of 5 logs of E. coli O157:H7, either acid-adapted or nonadapted, did not have a major effect on the growth of natural microbiota (Table 3), which showed similar increases in uninoculated samples at either temperature (data not shown). These results are in agreement with our previous studies describing the ability of aerobic meat spoilage bacteria to dominate in nonacid (water) meat decontamination washings (29, 30).

From the beginning to the end of storage at 4 or 10°C, the uninoculated 2% lactate or 2% acetate solution contained less than 1 log CFU/ml natural microbiota (data not shown). Thus, surviving populations on TSAYE (Table 3) from inoculated samples were very similar to those on TSAYE+G culture at inoculation (day 0) to an average value of 6.97 ± 0.12 in water meat washings. Potential alterations in acid-adapted cells (e.g., activation of energetically expensive mechanisms for adaptation of stationary-phase TSBYE) may have contributed to their subsequent decreased survival, compared to nonadapted cells, in water washings during storage at 10°C (Table 2). The corresponding declines of acid-adapted populations at 4°C were approximately 0.5 log lower (Table 2), because the low storage temperature would have suppressed any tendency of those lactate/water washings containing acid-adapted populations to a low pH after 24 h at 30°C.

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as yeasts with white colonies, most probably *Debaryomyces* or *Saccharomyces*, (i.e., spherical to elongated cells with budding; ascii with 1 to 2 ascospores) or *Candida* (similar cell morphology but without ascii formation) (23, 31). Compared to lactate-containing washings, the acetate-containing washings (1/1, 1/9, or 1/99) did not permit growth of natural microbe at 4°C (i.e., all colonies on countable TSAYE were *E. coli* O157:H7), while at 10°C, lower increases of natural microbe occurred at 1/9 and 1/99, and no increases occurred at 1/1, acetate/water washings, respectively (Table 3). This biota was composed of lactic acid bacteria, as most colonies were gram-positive, catalase-negative short rods in co-culture with yeasts that appeared in white colonies, while gram-negative bacterial colonies occasionally developed only in 1/99 acetate/water washings at 10°C.

The behavior of natural microbe in the acid meat decontamination washings observed in this study may have implications for the meat industry. Results clearly demonstrated that acidic meat rinses, diluted up to 10-fold with water washings, selectively inactivated or inhibited growth of the normal gram-negative psychrotrophic and aerobic flora of fresh meat; thus, packing plants that use organic acid treatments may harbor reduced numbers of competitive flora. In addition, the plant flora may change in composition, because acid-containing washings may select for specific types or groups of microorganisms, especially acid-tolerant spoilage (i.e., oxidative) yeasts able to assimilate lactate (11). Colonization of packing plants by lactic acid bacteria may be greater if acetic acid rather than lactic acid is used for meat decontamination. Conversely, gram-negative bacteria that survive meat decontamination may establish niches and increase in plants that use hot water or steam, or when organic acid rinses are mixed with water washes. This natural selection and the potential cross-contamination of beef with different bacteria or yeasts that may persist in the packing plant could alter the microbial ecology and spoilage pattern and might influence the safety of decontaminated beef, depending on the subsequent packaging and storage conditions. For example, yeasts would be expected to compete poorly and thus offer minimal protection against bacterial pathogens surviving on decontaminated meat due to their slower growth rates and low proliferation in vacuum packages of meat (11, 33). Instead, in aerobically stored fresh meat containing low numbers of competitive bacteria, yeasts may enhance the outgrowth of pathogens by assimilating lactate, thus contributing to the buffering capacity of meat and raising the pH while eliminating the residual antimicrobial effects of acid (11).

**Changes in pH of meat decontamination washings.**

The high initial pH of plain water washings remained constant throughout storage at 4°C, while it increased further at 10°C (Table 1), reflecting the increased growth of, and production of alkaline metabolites by, gram-negative bacteria at the elevated temperature (Table 3). In contrast, the low pH of 2% lactate or 2% acetic washings was unchanged throughout storage (14 days) under all conditions tested (Table 1). These results are in agreement with our previous findings (29). With respect to washing mixtures, no major pH changes were observed in 1/1 and 1/9 samples with either acid at either temperature or in acetate/water (1/99) washings stored at 4°C. In contrast, the pH increased proportionate to the temperature of storage in all lactate/water (1/99) washings at 4 or 10°C or in acetate/water (1/99) washings at 10°C (Table 1). This pH increase was associated with the observed increases in numbers of gram-negative bacteria or yeasts from days 7 to 14 of storage (Table 3).

Results of this study should be considered when selecting decontamination technologies for use in meat-packaging plants. That organic acid interventions may deliver significant reductions (e.g., 1 to 3 logs) in surface meat contaminants and assist plants in passing U.S. regulatory criteria has been shown by several studies in the laboratory or at the plant level (16, 32, 34, 35); therefore, their importance and practical usefulness should be recognized. However, potential long-term effects of organic acid interventions on the microbial ecology of meat-packaging plants and products should be considered in order to recognize and manage potential safety risks. This study demonstrates that pH conditions prevailing in decontamination waste fluids, as well as in the packing plant environment overall, may allow the survival of *E. coli* O157:H7, while they may also alter the composition and populations of natural flora, including that of meat. To validate and extrapolate our findings to the packing plant level, decontamination waste fluids collected from a commercial beef-packing plant that washes carcasses with water followed by misting with 2% lactic acid were shown to have a pH of 3.6 to 3.9 after mixing. According to the pH range of the lactate/water washings prepared in this study (Table 1), the pH of the commercially collected washings indicated more than a 10-fold dilution of the original lactate solution with water washings, a finding that supports safety concerns. Additional studies are therefore required to evaluate whether these conditions may enhance or maintain the acid tolerance response due to prolonged exposure of *E. coli* O157:H7 survivors to acidic waste fluids of sublethal pH (>3.5 to 4.5), where the more acid-sensitive natural meat microbe can not survive or grow. This could increase safety risks if naturally acid-adapted pathogens of potentially increased virulence (8, 15, 31) establish niches in plants that can serve as cross-contamination sources for meat (29, 35). Pathogen survival and associated potential safety risks may be greater if acetic acid is used for the decontamination of meat because of its lower effectiveness, compared to that of lactic acid, against *E. coli* O157:H7 (Table 2) and its increased effectiveness against the natural meat microbiota (Table 3).

Water washings may support the growth of meat spoilage bacteria in plants; however, this competitive flora has the potential to prevent the growth or reduce the number of *E. coli* O157:H7, provided that temperatures in the plant, or on the meat, are below 10°C (4, 24, 36). Most importantly, we recently showed (28) that exposure of *E. coli* O157:H7 to water washings from meat may induce acid sensitization of the pathogen, a phenomenon that, in prac-
tice, may lessen potential safety risks. Also, in another study (30), *L. monocytogenes* shifted from acid resistant after 1 day to very acid sensitive after 8 days of growth in unfiltered washings at 35°C, while this response was completely reversed when the same washings were filter sterilized prior to inoculation. Further studies are needed to address the observed acid sensitization and to clarify, overall, the complex microbial interactions and pathogen responses to acid stress in fresh meat and plant environments, which will allow this knowledge to be used in meat safety enhancement.

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