Survival or Growth of *Escherichia coli* O157:H7 in a Model System of Fresh Meat Decontamination Runoff Waste Fluids and Its Resistance to Subsequent Lactic Acid Stress

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A potential may exist for survival of and resistance development by *Escherichia coli* O157:H7 in environmental niches of meat plants applying carcass decontamination interventions. This study evaluated (i) survival or growth of acid-adapted and nonadapted *E. coli* O157:H7 strain ATCC 43895 in acetic acid (pH 3.6 ± 0.1) or in water (pH 7.2 ± 0.2) fresh beef decontamination runoff fluids (washings) stored at 4, 10, 15, or 25°C and (ii) resistance of cells recovered from the washings after 2 or 7 days of storage to a subsequent lactic acid (pH 3.5) stress. Corresponding cultures in sterile saline or in heat-sterilized water washings were used as controls.

In acetic acid washings, acid-adapted cultures survived better than nonadapted cultures, with survival being greatest at 4°C and lowest at 25°C. The pathogen survived without growth in water washings at 4 and 10°C, while it grew by 0.8 to 2.7 log cycles at 15 and 25°C, and more in the absence of natural flora. *E. coli* O157:H7 cells habituated without growth in water washings at 4 or 10°C were the most sensitive to pH 3.5, while cells grown in water washings at 15 or 25°C were relatively the most resistant, irrespective of previous acid adaptation. Resistance to pH 3.5 of *E. coli* O157:H7 cells habituated in acetic acid washings for 7 days increased in the order 15°C > 10°C > 4°C, while at 25°C cells died off. These results indicate that growth inhibition by storage at low temperatures may be more important than competition by natural flora in inducing acid sensitization of *E. coli* O157:H7 in fresh meat environments. At ambient temperatures in meat plants, *E. coli* O157:H7 may grow to restore acid resistance, unless acid interventions are applied to inhibit growth and minimize survival of the pathogen. Acid-habituated *E. coli* O157:H7 at 10 to 15°C may maintain a higher acid resistance than when acid habituated at 4°C. These responses should be evaluated with fresh meat and may be useful for the optimization of decontamination programs and postdecontamination conditions of meat handling.

*Escherichia coli* O157:H7 has become the primary target organism of food animal carcass decontamination interventions, which are of increasing commercial use in North America (3, 13, 21, 39, 41). In-plant applications have shown effectiveness of decontamination in reducing the percentage of carcass samples being *E. coli* O157:H7 positive from previsceration to postprocessing (2, 19). However, there is a need to evaluate the potential for development of acid resistance in *E. coli* O157:H7 during application of acid decontamination interventions in meat (31). Studies have shown that the pathogen may survive decontamination of meat with lactic or acetic acid (7, 16, 17), suggesting that survivors can exist and may potentially adapt to the residual organic acid in situ in commercial meat processing environments (31).

Berry and Cutter (6) demonstrated that previous acid adaptation of *E. coli* O157:H7 by culturing in media with 1% glucose (9) reduced the effectiveness of a 2% acetic acid solution to inactivate it on decontaminated beef. Recent studies from our laboratory have used meat decontamination runoff waste fluids of different pHs (acidic, acid-diluted, or nonacid-water spray washings) as a model system to evaluate responses of *E. coli* O157:H7 under conditions simulating those in meat plant environments (33, 34, 35, 37, 42). Similar to the case for fresh meat (6), acid adaptation enhanced survival of *E. coli* O157:H7 in acetic-containing washings stored at 4 or 10°C for up to 14 days (35). Under all conditions tested, nonadapted cell populations declined faster than acid-adapted cell populations, while declines increased as the acid concentration (percent) in the washings and the temperature of storage increased (35). At equal acid concentrations (percent) in the washings, declines of acid-adapted and nonadapted populations of *E. coli* O157:H7 were more dramatic in the presence of lactic acid than in the presence of acetic acid (33, 35). Conversely, in water meat washings *E. coli* O157:H7 survived without growth at 4°C and with minor, if any, growth at 10°C (33, 34, 35). Compared to acid-adapted cells, nonadapted cells of *E. coli* O157:H7 showed a greater potential for survival and a tendency to initiate growth in nonacid water meat washings at 10°C (35), while a temperature increase to 15°C had a major accelerating effect on pathogen growth, irrespective of acid adaptation (42).

Additional studies evaluated resistance of *E. coli* O157:H7 to lactic or acetic acid (pH 3.5 or 3.7) after habituation in water or in acid-containing meat washings at 10°C (34, 37). We found that cell populations of *E. coli* O157:H7 recovered from acid-diluted meat washings of pH 3.5 to 4.7 that permitted survival at 10°C were resistant to pH 3.5 adjusted with lactic acid,

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especially if they were previously acid adapted (37). Subsequent acid resistance was always higher in *E. coli* O157:H7 populations habituated in acetic acid-diluted washings, which had a higher pH and permitted greater survival of the pathogen than in lactic acid washings diluted to the same ratio with water washings (37). In contrast, habitation of *E. coli* O157:H7 in water meat washings of neutral pH at 10°C dramatically sensitized the pathogen cells to subsequent lactic (pH 3.5) or acetic (pH 3.7) acid stress (34, 37). We verified that sensitization to acid was not due to possible underestimation of plate counts following “clumping” or low recovery of acid-shocked *E. coli* O157:H7 cells on the enumeration media used (34, 37). Previous acid adaptation of the pathogen, meat nutrients and competitive natural flora present in the washings, and the 10°C storage temperature appeared to influence acid sensitization of *E. coli* O157:H7 (37). Therefore, in the present study, temperature variations and additional interfering factors in fresh meat environments were isolated in selected treatments, and their potential effects on *E. coli* O157:H7 responses to meat decontamination and postdecontamination acid stresses were evaluated.

**Materials and Methods**

Preparation of *E. coli* O157:H7 inocula. A rifampin-resistant (Rif<sup>R</sup>) (100 μg/ml) derivative of the meat outbreak *E. coli* O157:H7 strain ATCC 43895 was used. This strain was selected for use in the present and previous (33, 34, 35, 37) meat decontamination studies because of its high inherent acid resistance (5, 6); it was used because the Rif<sup>R</sup> derivative showed an acid resistance as high as that of its parental strain (36). The strain was activated by two consecutive transfers (30°C, 24 h) in 10 ml of Trypticase soy broth (BBL, Becton Dickinson Co., Sparks, MD) with 0.6% yeast extract (Difco, Becton Dickinson Co., Sparks, MD) (TSBYE). Non-acid-adapted and acid-adapted inocula were prepared by transferring 0.1 ml of the activated culture in 10 ml of glucose-free TSBYE (BBL) or glucose-free TSBYE with 1% added glucose (Sigma, St. Louis, MO), respectively, as described previously (35, 36). After 24 h of incubation at 30°C, the pH of the acid-adapted cultures was 5.0 ± 0.1 while the pH of nonadapted cultures was 6.8 ± 0.1. These cultures of *E. coli* O157:H7 were used to inoculate the meat washings.

Preparation of meat washings and inoculation. Water washings and 2% acetic acid washings were collected after spraying 2-kg portions of fresh, nondecontaminated top rounds of beef in a model spray washer, as described previously (35, 36). After 24 h of incubation at 30°C, the pH of the water washings and 2% acetic acid washings were measured using a digital pH meter (Accumet 50; Fisher Scientific, Houston, Tex.) equipped with a glass electrode (Hanna Instruments, Ann Arbor, Mich.). Colonies other than those of *E. coli* O157:H7 grown on countable TSAYE plates were tentatively characterized by simple rapid tests (32, 35). Colonies of *E. coli* O157:H7 shared a large, circular, and smooth appearance on TSAYE and TSAYE+rif, except for the reddish background in Rif<sup>R</sup> plates. Representative colonies were confirmed by an *E. coli* O157 latex agglutination test (Remel Diagnostic Reagents, Lenexa, Kan.).

**Assessment of acid tolerance.** Acid tolerance of *E. coli* O157:H7 strain ATCC 43895rif was assessed after 2 and 7 days of habituation at 4, 10, 15, or 25°C for all treatments. The challenge medium was TSBYE acidified to pH 3.5 with lactic acid (20–25% w/v; Sigma, St. Louis, MO) or acetic acid (20–25% w/v; Sigma, St. Louis, MO) prior to sterilization at 121°C for 15 min. Acetic acid was not used as an acidulant in this study because previous studies have shown rapid declines below the detection limit of *E. coli* O157:H7 when it is exposed to pH 3.5 with this acid (30, 36). Specifically, a 0.2-unit difference in challenge pH (3.5 to 3.7) of TSBYE acidified with acetic acid caused great variations in survival of acid-adapted and nonadapted cells of *E. coli* O157:H7 (34, 37). This suggested that reliability was limited. The challenge tests may be reduced as a result of slight differences in the pH in the volume of acetic acid added. Moreover, acetic acid acts primarily by its undissociated form, while the lower pH of lactic acid accounts more for the pH effects on bacteria (10, 30). Also, acetic acid may be partially lost during heat sterilization of challenge media due to its volatile nature. The pH of acidified TSBYE with lactic acid was checked after sterilization and before each use to be within 0.05 unit from the desired pH. 3.5. Acidified TSBYE was tempered at 25°C for 30 min and kept at this temperature during acid challenging. This involved addition of 1 ml of culture from each treatment to 9 ml of challenge medium. It was assumed that the initial populations of natural flora and *E. coli* O157:H7 at exposure to pH 3.5 corresponded with the TSAYE and TSAYE+rif counts, respectively, reduced by 1 log to account for the 1:9 dilution in acidified TSBYE. Growth of acid challenge bacteria was carried out in direct plate counts following “clumping” or low recovery of acid-adapted *E. coli* O157:H7 at 30°C for 24 h, and bacterial counts were determined on Salmonella-Shigella agar with 0.1% sodium thiosulfate (Sigma), 0.1% otherwise (37). Bacteria were exposed to pH 3.5 for 120 min, after which 1-ml samples were taken, serially diluted in 9 ml of 0.1% buffered peptone water, and plated on TSAYE or TSAYE+rif to determine populations of acid survivors of natural flora and *E. coli* O157:H7, respectively. Colonies on plates were enumerated after incubation at 30°C for 48 h and were characterized, as described above.

**Statistical analyses.** Two independent experiments were conducted, with two samples analyzed per replicate. Microbiological data were converted to log CFU per milliliter. Preliminary statistical analysis of fixed effects by using the mixed-model procedure of SAS (88) indicated that log CFU-per-milliliter populations were dependent on type of culture medium (TSAYE or TSAYE+rif). Therefore, data were reanalyzed separately using a 2 (inoculum type) by 5 (storage time) by 4 (washing type) by 4 (temperature) by 2 (replicate) factorial design (see Table 3). All statistical analyses were conducted with the SAS for fixed effects and for all interactions. Least-squares means in populations were separated by the analysis of variance mixed-model procedure of SAS (38). All differences were reported at a significance level of alpha = 0.05.
RESULTS

Behavior of habituated E. coli O157:H7 in meat washings. Acid-adapted cells survived better ($P < 0.05$) than nonadapted cells in acetic acid washings (pH 3.6 ± 0.1), while survival of both types of cells was greatest at 4°C and lowest at 25°C (Table 1). Overall, survival of E. coli O157:H7 in acetic acid washings was lower ($P < 0.05$) than that in nonacidic treatments, which included water meat washings (pH 7.2 ± 0.2), sterile water meat washings (pH 7.4 ± 0.2), and saline (pH 7.1 ± 0.2). As expected, no growth of E. coli O157:H7 occurred in saline at all storage temperatures, due to an absence of nutrients (Table 1). Thus, the saline treatment represented a typical starvation stress similar to those applied by others with studies on the growth potential of the natural flora (Table 2). Acid adaptation did not affect ($P > 0.05$) growth of E. coli O157:H7 in water meat washings at 15°C (Table 1), although its populations never reached those of the natural flora (Table 2). Acid adaptation did not affect ($P > 0.05$) growth of E. coli O157:H7 in water meat washings at 15°C, irrespective of acid adaptation. Moreover, acid-adapted cells declined by 0.8 to 2.7 log cycles in all water meat washings at 10°C.

TABLE 1. Changes in populations of inoculated Escherichia coli O157:H7 strain ATCC 43895rif+ in fresh meat decontamination washings and sterile saline during storage at different temperatures

| Treatment                        | Log CFU/ml ($n = 4$) after the indicated days of storage$^a$ |
|----------------------------------|-------------------------------------------------------------|
|                                  | 0       | 2       | 4       | 7       | 14      | 0       | 2       | 4       | 7       | 14      |
| Sterile saline                   |parens     |         |         |         |         |         |         |         |         |         |
| 4.9 A a                          | 4.9 A c  | 4.7 A d | 4.4 ABC d | 3.7 C d | 5.2 A a | 5.0 A c | 4.9 A cde| 4.6 ABC de| 3.9 BC d |
| 4.9 ABC a                       | 4.7 ABC d| 4.2 BCD d| 3.6 D g | 3.8 CD d| 5.2 A a | 5.1 A c | 5.0 AB cd| 4.9 AB cde| 4.8 AB c |
| 4.9 A a                         | 4.6 A d  | 4.7 A d | 5.0 A c  | 5.2 A a | 5.1 A c | 5.1 A cd| 5.1 A cd | 5.1 A c  |         |
| 4.9 ABC a                       | 5.0 AB cd| 4.9 ABC d| 4.8 AB d | 4.3 B d | 5.2 A a | 5.3 A c | 5.5 A bc | 5.4 A bc | 5.2 A bc |
| Sterile water meat washings      |         |         |         |         |         |         |         |         |         |         |
| 4.9 A a                         | 4.8 ABC d| 4.8 A d | 4.7 A d  | 5.0 A c  | 5.1 A c | 5.1 AB cd| 4.8 AB cde| 4.6 ABC cd|         |
| 4.9 ABC a                       | 4.7 ABC d| 4.4 BCD d| 4.0 CD e| 3.8 CD d| 5.2 A a | 5.0 A c | 5.0 AB cd| 5.2 AB cd| 5.3 A bc |
| 4.9 E a                         | 6.2 CD b | 7.1 ABC abc| 7.6 A a | 7.6 A a | 5.2 DE a| 6.4 BCD b| 7.2 BA a | 7.5 A a | 7.6 A a |
| 4.9 B a                         | 7.4 A a  | 7.4 A ab| 7.5 A a  | 7.5 A a | 5.2 BA a| 7.5 A a | 7.6 A a | 7.5 A a | 7.8 A a |
| Water meat washings              |         |         |         |         |         |         |         |         |         |         |
| 4.9 A a                         | 4.9 A c  | 4.8 A d | 4.8 A d  | 4.3 A cd| 5.2 A a | 5.1 A c | 4.9 A cde| 4.9 A cde| 4.7 A cd |
| 4.9 ABC a                       | 4.7 ABC d| 4.4 BCD d| 4.0 CD e| 4.1 B d| 5.2 A a | 5.0 A c | 4.8 AB cde| 4.8 AB cde| 4.5 AB cd |
| 4.9 A c                         | 5.6 ABC bc| 5.7 ABC bc| 5.8 AB bc| 5.9 AB b| 5.2 BA a| 6.2 A b | 6.2 A b | 6.3 A b | 6.0 A b |
| 4.9 B a                         | 7.4 A a  | 7.5 A a | 7.6 A a  | 6.8 A a | 5.2 B a | 7.6 A a | 7.6 A a | 7.5 A a | 7.2 A a |
| Acetic acid meat washings        |         |         |         |         |         |         |         |         |         |         |
| 4.9 A a                         | 4.8 ABC d| 4.8 A d | 4.6 ABC d| 3.8 CD d| 5.2 A a | 4.9 A c | 4.7 AB cde| 4.1 BC ef | 2.9 D c |
| 4.9 ABC a                       | 4.9 ABC c| 4.7 ABC d| 4.3 B d  | 2.9 D ef| 5.2 A a | 4.7 ABC d| 4.2 BC c | 3.4 CD fg | 1.4 E f  |
| 4.9 A a                         | 4.8 ACE d| 4.5 ABC d| 3.9 CD fg| 2.3 E f | 5.2 A a | 4.6 ABC e| 4.1 BC e | 3.2 DE g  | <1.0 F g  |
| 4.9 A a                         | 3.6 B c  | 2.1 C d  | <1.0 D h | <1.0 D g| 5.2 A a | 2.6 CD f | <1.0 D h | <1.0 D h  | <1.0 D h  |

$^a$ Means with different uppercase letters in the same row are significantly different ($P < 0.05$); means with different lowercase letters in the same column are significantly different ($P < 0.05$). Standard deviations range from 0.0 to 1.8.
acid washings stored at 4°C, most of the colonies grown on TSAYE plates (Table 2) were surviving cells of *Escherichia coli* O157:H7, since yeasts and lactic acid bacteria did not increase under refrigeration. In sterile meat washings and saline, populations on TSAYE (data not shown) were similar to those on TSAYE+rif (Table 1), confirming that rifampin did not affect the behavior of strain ATCC 43895rif+ (36).

The pH (data not shown in tabular form) of saline decreased slightly from 7.1 at day 0 to 6.8 by day 14. The pHs of all water washings at 4 and 10°C of heat-sterilized water washings at 15 and 25°C fluctuated between 6.4 and 7.4 from day 2 to 14. The pHs of water washings at 15 and 25°C increased from 7.2 to 7.7, evidently due to the predominance of *Pseudomonas*-like bacteria. The pHs of acetic acid washings ranged from 3.5 to 3.8 at all incubation temperatures.

**Resistance of *E. coli* O157:H7 to a subsequent acid stress.** Survival patterns of *E. coli* O157:H7 exposed to pH 3.5 following habituation for 2 or 7 days in meat washings or saline at 4, 10, 15, and 25°C were significantly different (*P < 0.05*), depending on treatment, habituation time, and, mainly, habituation temperature (Table 3). Specifically, cells starved in saline for 2 days could resist pH 3.5. Their survival was greater (*P < 0.05*) at 4°C than at 25°C and was intermediate at 10 and 15°C, while it was not enhanced by previous acid adaptation (*P > 0.05*) at any temperature. Thus, based on these findings, a 2-day period of starvation under refrigeration seemed to en-

### TABLE 2. Changes in populations of natural flora in meat decontamination washings inoculated with *Escherichia coli* O157:H7 strain ATCC 43895rif+ and stored at different temperatures

| Treatment                     | Storage temp (°C) | Log CFU/ml (n = 4) after the indicated days of storagea |
|-------------------------------|-------------------|--------------------------------------------------------|
|                               |                   | Acid-adapted inoculum | Nonadapted inoculum |
|                               |                   | 0  | 2  | 4  | 7  | 14 | 0  | 2  | 4  | 7  |
| Water meat washings           |                   | 4  | 4.9 C a | 4.9 C c | 4.9 C b | 6.5 B b | 8.0 A a | 5.1 C a | 5.1 C bc | 5.1 C b | 6.7 B b | 7.9 A a |
|                               |                   | 10 | 4.9 C a | 5.3 C bc | 7.4 B a | 8.4 A a | 8.7 A a | 5.1 C a | 5.1 C bc | 7.4 B a | 8.4 A a | 8.6 A a |
|                               |                   | 15 | 4.9 C a | 6.7 B b | 8.4 A a | 8.6 A a | 8.7 A a | 5.1 C a | 6.5 B b | 8.4 A a | 8.7 A a | 8.5 A a |
|                               |                   | 25 | 4.9 B a | 8.3 A a | 8.7 A a | 8.8 A a | 8.2 A a | 5.1 B a | 8.5 A a | 8.6 A a | 8.7 A a | 8.6 A a |
| Acetic acid meat washings     |                   | 4  | 4.9 A a | 4.9 A c | 4.8 A bc | 4.6 A c | 4.0 AB c | 5.1 A a | 4.9 A c | 4.6 A b | 4.1 AB c | 2.9 B c |
|                               |                   | 10 | 4.9 A a | 4.9 A c | 4.8 A bc | 4.3 AB cd | 5.6 A b | 5.1 A a | 4.8 A c | 4.6 AB b | 3.4 B c | 5.6 A b |
|                               |                   | 15 | 4.9 B a | 4.8 B c | 4.7 B bc | 4.3 B cd | 4.8 B bc | 5.1 A a | 4.6 B cd | 4.9 B b | 5.4 B bc | 7.1 A ab |
|                               |                   | 25 | 4.9 AB a | 3.7 ABC c | 4.0 ABC c | 3.3 BC d | 3.9 ABC c | 5.1 A a | 3.1 C d | 3.2 C c | 3.7 AB c | 4.0 ABC c |

* Means with different uppercase letters in the same row are significantly different (*P < 0.05*); means with different lowercase letters in the same column are significantly different (*P < 0.05*). Standard deviations range from 0.0 to 3.4.

### TABLE 3. Surviving populations of *Escherichia coli* O157:H7 strain ATCC 43895rif+ after exposure to pH 3.5 with lactic acid

| Treatment                | Storage temp (°C) | Log CFU/ml (n = 4)b |
|--------------------------|-------------------|---------------------|
|                          |                   | Acid-adapted inoculum | Nonadapted inoculum |
|                          |                   | 0  | 2  | 4  | 7  | 14 | 0  | 2  | 4  | 7  |
| Sterile saline           |                   | 4  | 3.9 A c | 3.3 A b | 3.4 A cde | 2.1 B c | 4.0 A d | 3.3 A b | 3.6 A cd | 2.1 B cd |
|                          |                   | 10 | 3.7 AB c | 2.6 B c | 2.7 BC e | 1.1 D d | 4.1 A d | 2.7 BC e | 3.9 A c | 2.3 Cd |
|                          |                   | 15 | 3.6 AB cd | 2.4 B c | 4.0 A bc | 2.0 CD d | 4.1 A d | 2.8 BC c | 4.1 A cd | 2.7 BC c |
|                          |                   | 25 | 4.0 A c | 1.6 CD e | 3.8 AB bcd | 2.5 CD e | 4.3 A d | 1.6 D d | 4.4 A bc | 2.9 BC c |
| Sterile water meat washings |                   | 4  | 3.8 A c | <1.0 B e | 3.7 A d | <1.0 B e | 4.1 A d | <1.0 B e | 3.8 A cd | <1.0 B f |
|                          |                   | 10 | 3.7 AB c | <1.0 B e | 3.0 B d | <1.0 C e | 4.2 A cd | <1.0 C e | 4.2 A c | <1.0 C f |
|                          |                   | 15 | 5.2 B b | 1.9 C cd | 6.6 A a | 6.1 AB a | 5.4 B b | 2.1 C cd | 6.5 A a | 6.2 AB a |
|                          |                   | 25 | 6.4 A a | 5.9 A a | 6.5 A a | 6.3 A a | 6.5 A a | 6.0 A a | 6.5 A a | 6.4 A a |
| Water meat washings       |                   | 4  | 3.9 A c | <1.0 B e | 3.8 A bcd | <1.0 B e | 4.1 A d | <1.0 B e | 3.9 A cd | <1.0 B f |
|                          |                   | 10 | 3.7 A c | <1.0 B e | 3.2 A d | <1.0 B e | 4.0 A d | <1.0 B e | 3.8 A cd | <1.0 B f |
|                          |                   | 15 | 4.6 ABC bc | 2.1 CD d | 4.8 ABC c | 4.1 C b | 5.2 AB bc | 2.2 CD c | 5.3 A b | 4.2 BC b |
|                          |                   | 25 | 6.4 AB a | 5.5 BC a | 6.6 A a | 5.9 ABC a | 6.6 A a | 5.3 C a | 6.5 A a | 5.8 ABC a |
| Acetic acid meat washings |                   | 4  | 3.8 A c | <1.0 B e | 3.6 A cde | <1.0 C e | 3.9 A d | 1.5 B d | 3.1 A de | 1.1 B e |
|                          |                   | 10 | 3.9 A c | 1.2 D d | 3.3 AB cde | 1.3 D d | 3.7 A d | 1.6 CD d | 2.4 BC e | 1.3 D c |
|                          |                   | 15 | 3.8 A c | 2.6 BC bc | 2.9 ABC de | 1.4 CD d | 3.6 AB d | 2.4 CD bcd | 2.2 CE d | 1.4 D de |
|                          |                   | 25 | 2.6 A d | 1.1 B d | <1.0 C f | <1.0 C e | 1.6 AB e | <1.0 C f | <1.0 C f | <1.0 C f |

a Populations exposed to pH 3.5 were acid-adapted or nonadapted *E. coli* O157:H7 inoculated with or without growth in meat decontamination washings or sterile saline for 2 or 7 days of storage at 4, 10, 15, or 25°C.
b Means with different uppercase letters in the same row are significantly different (*P < 0.05*); means with different lowercase letters in the same column are significantly different (*P < 0.05*). Standard deviations range from 0.0 to 1.5.
hance survival of \textit{E. coli} O157:H7 after a subsequent acid stress (pH 3.5). This survival pattern was, however, reversed upon a more extended 7-day period of starvation in saline; while the acid resistance of cells starved at 25°C increased, that of cells starved at 4°C decreased. Acid-adapted cells starved in saline at 10°C for 7 days had the lowest (\(P < 0.05\)) survival at pH 3.5 (Table 3). This, however, was due to their greater (\(P < 0.05\)) death compared to other cells starved in saline for 7 days (Table 1) and not because they became more sensitive to pH 3.5.

Acid-adapted and nonadapted \textit{E. coli} O157:H7 habituated in water meat washings for 2 or 7 days showed major (\(P < 0.05\)) differences in survival at pH 3.5 compared to cells starved in saline (Table 3). These differences occurred despite the fact that both washings and saline had a neutral pH. The most prominent response was that nongrowing \textit{E. coli} O157:H7 cells in water washings at 4 or 10°C became very acid sensitive after 2 days and continued to be so after 7 days. This acid sensitization was evident for cells from all water washings at 4 or 10°C, irrespective of previous acid adaptation or presence of natural flora (Table 3). The only exception was the nonadapted cells in one of the replicates, which, as mentioned above, grew only in heat-sterilized water washings after 14 days at 10°C. When those cells were exposed to pH 3.5, they survived at 1.9 log CFU/ml (data not shown). Consistent with this finding, acid-adapted and nonadapted \textit{E. coli} O157:H7 cells grown in the water meat washings at 15 or 25°C were relatively the most resistant to pH 3.5 (Table 3). It should be stressed that \textit{E. coli} O157:H7 cell populations after 2 days at 15°C showed greater reductions at pH 3.5 than those at 25°C (Table 3). This was probably because cells were still in exponential phase in the washings on day 2 (Table 1) but were not on day 7, when they showed increases in resistance similar to those for cells grown at 25°C (Table 3). Previous acid adaptation of cells grown in water washings at 15 and 25°C did not influence their subsequent acid resistance. Also, the dominant growth of natural flora (Table 2) and the resulting increases in pH did not appear to induce an acid sensitization in \textit{E. coli} O157:H7 at 15 and 25°C (Table 3).

After 2 days of habituation in acetic acid washings at 4°C, acid-adapted survivors were the most sensitive at pH 3.5 (\(P < 0.05\)) and continued to be so after 7 days (Table 3). Based on net log cycle population reductions, day 7 survivors from acetic acid washings were more resistant to pH 3.5 in the order 15°C > 10°C > 4°C, with nonadapted cells being slightly more resistant than acid-adapted cells. These responses of \textit{E. coli} O157:H7 to a subsequent lactic acid stress after 2 or 7 days of habituation in acidic meat washings (Table 3) were opposite to their responses to the initial acetic acid stress. Indeed, as mentioned above, survival in acetic acid washings was increased in the order 4°C > 10°C > 15°C > 25°C, with acid-adapted cells always being more resistant than nonadapted cells (Table 1). Since \textit{E. coli} O157:H7 died off after 7 days of habituation in acetic acid washings at 25°C, evaluation of its subsequent acid resistance to pH 3.5 was not possible.

**DISCUSSION**

Of particular interest was the finding that absence of growth of \textit{E. coli} O157:H7 in water meat washings of neutral pH resulted in sensitization of the cells to a subsequent lethal acid stress, while growth was required for the cells to maintain or restore a high acid resistance. Growth of \textit{E. coli} O157:H7 in water washings was affected primarily by the storage temperature. Naturally, it was absent at 4°C, was occasional and delayed at 10°C, and was significant at ≈15°C, confirming our previous data (33, 34, 35, 37). The type and concentration of nutrients are known to have a strong influence on the growth potential of \textit{E. coli} O157:H7 at incubation temperatures ranging from 5.5 to 9.5°C (22). Also, the minimum growth temperature of many \textit{E. coli} O157:H7 strains under optimal culturing conditions in broth is 8°C (28). This temperature limit may increase to approximately 10°C under low-nutrient conditions (22) and with competition by the natural flora in meat washings. The results further indicated that when environmental temperatures are close to 10°C, acid adaptation may reduce the ability of \textit{E. coli} O157:H7 to maintain or restore acid resistance by reducing its potential for growth in nonacid meat decontamination washings.

The acid sensitization of \textit{E. coli} O157:H7 following habituation without growth in water washings at 4 and 10°C is difficult to explain. It appeared to be independent of the presence of natural flora, because it was also demonstrated in cells habituated in heat-sterilized water meat washings at 4 and 10°C. However, it was somehow enhanced by meat residual components in the washings, given that \textit{E. coli} O157:H7 cells starved in saline for 2 or 7 days at 4 or 10°C remained acid resistant. Acid sensitization was also correlated with cell population reductions of \textit{E. coli} O157:H7 under low-temperature and high-pH conditions in meat washings. Conner and Kotrola (15) reported similar declines in viability of \textit{E. coli} O157:H7 in normal TSBYE incubated at 4°C for up to 56 days. Arnold and Kaspar (1) reported on the ability of exponential-phase cells of \textit{E. coli} O157:H7 strain ATCC 43895 to increase their acid tolerance upon starvation in phosphate-buffered saline (PBS) at 25°C but not at 4°C, where a 100-fold decrease in viability occurred at 48 h. Death in PBS at 4°C was accompanied by a >10-fold to 100-fold loss in viability upon a subsequent exposure of starved \textit{E. coli} O157:H7 survivors to an acid challenge in gastric fluid (pH 1.5) (1). Based on the results of a study by Broeze et al. (8), Arnold and Kaspar (1) suggested that the inability of \textit{E. coli} O157:H7 to synthesize proteins at below 8°C might have been responsible for its death in PBS at 4°C and subsequent acid sensitization. Indeed, addition of chloramphenicol in PBS at 25°C to prevent protein synthesis sensitized the pathogen to acid (1). Thus, the slow death and acid sensitization of \textit{E. coli} O157:H7 starved in saline and in water meat washings at 4 and 10°C (Tables 1 and 3) may have been due to an inability of the pathogen cells to synthesize proteins.

Several studies have reported that low temperatures and acid adaptation enhance survival of \textit{E. coli} O157:H7 in acidic foods (6, 14, 24, 26). However, few studies have evaluated the potential for survival of acid-stressed or acid-adapted \textit{E. coli} O157:H7 in situ in foods after a subsequent food- or host-related acid stress (37, 43). Most of the existing knowledge on this issue is from studies with laboratory media (4, 15, 20, 23, 25). Lin et al. (25) reported that once induced, acid resistance mechanisms of \textit{E. coli} O157:H7 remain active for prolonged periods of cold storage at 4°C. In addition, Conner and Kotrola (15) emphasized that loss of viability of \textit{E. coli} O157:H7 at 4°C...
was significantly greater in TSBYE of neutral pH than in TS-BYE acidified with organic acids. Elhanafi et al. (20) reported that cold (4°C) and cold and mildly acidic (4°C; pH 5.5 with lactic acid) stresses significantly decreased subsequent tolerance of E. coli O157:H7 to pH 2.0 with HCl in TSB or to simulated gastric fluid (pH 1.5); however, a larger decrease in acid tolerance was observed after cold stress than after cold-acid stress (20). Likewise, there was a larger decrease in acid resistance of E. coli O157:H7 after habitation in water meat washings than after habitation in organic acid-diluted meat washings at 10°C (37) (Table 3). This study, however, did not confirm potential increases in subsequent acid resistance of acetic-acid-habituated cells at 4°C compared to those habituated at 10 and 15°C. In contrast, as mentioned above, resistance of acetic-acid-habituated cells to pH 3.5 increased in the order 15°C > 10°C > 4°C, despite the fact that their resistance to acetic acid during acid habitation in the washings was the opposite (Table 1). This reversal in acid resistance of E. coli O157:H7 may be associated with the ability of cells to maintain synthesis of acid shock proteins and increases in the proportions of saturated fatty acids in their membranes (1, 25, 45) to cope with sequent acid stresses when habitated at temperatures above, but not below, their minimum growth limit. Additional studies including evaluation of potential alterations at the cellular level are required to elucidate these acid stress responses of E. coli O157:H7.

Potential implications. Based on these results, a potential for a rapid loss of acid resistance by E. coli O157:H7 at temperatures of ≤10°C may exist under commercial conditions. This may happen particularly when acid decontamination interventions with meat are not employed or if use of such acid-based interventions is limited in favor of hot water sprays, which can also inactivate or remove bacteria from the meat surface (12, 39, 41). Since residual killing or inhibitory effects of hot water sprays during storage of decontaminated meat or in washings are essentially absent (18, 41), potential adaptation and resistance development by long-term exposure of E. coli O157:H7 cells to acid could be prevented. It should be stressed, however, that acid responses of pathogens in model systems, such as meat washings or slurries, do not necessarily reflect those that could be expressed on solid meat surfaces (44). Moreover, constant maintenance of meat at temperatures ≤10°C to induce acid sensitization in E. coli O157:H7 is not always feasible in commercial meat processing environments. For example, decontamination sprays are generally applied under ambient temperatures, while water-decontaminated or untreated meat may support growth of E. coli O157:H7 under abusive storage conditions during further processing or at home. Under these conditions, the meat background flora may retard, but it cannot completely inhibit, growth of E. coli O157:H7 (27), while, according to this study, the pathogen may be able to restore acid resistance following its growth on fresh meat. Thus, both immediate and residual antimicrobial effects of organic acid decontamination sprays may be more useful for controlling E. coli O157:H7 upon storage of acid-treated meat under potentially abusive temperatures rather than under refrigeration. Overall, controlling E. coli O157:H7 in the meat industry will continue to be difficult, because acid stress responses of this pathogen are still poorly understood or are unpredictable (14, 29, 31). Therefore, irrespective of decontamination, fresh meat should be refrigerated promptly, followed by adequate cooking, to ensure safety and consumer health (40).

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