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Research Note

Stationary-Phase Acid Resistance and Injury of Recent Bovine *Escherichia coli* O157 and non-O157 Biotype I *Escherichia coli* Isolates†

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ABSTRACT

Stationary-phase acid resistance and the induction of acid resistance were assessed for recent bovine carcass isolates of *Escherichia coli,* including 39 serotype O157 strains and 20 non-O157 strains. When grown to stationary phase in the absence of glucose and without prior acid exposure, there was a range of responses to a pH challenge of 6 h at pH 2.5. However, populations of 53 of the 59 *E. coli* isolates examined were reduced by less than 2.00 log CFU/ml, and populations of 24 of these isolates were reduced by less than 1.00 log CFU/ml. In contrast, there was little variation in population reductions when the *E. coli* were grown with glucose and preadapted to acidic conditions. With few exceptions, acid adaptation improved survival to the acid challenge, with 57 of the 59 isolates exhibiting a log reduction of less than 0.50. Differences in acid resistance or the ability to adapt to acidic conditions between *E. coli* O157:H7 and non-O157 commensal *E. coli* were not observed. However, we did find that the *E. coli* O157 were disposed to greater acid injury after the low pH challenge than the non-O157 *E. coli,* both for cells that were and were not adapted to acidic conditions before the challenge. The enhancement of low pH survival after acid adaptation that was seen among these recent natural isolates of *E. coli* O157 further supports the idea that the previous environment of this pathogen should be a consideration when designing microbial safety strategies for foods preserved by low pH and acid.

Acid resistance of *Escherichia coli* O157:H7 and other foodborne pathogenic bacteria has been an active research area, because of the effect this resistance may have on the ability of these organisms to survive in acidic foods or to survive acid food processing and preservation treatments and to ultimately be ingested and potentially cause disease. *E. coli* O157:H7 foodborne infections have been transmitted most often via raw or undercooked beef and milk *(13, 15, 19, 29)*. However, outbreaks of *E. coli* O157 disease also have been associated with apple cider, fermented sausage, yogurt, and mayonnaise-based dressings, which are products that, all or in part, rely upon low pH and acid for preservation and safety *(5, 14, 36*; as cited in *48)*. Survival for extended time has been reported for this organism in acidic foods *(11, 22, 24, 33, 35, 42, 48)*. Furthermore, prior adaptation to acid or low-pH environments may improve the survival of *E. coli* O157 in acid foods *(30, 45)*. In addition, acid adaptation may provide cross-protection against the adverse effects of heat, irradiation, various preservatives, and other processes that are used to inactivate and inhibit microorganisms in foods *(7–9, 23, 40, 41)*.

We have previously demonstrated that both the degree of acid resistance of *E. coli* O157:H7 and the adaptation to acidic conditions by this pathogen can negatively affect the ability of acetic acid spray washes to reduce this organism in beef carcass tissue *(4)*. The group of *E. coli* O157 strains that were examined in the previous study were a small, generally well-studied collection primarily composed of clinical and ground beef isolates. The objective of the current work was to determine the variation of acid resistance and acid adaptability among those *E. coli* O157 strains that are likely to contaminate beef carcasses, by assessing the acid resistance characteristics of recent bovine isolates of this pathogen. Recent bovine isolates of non-O157 biotype I *E. coli* were included in these analyses, for purposes of comparison.

MATERIALS AND METHODS

***E. coli* isolates.** All *E. coli* O157 and non-O157 isolates (39 and 20 strains, respectively; Table 1) were recovered from commercially slaughtered beef carcasses during or after the carcass dressing process. The isolation of the *E. coli* O157 strains has been described elsewhere *(21)*, and selected isolates were used in the current study. Similar sponge and swab procedures were used to sample carcasses for generic *E. coli* during processing at a local cow and bull processing facility. The samples were transported to

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TABLE 1. *Populations expressed as log CFU/ml of stationary-phase* E. coli *O157 and non-O157* E. coli *grown with and without 1% glucose after 0 and 6 h of exposure to pH 2.5*[a]

E. coli strain, serotype, and/or PFGE subtype[b]	Length of exposure (h)	Cells cultured with 1% glucose		Cells cultured without glucose	
		TSA[c]	SMAC[c]	TSA	SMAC
1A-5, non-O157	0	6.82 (0.19)	6.87 (0.06)	6.84 (0.07)	6.86 (0.06)
	6	6.63 (0.03)	6.33 (0.30)	6.64 (0.16)	6.23 (0.23)
97AC1, O157, subtype 1d	0	6.77 (0.09)	6.58 (0.18)	6.70 (0.03)	6.72 (0.08)
	6	6.77 (0.07)	6.15 (0.48)	6.40 (0.04)	5.51 (0.15)
258AC1, O157, subtype 44	0	6.60 (0.13)	6.66 (0.24)	6.62 (0.08)	6.79 (0.08)
	6	6.20 (0.35)	5.02 (0.47)	6.26 (0.45)	5.12 (0.82)
3A-1, non-O157	0	6.62 (0.07)	6.83 (0.14)	6.68 (0.06)	7.00 (0.05)
	6	6.55 (0.05)	6.29 (0.30)	6.27 (0.06)	5.92 (0.35)
5A-1, non-O157	0	6.59 (0.10)	6.76 (0.14)	6.74 (0.05)	6.95 (0.07)
	6	6.58 (0.02)	6.20 (0.18)	6.30 (0.05)	5.98 (0.06)
294RC1, O157, subtype 7a	0	6.77 (0.12)	6.77 (0.03)	6.79 (0.11)	6.52 (0.24)
	6	6.69 (0.02)	6.44 (0.31)	6.33 (0.64)	5.57 (0.58)
3C-3, non-O157	0	6.82 (0.16)	7.01 (0.13)	6.93 (0.28)	7.05 (0.11)
	6	6.68 (0.04)	6.82 (0.15)	6.42 (0.10)	6.62 (0.17)
28RC1, O157, subtype 17	0	7.01 (0.06)	6.88 (0.14)	6.58 (0.03)	6.66 (0.05)
	6	6.96 (0.12)	6.73 (0.17)	6.06 (0.16)	5.18 (0.17)
233AC1, O157, subtype 6d	0	6.68 (0.06)	6.55 (0.16)	6.65 (0.06)	6.67 (0.05)
	6	6.62 (0.08)	5.99 (0.19)	6.11 (0.10)	5.37 (0.20)
127AC2, O157, subtype 39c	0	6.82 (0.22)	6.72 (0.08)	6.56 (0.04)	6.65 (0.08)
	6	6.70 (0.03)	6.17 (0.21)	5.99 (0.33)	5.25 (0.22)
96AC1, O157, subtype 8b	0	6.88 (0.22)	6.64 (0.08)	6.72 (0.04)	6.65 (0.18)
	6	6.73 (0.02)	6.24 (0.44)	6.13 (0.10)	5.39 (0.18)
52AC2, O157, subtype 14b	0	6.65 (0.05)	6.66 (0.09)	6.58 (0.05)	6.66 (0.07)
	6	6.61 (0.04)	6.07 (0.10)	5.98 (0.10)	4.84 (0.22)
1C-3, non-O157	0	7.22 (0.17)	7.15 (0.03)	7.14 (0.23)	7.16 (0.07)
	6	6.74 (0.08)	6.60 (0.10)	6.54 (0.04)	6.23 (0.04)
239PoC1, O157, subtype 29a	0	6.64 (0.15)	6.46 (0.26)	6.62 (0.02)	6.64 (0.13)
	6	6.48 (0.05)	4.76 (0.50)	5.90 (0.44)	4.99 (0.50)
3A-2, non-O157	0	6.70 (0.07)	6.88 (0.12)	6.99 (0.22)	7.01 (0.07)
	6	6.60 (0.09)	6.21 (0.21)	6.26 (0.05)	5.95 (0.06)
1A-2, non-O157	0	6.95 (0.28)	6.98 (0.07)	6.83 (0.09)	6.98 (0.14)
	6	6.79 (0.08)	6.53 (0.32)	6.08 (0.06)	5.82 (0.02)
281AC1, O157, subtype 46b	0	7.15 (0.03)	6.90 (0.11)	6.67 (0.03)	6.74 (0.07)
	6	7.12 (0.10)	6.47 (0.19)	5.81 (0.13)	4.66 (0.15)
143AC1, O157, subtype 8c	0	6.69 (0.06)	6.62 (0.28)	6.64 (0.11)	6.64 (0.06)
	6	6.65 (0.04)	4.51 (0.10)	5.74 (0.03)	4.66 (0.15)
2A-1, non-O157	0	6.73 (0.04)	6.97 (0.07)	6.94 (0.17)	7.09 (0.25)
	6	6.76 (0.06)	6.53 (0.08)	6.04 (0.17)	5.88 (0.28)
3B-2, non-O157	0	7.03 (0.04)	7.03 (0.06)	7.05 (0.23)	7.07 (0.07)
	6	6.67 (0.12)	6.65 (0.39)	6.13 (0.05)	6.02 (0.03)
185AC2, O157, subtype 4c	0	6.68 (0.02)	6.69 (0.04)	6.66 (0.02)	6.74 (0.06)
	6	6.64 (0.02)	5.34 (0.34)	5.72 (0.20)	4.85 (0.36)
2A-3, non-O157	0	6.74 (0.05)	6.91 (0.08)	7.06 (0.08)	7.10 (0.06)
	6	6.60 (0.04)	6.07 (0.18)	6.08 (0.13)	5.74 (0.09)
1D-4, non-O157	0	6.86 (0.15)	7.12 (0.11)	6.84 (0.25)	7.07 (0.07)
	6	6.83 (0.24)	6.72 (0.12)	5.86 (0.15)	5.94 (0.27)
1A-4, non-O157	0	7.05 (0.04)	7.13 (0.06)	7.10 (0.13)	7.09 (0.04)
	6	6.71 (0.06)	5.67 (0.68)	6.11 (0.02)	5.52 (0.30)
2A-4, non-O157	0	7.03 (0.27)	7.11 (0.04)	6.86 (0.27)	7.01 (0.06)
	6	7.00 (0.22)	7.07 (0.08)	5.84 (0.20)	5.51 (0.14)
94AC2, O157, subtype 25	0	6.88 (0.33)	6.73 (0.08)	6.63 (0.04)	6.69 (0.06)
	6	6.78 (0.18)	5.86 (0.94)	5.60 (0.14)	4.94 (0.22)
1B-3, non-O157	0	7.03 (0.12)	7.03 (0.18)	7.22 (0.13)	7.36 (0.18)
	6	6.66 (0.08)	5.35 (0.73)	6.16 (0.05)	5.48 (0.25)
344AC4, O157, subtype 34	0	6.73 (0.06)	6.72 (0.11)	6.62 (0.05)	6.61 (0.05)
	6	6.87 (0.05)	6.56 (0.08)	5.55 (0.08)	4.65 (0.06)

TABLE 1. *Continued*

E. coli strain, serotype, and/or PFGE subtype[b]	Length of exposure (h)	Cells cultured with 1% glucose		Cells cultured without glucose	
		TSA[c]	SMAC[c]	TSA	SMAC
245RC1, O157, subtype 26	0	6.94 (0.31)	6.73 (0.16)	6.71 (0.32)	6.71 (0.13)
	6	6.76 (0.04)	5.49 (1.15)	5.64 (0.33)	4.36 (0.61)
326RC3, O157, subtype 13b	0	6.69 (0.06)	6.67 (0.10)	6.57 (0.07)	6.59 (0.14)
	6	6.68 (0.03)	4.49 (0.38)	5.47 (0.17)	3.62 (0.17)
315RC3, O157, subtype 46b	0	7.12 (0.11)	6.86 (0.31)	6.65 (0.08)	6.76 (0.11)
	6	7.00 (0.24)	6.37 (0.21)	5.55 (0.10)	4.59 (0.16)
134AC2, O157, subtype 40	0	7.23 (0.06)	7.09 (0.05)	6.79 (0.19)	6.82 (0.03)
	6	7.10 (0.11)	6.47 (0.27)	5.66 (0.07)	4.68 (0.16)
08AB1, O157, subtype 32	0	6.77 (0.06)	6.65 (0.05)	6.60 (0.07)	6.69 (0.09)
	6	6.70 (0.06)	6.17 (0.03)	5.47 (0.02)	4.66 (0.04)
236AC1, O157, subtype 38	0	6.78 (0.04)	6.73 (0.16)	6.70 (0.07)	6.59 (0.10)
	6	6.67 (0.02)	6.22 (0.18)	5.54 (0.08)	4.38 (0.36)
338AC1, O157, subtype 39a	0	7.15 (0.12)	6.79 (0.11)	6.64 (0.07)	6.67 (0.04)
	6	6.84 (0.06)	6.19 (0.19)	5.47 (0.05)	4.41 (0.06)
241AC1, O157, subtype 6b	0	7.19 (0.04)	6.71 (0.09)	6.53 (0.07)	6.48 (0.01)
	6	6.85 (0.02)	6.39 (0.06)	5.33 (0.05)	4.19 (0.23)
1E-1, non-O157	0	6.90 (0.18)	7.17 (0.23)	6.86 (0.17)	7.13 (0.08)
	6	6.64 (0.02)	7.13 (0.10)	5.66 (0.03)	5.66 (0.14)
313AC1, O157, subtype 47b	0	7.16 (0.08)	7.02 (0.06)	6.68 (0.01)	6.76 (0.03)
	6	7.00 (0.14)	6.19 (0.28)	5.47 (0.10)	4.23 (0.27)
346AC1, O157, subtype 29c	0	6.97 (0.16)	6.87 (0.06)	6.65 (0.04)	6.78 (0.02)
	6	6.81 (0.01)	6.40 (0.19)	5.42 (0.07)	4.73 (0.11)
245AC1, O157, subtype 10	0	7.16 (0.12)	6.64 (0.18)	6.69 (0.11)	6.69 (0.03)
	6	6.94 (0.02)	6.48 (0.03)	5.44 (0.03)	4.29 (0.03)
1C-1, non-O157	0	7.06 (0.24)	6.94 (0.32)	7.04 (0.17)	7.12 (0.11)
	6	6.88 (0.10)	6.99 (0.02)	5.77 (0.05)	5.72 (0.06)
132AC3, O157, subtype 18	0	6.79 (0.07)	6.66 (0.12)	6.63 (0.08)	6.68 (0.03)
	6	6.64 (0.03)	5.35 (0.91)	5.35 (0.05)	4.29 (0.07)
150AC2, O157, subtype 30b	0	6.59 (0.03)	5.76 (0.27)	6.41 (0.08)	6.20 (0.04)
	6	6.46 (0.19)	4.28 (0.10)	5.09 (0.29)	4.21 (0.54)
349AC1, O157, subtype 9b	0	6.82 (0.13)	6.70 (0.08)	6.69 (0.06)	6.74 (0.08)
	6	6.58 (0.04)	5.85 (0.33)	5.36 (0.07)	4.49 (0.09)
345AC3, O157, subtype 36	0	7.01 (0.19)	6.77 (0.06)	6.66 (0.06)	6.71 (0.03)
	6	6.85 (0.12)	6.42 (0.03)	5.33 (0.06)	4.63 (0.13)
2E-3, non-O157	0	6.98 (0.21)	7.16 (0.03)	7.09 (0.10)	7.18 (0.09)
	6	6.67 (0.05)	6.77 (0.28)	5.74 (0.07)	5.97 (0.06)
271AC1, O157, subtype 41	0	6.76 (0.10)	6.79 (0.07)	6.58 (0.08)	6.71 (0.09)
	6	6.78 (0.02)	5.72 (0.46)	5.16 (0.17)	4.22 (0.41)
198AC1, O157, subtype 33	0	6.75 (0.02)	6.85 (0.11)	6.63 (0.06)	6.67 (0.11)
	6	6.60 (0.04)	5.74 (0.33)	5.21 (0.07)	4.26 (0.05)
302RC1, O157, subtype 16	0	7.22 (0.06)	6.35 (0.17)	6.62 (0.05)	6.67 (0.06)
	6	7.07 (0.13)	5.50 (0.06)	5.14 (0.03)	3.49 (0.06)
263PoC1, O157, subtype 11	0	7.12 (0.21)	6.83 (0.07)	6.67 (0.03)	6.78 (0.11)
	6	6.73 (0.07)	5.68 (0.57)	5.12 (0.04)	4.28 (0.20)
214AC3, O157, subtype 23b	0	6.80 (0.04)	6.47 (0.34)	6.67 (0.20)	6.62 (0.14)
	6	6.64 (0.07)	4.49 (0.56)	5.09 (0.06)	3.43 (0.13)
324AC1, O157, subtype 22	0	7.24 (0.05)	6.76 (0.12)	6.67 (0.07)	6.62 (0.01)
	6	6.90 (0.03)	6.08 (0.14)	5.02 (0.11)	3.99 (0.05)
348AC1, O157, subtype 24	0	6.74 (0.03)	6.51 (0.07)	6.47 (0.16)	6.42 (0.06)
	6	6.71 (0.14)	3.95 (1.08)	4.52 (0.05)	2.15 (0.54)
317RC5, O157, subtype 3	0	7.07 (0.11)	6.87 (0.04)	6.79 (0.04)	6.83 (0.13)
	6	6.75 (0.09)	4.88 (0.40)	4.76 (0.38)	3.31 (0.17)
263AC1, O157, subtype 8e	0	6.71 (0.05)	6.64 (0.14)	6.64 (0.03)	6.64 (0.13)
	6	6.65 (0.04)	4.23 (0.50)	4.39 (0.02)	3.40 (0.19)

TABLE 1. *Continued*

E. coli strain, serotype, and/or PFGE subtype[b]	Length of exposure (h)	Cells cultured with 1% glucose		Cells cultured without glucose	
		TSA[c]	SMAC[c]	TSA	SMAC
340AC3, O157, subtype 28	0	7.11 (0.05)	6.91 (0.23)	6.87 (0.21)	6.71 (0.14)
	6	6.80 (0.04)	6.07 (0.10)	3.66 (0.06)	3.14 (0.12)
5B-2, non-O157	0	7.08 (0.11)	7.07 (0.11)	7.03 (0.20)	7.11 (0.13)
	6	6.80 (0.13)	6.24 (0.22)	3.53 (0.13)	4.09 (0.88)
3D-1, non-O157	0	7.07 (0.35)	6.90 (0.08)	7.27 (0.13)	7.20 (0.08)
	6	1.89 (0.69)	<1.30[d]	1.31 (0.02)	<1.30
3D-3, non-O157	0	7.07 (0.13)	6.83 (0.20)	7.12 (0.10)	7.17 (0.03)
	6	<1.30	<1.30	<1.30	<1.30

[a] Values are means of two independent trials with all determinations done in duplicate. Values in parentheses are standard deviations.
[b] PFGE subtype designations of Barkocy-Gallagher et al. *(3).*
[c] TSA, tryptic soy agar; SMAC, MacConkey sorbitol agar.
[d] The minimum level of detection was 1.30 log CFU/ml.

the laboratory for immediate processing and plating onto *E. coli*/coliform Petrifilm plates (3M Microbiology Products, St. Paul, Minn.). After incubation at 35°C for 24 h, five characteristic *E. coli* colonies (blue colonies with gas production) were selected from each positive sample and streaked for isolation onto tryptic soy agar (TSA; Difco, Becton Dickinson Microbiology Systems, Sparks, Md.). All isolates were subcultured minimally before freezing at −20°C in 30% glycerol stocks.

Biochemical and genetic characterization of *E. coli* isolates. The biochemical, serological, and genetic characteristics of the *E. coli* O157 bovine isolates have been described elsewhere *(21).* All of the *E. coli* O157 strains were shown to be H7-positive or to carry $fliC_{H7}$, as well as at least one *stx* gene, *eae,* and $hlyA_{EHEC}$ *(3, 21).* These *E. coli* O157 isolates were analyzed by *Xba*I pulsed-field gel electrophoresis (PFGE) genotyping *(3).* The 39 *E. coli* O157 isolates examined in the current study were chosen as representatives of unique PFGE subtype groups.

As part of a separate study, the generic *E. coli* beef carcass isolates described above were examined by PFGE genotyping of *Not*I-digested genomic DNA (G. A. Barkocy-Gallagher, G. R. Siragusa, and M. Koohmaraie, unpublished data). The 20 isolates used in the present study represent 20 different PFGE subtype groups. These isolates were characterized and confirmed as *E. coli* biotype I using API 20E microbial identification kits (bioMérieux, Inc., St. Louis, Mo.) and by the demonstration of lactose fermentation with gas production, indole production, the inability to utilize citrate as a sole carbon source, negative Voges-Proskauer reaction results, and positive methyl red reaction results *(27).* The *E. coli* biotype I isolates were screened using Oxoid *E. coli* O157 latex test reagents and gave negative agglutination reactions (Oxoid Limited, Basingstoke, UK). These non-O157 *E. coli* isolates were further screened by PCR for the presence of the enterohemorrhagic *E. coli* (EHEC) and *E. coli* O157 genes, including stx_1, stx_2, *eae,* EHEC *hlyA,* and $rfbE_{O157}$, using the primers and multiplex PCR assay described by Paton and Paton *(38).*

Determination of stationary-phase acid resistance characteristics of *E. coli.* The pH-dependent and -independent stationary-phase acid resistance of both O157 and non-O157 *E. coli* were determined essentially as described by Buchanan and Edelson *(6).* To produce stationary-phase cells that were adapted and not adapted to low pH, cells were cultivated in tryptic soy broth (TSB) with 1% glucose and without glucose, respectively, at 37°C for 18 h. The final pH of cultures grown in TSB that contained glucose was 4.3 to 5.0, whereas the final pH of cultures grown in TSB without glucose was 6.2 to 6.9.

For the acid-challenge experiments, 0.1-ml volumes of the 18-h cultures were inoculated into 10-ml volumes of brain heart infusion broth that had been adjusted to pH 2.5 with concentrated HCl (BHI-2.5) *(6).* The BHI-2.5 tubes were incubated statically at 37°C. Viable cell numbers were determined at 0 and 6 h after serially diluting the BHI-2.5 samples, if necessary, in buffered peptone water and plating in duplicate on both TSA and MacConkey sorbitol agar (SMAC), using a model D spiral plater (Spiral Systems Instruments, Bethesda, Md.). Experiments were duplicated on separate days for both acid-adapted and nonadapted cells of each O157 and non-O157 *E. coli* strain.

Statistical analysis. Bacterial populations were converted to log CFU per ml values before statistical analyses. The Welch's corrected unpaired *t* test was performed on log reductions and log-reduction differences, using the InStat statistical analysis package (version 3.00; GraphPad Software, Inc., San Diego, Calif.).

RESULTS AND DISCUSSION

In a previous study, we examined the effect of the acid adaptation of *E. coli* O157:H7 on the ability of 2% acetic acid spray washes to reduce levels of this pathogen on beef carcass surface tissue *(4).* To identify strains for use in that previous work, a small collection of *E. coli* O157:H7 isolates was screened to assess their stationary-phase acid resistance and ability to induce acid tolerance, using the same procedures used in the current work. On the basis of the degrees of survival and injury of stationary-phase cells (grown both with and without glucose) after the exposure to pH 2.5 for 6 h, the *E. coli* O157:H7 strains were categorized as either acid resistant, acid adaptable, or acid sensitive *(4).* We found that differences in acid resistance among different isolates and adaptation to acidic conditions by an individual adaptable isolate were both detectable when comparing *E. coli* O157:H7 populations that remained on beef tissue after acetic acid spray washing. The *E. coli* O157:H7 isolates used in our previous work primarily were well-characterized laboratory and type strains. In the current study, we sought to evaluate the stationary-phase acid resistance and adaptation characteristics of a

larger collection of *E. coli* O157 that had been more recently isolated from cattle.

Populations of stationary-phase *E. coli* O157 and non-O157 *E. coli* grown in the presence and absence of 1% glucose after 0 and 6 h of exposure to pH 2.5 are summarized in Table 1. Our primary means of comparing the inherent acid resistances of the *E. coli* strains was to compare their stationary-phase responses to a 6-h pH challenge after growth in TSB without glucose, thereby comparing their pH-independent acid resistance. In Table 1, the *E. coli* strains are ranked in descending order from the greatest resistance (least log reduction) to the least resistance (greatest log reduction), as calculated from populations determined on TSA of *E. coli* cultured without glucose. There was no obvious correlation between subtype cluster and acid resistance. When the log reductions of these stationary-phase, non–acid-adapted cells are compared, there is a range of acid resistance responses. Twenty-four of the 59 *E. coli* strains were reduced by less than 1.0 log after 6 h of exposure to pH 2.5. An additional 29 isolates were reduced by greater than 1.0 log but less than 2.0 log. Only six isolates, which included three *E. coli* O157 and three non-O157 *E. coli,* were reduced by greater than 2.0 log by the acid challenge conditions, and only one isolate (non-O157 *E. coli* 3D-3) was reduced to below detectable levels in 6 h.

In contrast to the responses seen after cultivation without glucose, when grown in glucose and, therefore, adapted to acidic conditions, there was little variation in acid resistance among the *E. coli* strains, as determined on TSA (Table 1). With only two exceptions, all glucose-grown *E. coli* were strongly tolerant of the acid challenge: 57 of 59 isolates showed less than a 0.50-log reduction. The two exceptions were two non-O157 *E. coli,* strains 3D-1 and 3D-3, which were reduced by 5.2 log and to below detectable levels, respectively. Thus, with few exclusions, the preadaptation to acidic conditions was important to improved *E. coli* survival upon subsequent exposures to acid or low pH. In addition, and in accordance with our previous criteria, the large majority of the *E. coli* bovine isolates examined were categorized as acid resistant (*4*).

The reasons for the acid-sensitive phenotypes of non-O157 *E. coli* strains 3D-1 and 3D-3 are unknown. Greater sensitivity to low pH was exhibited by these two strains regardless of cultivation in glucose and resultant adaptation to acidic conditions, which suggests that these strains may have defects in *rpoS* or other *rpoS*-regulated genes that are involved in stationary phase–dependent acid resistance *(1, 16, 43).* Waterman and Small *(47)* reported the occurrence of mutant *rpoS* genes in recently isolated populations of Shiga toxin–producing *E. coli.* In addition to the acid resistance system that requires the alternative sigma factor RpoS, two other acid resistance systems have been described for *E. coli,* that have also been identified as active in stationary-phase cells (*12, 26, 31, 32*). One of these systems is glutamate dependent and is thought to use an inducible glutamate decarboxylase; the second of these systems is arginine dependent and uses an inducible arginine decarboxylase. It is possible that the greater range of acid challenge responses seen for *E. coli* that were cultivated without glucose is reflective of differing contributions of each of these separate acid resistance systems.

Among the 20 non-O157 *E. coli* isolates, PCR results showed that two isolates harbored some EHEC virulence factor genes. *E. coli* 1A-5 possesses the stx_2 gene, and *E. coli* 3A-1 possesses both stx_1 and stx_2 genes and the EHEC *hlyA* gene (data not shown). A recent report documented the common occurrence of *E. coli* bearing Shiga toxin genes and other virulence genes on commercially processed beef carcasses *(2).* When comparing log reductions of *E. coli* O157:H7 and non-O157 *E. coli* populations, we did not observe statistically significant differences in log reductions within either acid adaptation group (cultivated with or without glucose) on either medium (TSA or SMAC; $P > 0.05$). Prior to the current work, there have been few studies that have examined the acid resistance characteristics of appreciable numbers of nonpathogenic or generic strains of *E. coli (20, 25, 32).* However, most of the studies that have compared the acid resistances of these two groups of *E. coli* have found that both commensal and pathogenic strains of *E. coli* typically were similarly acid resistant *(6, 20, 25, 32).* Thus, the present results further emphasize that, although *E. coli* O157 can be highly acid tolerant, this characteristic is not unique to pathogenic strains of *E. coli.*

The only difference in acid resistance characteristics that we observed between the *E. coli* O157:H7 and non-O157 *E. coli* strains was related to the extent of injury suffered by the *E. coli* after the 6-h exposure to pH 2.5. Plating on both nonselective and selective media has been previously demonstrated as useful for appraising *E. coli* acid resistance in terms of both total viable as well as sublethally injured cells *(4, 6).* Both the percent injury and log reduction differences were calculated from bacterial counts on TSA and SMAC after the acid challenge [Table 2; log reduction difference = (log reduction calculated from colony counts on SMAC) − (log reduction calculated from colony counts on TSA)]. As measured by the inability of sublethally injured cells to form colonies on the selective SMAC, the *E. coli* O157 strains were more severely injured by the acid challenge than were the non-O157 *E. coli* strains (Table 2). For *E. coli* cultivated in glucose, 81% of the viable *E. coli* O157 strains were injured, and 38% of the viable non-O157 *E. coli* strains were injured. The log reduction differences for these two groups of *E. coli* were different ($P \leq 0.003$). Percent injury results for *E. coli* that were grown without glucose were similar; O157 and non-O157 *E. coli* were 89 and 36% injured, respectively, and the log reduction differences were different ($P \leq 0.0001$). Of interest, cultivation with or without glucose did not affect log reduction differences within either O157 or non-O157 *E. coli* ($P > 0.05$). To our knowledge, differences in the degree of injury imposed by low pH exposures between the two general groupings of pathogenic and commensal *E. coli* have not been reported. The acid resistance and adaptation of *E. coli* O157:H7 have been the topic of numerous studies, and some of these works have reported differences in the abilities of *E. coli* O157 and generic non-O157 *E. coli* to survive exposures to low pH *(1, 35).* However,

TABLE 2. *Mean percent injury and mean log reduction differences of 39* E. coli *O157 and 18 non-O157* E. coli *grown with and without 1% glucose after 6 h of exposure to pH 2.5, as indicated by the inability of injured cells to form colonies on selective SMAC compared with nonselective TSA*[a]

	Cells cultured with 1% glucose		Cells cultured without glucose	
E. coli group	% injury[b]	Log reduction difference[c]	% injury	Log reduction difference
O157	81	0.83	89	1.06
Non-O157[d]	38	0.39	36	0.33

[a] TSA, tryptic soy agar; SMAC, MacConkey sorbitol agar.

[b] % injury = [1 − (colony counts on SMAC after 6 h low pH exposure/colony counts on TSA after 6 h low pH exposure)] × 100. Negative percent injury values were valued as zero for calculation of mean percent injury.

[c] Log reduction difference = (log reduction calculated from colony counts on SMAC) − (log reduction calculated from colony counts on TSA). Negative log reduction differences were valued as zero for calculation of mean log reduction differences.

[d] Because of their atypical acid resistance response (see text), non-O157 *E. coli* strains 3D-1 and 3D-3 were excluded from these analyses, to avoid skewing the results.

as previously mentioned, most studies that have compared large numbers of strains of O157 and non-O157 *E. coli* have not reported differences in acid resistances *(6, 20, 25, 32)*.

The problems associated with the reduced ability to detect sublethally injured bacteria in foods are well recognized. For example, if a pathogen is sublethally injured and cannot be detected in a food product after a thermal or other preservation process, the process may be incorrectly assessed as adequate to eliminate the target pathogen. As another example, depending on the environment of the food, a previously undetected, injured pathogen may be able to repair itself and proliferate in the food that previously may have been tested and thought to be pathogen free. Foodborne bacteria may become acid-injured during fermentation processes or by the addition of various organic acids that are commonly used as food preservatives. The increased sensitivity of acid-injured *E. coli* to selective media containing bile salts or other agents has been reported for both *E. coli* O157 and nonpathogenic *E. coli (39, 44)*. In addition, considerable efforts have been made to develop media for the improved detection of injured *E. coli* O157 (e.g., see *(17, 28, 34)*). Any potential food safety consequences of differences in acid injury of *E. coli* O157 and commensal nonpathogenic *E. coli* are not known. However, these data imply that generic *E. coli* may be an appropriately conservative surrogate for challenge studies involving food preservation by acid, which was suggested by Duffy et al. *(20)* for salami fermentation.

The reasons for the differences in the degree of injury from acid exposure between the bovine isolates of O157 and non-O157 *E. coli* are unknown, but it will be interesting to learn whether this is also true of *E. coli* from other sources. Certainly, the differences may be related to distinct ecological specializations of these two groups of *E. coli*. For example, Naylor et al. *(37)* recently reported that *E. coli* O157:H7, but not other *E. coli* serotypes, specifically colonize a distinct region of the bovine gastrointestinal tract, because of a tropism for the mucosal tissue at the terminal rectum. However, although the pH and volatile fatty acid content of lower intestinal tract contents do vary depending on the composition of the cattle ration *(10, 18, 46)*, the pH of these contents typically are similar throughout the large intestine to the rectum of any given animal *(46)*. Further work will be needed to understand the biological relevance, if any, of the difference in acid injury between bovine O157 and non-O157 *E. coli*.

To summarize, and as would be expected given their natural residence in the mammalian gastrointestinal tract, the large majority of the 59 recent bovine isolates of both O157 and non-O157 *E. coli* were found to have the capacity to survive exposures to low-pH environments. In addition, we found little variation of acid resistance and adaptability among these isolates. Without adaptation to acidic conditions, 41% of the 59 stationary-phase *E. coli* were reduced by less than 1.0 log after an acid challenge of pH 2.5 for 6 h. With only two exceptions, cultivation in glucose, with its accompanying depression of pH and preadaptation of the cells, considerably improved the survival of the stationary-phase *E. coli*; populations of 57 of the 59 isolates were reduced by less than 0.5 log. When O157 and non-O157 *E. coli* were compared, log reductions of viable cells after the acid challenge were not different. However, the *E. coli* O157 were more highly acid-injured than were the non-O157 *E. coli*. That acid adaptation enhances the low-pH survival of the majority of bovine isolates of *E. coli* O157 underscores the concept that the previous environment of microbial pathogens should be a consideration when designing and testing safety and preservation systems for beef.

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