

Fate of Field-Isolated *Escherichia coli* O157 in Ground Beef at Different Storage Temperatures[†]

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ABSTRACT

The survival of six *Escherichia coli* O157 strains, including five strains recently isolated from beef carcasses and strain ATCC 43895, was evaluated at 0, 1, 7, and 14 days in ground beef held at -20, 1, 4, and 7°C. Only small losses in cell numbers occurred at -20 and 1°C; in general, cell numbers decreased during the first day of storage and then remained unchanged through day 14. At -20°C, statistically significant reductions in cell numbers were observed only for strains 55AC1 and 299AB3 due to greater losses in the first day. At 1°C, strain 131AC1 did not decrease in cell numbers during the first day of storage, but both this strain and strain 55AC1 experienced statistically significant reductions in viable cell numbers by day 14, primarily due to losses after day 7. At 4°C, after an initial loss of cell numbers for four strains, minor increases were observed for all six strains by day 14. The differences were statistically significant for strains 114AC1, 299AB3, and ATCC 43895, but were small enough to question whether they reflect actual growth. When the inoculated ground beef was stored at 7°C for 14 days, growth of all six strains was statistically significant, with populations increasing between 0.9 and 1.5 log₁₀ CFU/g. This study demonstrates that there are small differences in the abilities of various *E. coli* O157 strains to survive and sometimes grow in fresh ground beef at cold storage temperatures, but overall these differences do not appear to be meaningful. The differences cannot be attributed to recency of isolation, since strain ATCC 43895 behaved similarly to recently isolated strains. Storage temperatures of 4°C or below limited growth of *E. coli* O157 isolates, but did not have a noteworthy effect on survival.

Cattle are a major reservoir of *Escherichia coli* O157:H7, an organism associated with disease that is often due to consumption of undercooked ground beef. Recently, we recovered *E. coli* O157:H7 and nonmotile isolates (*E. coli* O157) from feces, hides, and carcasses at commercial beef processing plants (7). Fingerprinting of these isolates revealed substantial variation among their genomes (3). It has been suggested that genomic differences among *E. coli* O157:H7 isolates may correlate with their ability to cause disease in humans (9). However, the strains may not differ in virulence; instead, they may differ in their ability to arrive at the human host. For example, some strains may survive better during ground beef processing, storage, and distribution.

Ground beef is typically kept either frozen or refrigerated, but may be exposed to slightly higher "abusive" temperatures during storage or distribution. To date, researchers investigating growth of *E. coli* O157 in ground beef at low temperatures have used a variety of laboratory strains whose genomic differences were not determined (1, 2, 5, 6, 13–16). In addition, in most of these studies, the

strains were genetically altered, which could have affected their ability to compete with other microorganisms (1, 5, 6, 11–13, 16). Furthermore, survival and growth of recently isolated *E. coli* O157 strains may be different than that of strains analyzed after substantial passaging in the laboratory. The present study was designed to monitor the fate of five, genomically different, recently isolated *E. coli* O157 strains and strain ATCC 43895 in ground beef at probable storage temperatures (-20, 1, 4°C) and under potential temperature abuse conditions (7°C).

MATERIALS AND METHODS

Cultures and cell suspension. Six *E. coli* O157 strains were used in this study (Table 1), including strain ATCC 43895 and five strains recovered previously from beef carcasses before evisceration (7). The five "field" strains were genetically different from one another and have been passaged in the laboratory no more than 5 to 10 times. They represent the major subtypes within three relatedness clusters identified by genomic fingerprinting of the *E. coli* O157 isolates recovered at beef processing plants (3). Cluster B is the largest cluster and includes a large subcluster, designated B1. Cluster C is closely related to cluster B, whereas cluster A is more distantly related and includes the fewest isolates. *E. coli* O157 strain ATCC 43895 also was fingerprinted and assigned to cluster B1 (data not shown). Inocula were prepared by growing cultures at 37°C for 18 h in tryptic soy broth (TSB; Difco Laboratories, Detroit, Mich.), then diluting to reach approximately 6.0 log₁₀ CFU/ml.

Meat samples. Fresh ground beef (80% lean and 20% fat) was obtained from a local grocery store. The meat was divided

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[†] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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TABLE 1. *E. coli* O157 isolates used in this study^a

Isolate	Motility	<i>stx1</i>	<i>stx2</i>	Cluster	Subtype
55AC1	+	+	+	B (not B1)	23b
114AC1	+	-	+	B1	1d
131AC1	+	-	+	C	39c
237AC1	+	+	+	B1	6d
299AB3	-	-	+	A	46b
ATCC 43895	+	+	+	B1	ND

^a Cluster and subtype refer to designations in Barkocy-Gallagher et al. (3) and are based on genomic fingerprinting with relatedness analyses. B1 is a large subcluster within B. *stx1* and *stx2* are the genes encoding Shiga toxins 1 and 2, respectively. ND, not determined; +, positive; -, negative.

into 100-g patties, placed into sterile stomacher bags (Seward Medical, London, UK), and inoculated with 1 ml of diluted culture, resulting in approximately 4.0 log₁₀ CFU/g. The meat was kneaded for 3 min using gloved hands, then formed into a 2 by 17 by 7-cm patty. Samples were incubated in the bags at -20, 1, 4, or 7°C for up to 14 days.

Bacterial enumeration. Duplicate samples were tested after 0, 1, 7, and 14 days of storage. Frozen samples were thawed at room temperature for approximately 30 min before analysis. An equal volume of buffered peptone water (100 ml) was added to each patty and the samples were stomached (Stomacher 400, Seward Medical) for 2 min. *E. coli* O157 counts were determined by plating in duplicate on MacConkey sorbitol agar containing 0.5 mg/liter of cefixime and 2.5 mg/liter of potassium tellurite (Dynal Inc., Lake Success, N.Y.). Only those colonies with the pale, "fried-egg" morphologic structure typical of *E. coli* O157 were counted. Random positive colonies and colonies with questionable phenotypes were tested to confirm their identity using *E. coli* O157 DrySpot latex tests (Oxoid, Inc., Basingstoke, Hampshire, UK). Colony counts from duplicate plates were averaged.

Statistical analysis. Experiments were replicated three times. Least squares means of log₁₀ CFU/g were evaluated using the general linear model procedure of SAS statistical software (version 6.12, SAS Institute, Inc., Cary, N.C.). Statistically significant differences are defined as $P \leq 0.05$.

RESULTS AND DISCUSSION

Survival in ground beef was examined at four temperatures for six individual *E. coli* O157 strains (Table 1). At -20°C, essentially all of the decreases in cell numbers were small and occurred within the first day of storage, suggesting that the only lethal event was the initial cold shock (exposure to -20°C) and that *E. coli* O157:H7 is very cryotolerant. Minor differences were observed among the strains. Strains 55AC1 and 299AB3 suffered statistically significant losses in cell numbers: a reduction of 0.3 and 0.4 log₁₀ CFU/g, respectively, after 14 days of storage (Fig. 1). In addition, the log₁₀ reduction at day 14 for strain 55AC1 (0.5 log₁₀ CFU/g) was significantly greater than the log₁₀ reductions of all other strains except 299AB3; the log₁₀ reduction in strain 299AB3 (0.3-log₁₀ CFU/g decrease) was significantly different only than that of strain 114AC1 (0.0-log₁₀ CFU/g decrease).

Similar results were obtained by Uyttendaele et al.

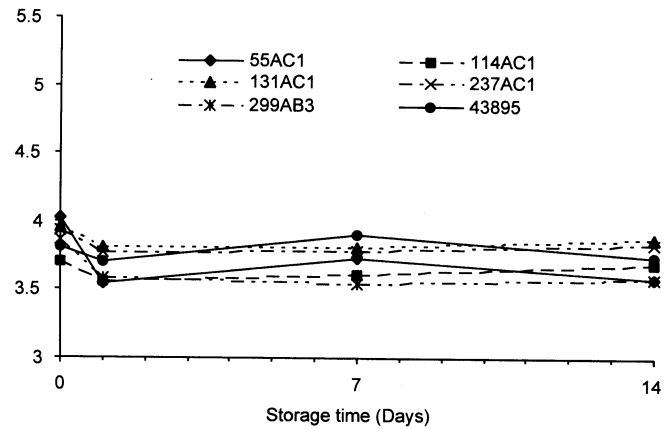


FIGURE 1. The growth patterns of individual *E. coli* O157 strains in ground beef at -20°C. The standard error of the least squares means was 0.1. The least significant difference was 0.3 ($P = 0.05$).

(15), who examined *E. coli* O157:H7 survival in ground beef stored at -18°C. In contrast, Sage and Ingham (14) reported up to a 2-log₁₀ decrease in *E. coli* O157:H7 counts from similarly inoculated ground beef stored at -20°C for 24 h. The evident differences in the latter study were the strains used, the thawing methods, and the plating media. The small but noticeable differences we found between strains suggest that strain differences could, at least in part, have accounted for the differing results. In addition, both thawing methods and plating media can have a notable effect on recovery of *E. coli* O157:H7 from frozen ground beef (4, 14). Our use of selective media was necessary because of the natural microflora present in the ground beef. Presumably, the use of nonselective media would have shown a slightly higher recovery of cells, reinforcing the cryotolerance of these *E. coli* O157:H7 strains.

Similar to the results obtained at -20°C, the log₁₀ reductions in *E. coli* O157 cell numbers at 1°C were small, and most of the loss in cell viability occurred within the first day, with two exceptions (Fig. 2). Strains 131AC1 and 55AC1 continued to decrease in cell numbers after day 7, with strain 131AC1 experiencing a more gradual decrease

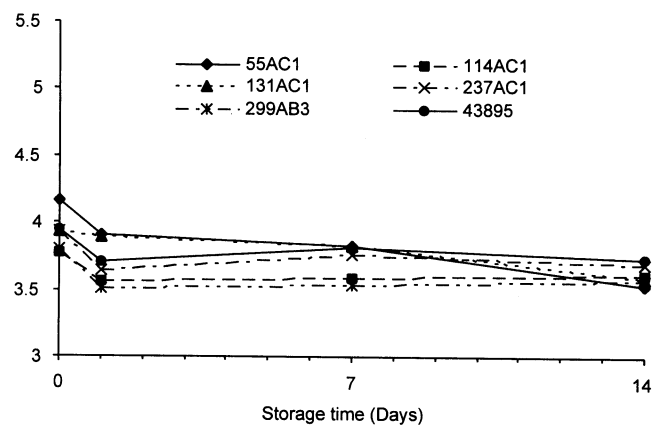


FIGURE 2. The growth patterns of individual *E. coli* O157 strains in ground beef at 1°C. The standard error of the least squares means was 0.1. The least significant difference was 0.3 ($P = 0.05$).

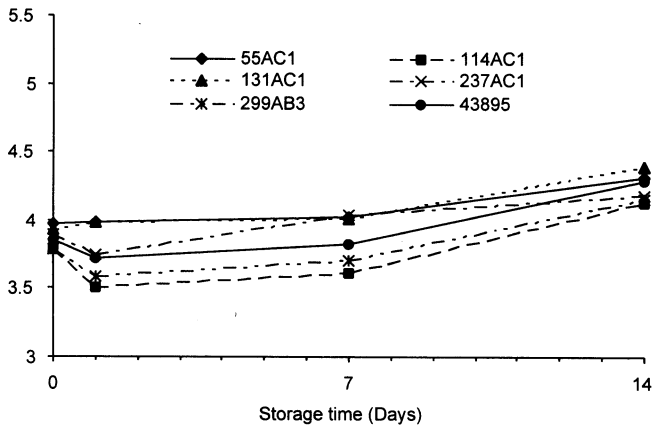


FIGURE 3. The growth patterns of individual *E. coli* O157 strains in ground beef at 4°C. The standard error of the least squares means was 0.2. The least significant difference was 0.5 ($P = 0.05$).

in cell numbers throughout the incubation period. Both of these strains suffered relatively small but statistically significant losses in cell numbers by day 14, with \log_{10} reductions of 0.3 and 0.6 CFU/g, respectively. Previously, Ajjarapu and Shelef (1) reported a somewhat larger reduction of approximately 1 \log_{10} CFU/g for *E. coli* O157:H7 in ground beef stored at 2°C for 18 days. However, direct comparisons between the studies cannot be made due to differences in ground beef storage time, experimental protocol, storage temperatures, and strains tested. In particular, *E. coli* O157 survival in the previous study could have been affected by the presence of the green fluorescent protein (GFP)-expressing plasmid in the *E. coli* O157:H7 strain tested. Strains with and without the plasmid had similar growth kinetics in Luria broth at 37 and 42°C (8), but there may still be differences in their growth at low temperatures and/or in ground beef. Furthermore, the presence of the plasmid could have affected the competitiveness of the bacteria (11).

Survival in ground beef also was examined for the six *E. coli* O157 strains at 4°C (Fig. 3). All six strains had a small overall increase in numbers by day 14, even though four of them appeared to experience minor decreases in cell numbers after 1 day (strains 114AC1, 237AC1, 299AB3, and ATCC 43895). The subsequent slight increases in cell numbers from day 1 to day 14 were statistically significant for strains 114AC1, 299AB3, and ATCC 43895, but are so small that they may not reflect meaningful growth. These data may differ from the results of Dorsa et al. (5), who showed a minor, statistically insignificant decrease in numbers of *E. coli* O157:H7 in ground beef stored for 14 days at 4°C. However, the streptomycin-resistance mutation in the strain used by Dorsa et al. (5) could have affected its ability to successfully compete with other organisms (12). Nevertheless, both studies agree that *E. coli* O157 survives well at 4°C.

When the inoculated ground beef was stored at 7°C for 14 days, all six *E. coli* O157 strains demonstrated statistically significant and substantial growth by day 14 (Fig. 4). Only strain 114AC1 demonstrated statistically significant

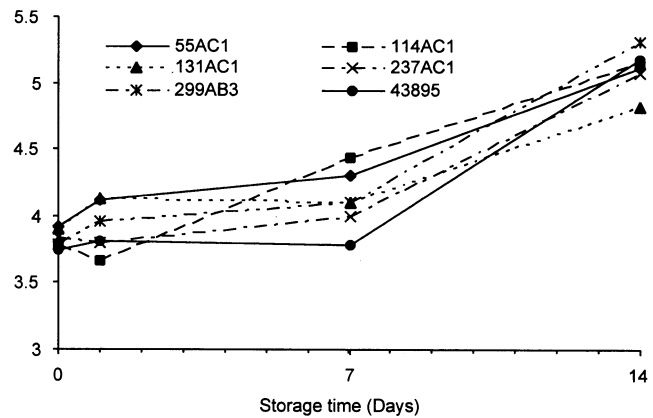


FIGURE 4. The growth patterns of individual *E. coli* O157 strains in ground beef at 7°C. The standard error of the least squares means was 0.2. The least significant difference was 0.5 ($P = 0.05$).

growth before day 7. The overall log increase in cell numbers by day 14 did not vary significantly among the strains; it ranged from 0.9 to 1.5 \log_{10} CFU/g for strains 131AC1 and 299AB3, respectively. In contrast, a slight decrease in *E. coli* O157 cell numbers was observed previously for a three-strain pool inoculated into ground beef and stored at 7°C for 11 days (15). We did not determine *E. coli* O157 counts between days 7 and 14, so the observed growth may have occurred largely after day 11.

The growth and survival of *E. coli* O157:H7 at low temperatures have also been examined by inoculation of irradiated ground beef. After storage at -20°C for 2 weeks, less than 0.5- \log_{10} CFU/g reductions in cell numbers were observed by Ansary et al. for three strains (2), which is similar to the results seen herein. These authors also reported a reduction of approximately 1 \log_{10} CFU/g by 14 days for a pool of strains inoculated into ground beef and stored at 2°C, which is a somewhat larger reduction than those presented herein for *E. coli* O157 in fresh ground beef stored at 1°C. The differences could be due to the effects of prior irradiation on the meat (1), the slightly different temperatures used, or strain differences. In a study by Palumbo et al. (13), rifampin-resistant strains were used to suggest that *E. coli* O157 survival or growth in ground beef at 5 and 8°C depended on low levels of competitive background microflora, in this case brought about by prior irradiation of the meat. However, the rifampin-resistance mutation could have made the cells more susceptible to competitive inhibition and therefore artificially less able to grow in fresh ground beef at low temperatures (10, 12). We did not determine total aerobic cell counts during our study, but preliminary work indicated that background flora levels (total mesophilic counts) in ground beef from the same store were similar to those reported by Palumbo et al. (13) (data not shown).

In summation, the growth and survival of *E. coli* O157 were examined in fresh, commercially available ground beef held at low temperatures reflective of storage or temperature abuse. The ground beef was inoculated with six individual *E. coli* O157 strains, five of which were field

strains recently isolated from bovine carcasses (7). Overall, the survival patterns for strain ATCC 43895 did not differ from those of the field strains. It had been hypothesized that the field strains might behave differently because of minimal passaging, since it is generally accepted that laboratory passaging may change phenotype and even minimal passaging can affect the *E. coli* O157 genome (3). Although it is still possible that *E. coli* O157 strains that have never been passaged in the laboratory may behave differently, these experiments suggest otherwise.

To some extent, the strains in this study behaved differently than each other, but the differences were small. On the whole, no one strain or genomic cluster stood out as being more or less successful in persisting at low temperatures. The three strains in subcluster B1 appeared to survive slightly better than the other strains at -20 and 1°C , but because of the number of strains tested, far-reaching conclusions are not warranted. In addition, strain 55AC1, which is the most closely related to the strains in cluster B1, demonstrated the poorest survival at these temperatures. Clearly, the data confirm that storage temperatures at or below 4°C are very important for limiting the growth of any *E. coli* O157 in ground beef and that freezing cannot be expected to eliminate the organism in this product.

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