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Fate of *Escherichia coli* O157:H7 as Affected by pH or Sodium Chloride and in Fermented, Dry Sausage

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The influence of pH adjusted with lactic acid or HCl or sodium chloride concentration on survival or growth of *Escherichia coli* O157:H7 in Trypticase soy broth (TSB) was determined. Studies also determined the fate of *E. coli* O157:H7 during the production and storage of fermented, dry sausage. The organism grew in TSB containing ≤6.5% NaCl or at a pH of 4.5 to 9.0, adjusted with HCl. When TSB was acidified with lactic acid, the organism grew at pH 4.6 but not at pH 4.5. A commercial sausage batter inoculated with 4.8×10^4 *E. coli* O157:H7 per g was fermented to pH 4.8 and dried until the moisture/protein ratio was ≤1.9:1. The sausage chubs were then vacuum packaged and stored at 4°C for 2 months. The organism survived but did not grow during fermentation, drying, or subsequent storage at 4°C and decreased by about 2 $\log_{10}$ CFU/g by the end of storage. These studies reveal the importance of using beef containing low populations or no *E. coli* O157:H7 in sausage batter, because when initially present at 10^4 CFU/g, this organism can survive fermentation, drying, and storage of fermented sausage regardless of whether an added starter culture was used.

Escherichia coli O157:H7, first recognized as a pathogen in 1982, is an important cause of hemorrhagic colitis and hemolytic-uremic syndrome. Several food-borne outbreaks of *E. coli* O157:H7 infection have been linked epidemiologically to the consumption of undercooked ground beef and raw milk, suggesting that dairy cattle may be a reservoir for this organism. *E. coli* O157:H7 has been isolated from retail ground beef, poultry, pork, and lamb (4) and from fecal samples of young animals from herds associated with cases of hemolytic-uremic syndrome (2, 7).

The influence of pH, NaCl, and $NaNO_2$ on the growth of enteropathogenic *E. coli* has been studied (5); however, there are no published data on the influence of acid conditions, NaCl concentration, or the environment of fermented, dry sausage on *E. coli* O157:H7. Previous studies (4, 10) revealed that the maximum temperature range for prolific growth of *E. coli* O157:H7 is <44.5°C, which is lower than that of most other *E. coli*.

The purpose of this study was to determine survival and growth characteristics of *E. coli* O157:H7 in culture medium at different NaCl concentrations or pH values (adjusted with HCl or lactic acid) and in fermented, dry sausage.

MATERIALS AND METHODS

Preparation of bacterial inocula. A five-strain mixture of *E. coli* O157:H7 (including 932 and CL8 [both human isolates], 933 [meat isolate], EC 204P [pork isolate], and EC 505B [beef isolate]) was used for all inoculation studies. Strains were grown individually in 10-ml Trypticase soy broth (TSB; BBL Microbiology Systems, Cockeysville, Md.) at 37°C for 16 h. Cells were sedimented at 2,500 × *g* for 20 min and then suspended in 10 ml of 0.1 M phosphate-buffered saline (PBS), pH 7.2. The optical density at 640 nm of the cell suspension was determined and adjusted to 0.5 (ca. 10^8 CFU/ml) with PBS. Approximately equal populations of each cell suspension were combined and diluted to appropriate populations with PBS, and *E. coli* O157:H7 counts were determined by plating the five-strain mixture on Trypticase soy agar (TSA).

Effect of sodium chloride and pH on growth of *E. coli* O157:H7 in broth. (i) NaCl. TSB (250-ml portions) was adjusted to seven different NaCl concentrations (0.5, 1.5, 2.5, 4.5, 6.5, 8.5, and 10.5% [wt/vol]). TSB (10 ml per tube) was dispensed in screw-cap tubes (165 by 16 mm) and autoclaved at 121°C for 20 min. Cultures were prepared as described above and adjusted to yield ca. 500 CFU/ml of TSB when inoculated with 0.1 ml of culture suspension. Inoculated tubes were incubated at 37°C. At appropriate times, cultures were serially (1:10) diluted in 0.1 M PBS, and *E. coli* O157:H7 was enumerated in duplicate by surface plating on TSA. At each sampling time, pH was also determined. Sampling was discontinued after growth reached the stationary phase or after cells could no longer be detected by direct plating (<10 CFU/ml). Generation times (or no growth or death) were derived from the exponential growth rate and determined in triplicate.

(ii) pH. TSB (250-ml portions) was adjusted to appropriate pH values, using 85% lactic acid (pH 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0), 6 N HCl (pH 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0), or 5 M NaOH (pH 7.5, 8.0, 8.5, and 9.0). Untreated TSB (pH 7.3) was used as the control. To minimize changes in pH that occur during autoclaving, media were filter sterilized by passage through 0.2-μm-pore-size filter flask units (Nalgene sterilization filter units with nylon membrane; Nalge Company, Rochester, N.Y.), allowed to equilibrate for 24 h at 37°C, and then dispensed (10 ml per sterile screw-cap tube). TSB at pH <5.0 was inoculated with 5×10^4 *E. coli* O157:H7 CFU/ml, whereas broth at pH ≥5.0 was inoculated at ca. 500 *E. coli* O157:H7 CFU/ml. Cultures were incubated at 37°C and assayed as described above for the NaCl study.

Effect of fermentation and drying on *E. coli* O157:H7 in a fermented, dry sausage. (i) Bacterial cultures. A dextrose-

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fermenting *Pediococcus acidilactici* bacterial culture (LACTACEL 115; Microlife Technics, Sarasota, Fla.) was used as the starter culture and was added to the sausage batter at levels recommended by the manufacturer (ca. 10^7 CFU/g). A five-strain mixture of *E. coli* O157:H7 was prepared as described above.

(ii) Inoculation and processing of sausage. Sausage batter (provided by a sausage manufacturer) was prepared from meat (23% beef–77% pork) with a target fat content of 22%. Batter (27.2-kg batch) included glucose (170 g), commercial spice premix (816 g), and a commercial cure mixture (508 g), which was formulated to provide 156 μg of $NaNO_2$ per g and 3.5% NaCl in the batter. The meat was inoculated with ca. 5×10^4 CFU of *E. coli* O157:H7 (five-strain mixture in 272 ml of 0.1 M PBS per 27.2 kg of meat) per g and mixed (Buffalo model 2VSS mixer; John E. Smith's Sons Co., Buffalo, N.Y.) for 3 min. About one-third of the sausage batter was removed from the mixer to be used to prepare sausage without starter culture, and LACTACEL 115 starter culture (10.2 g of resuspended culture in 94 ml of sterile H_2O per 20.2 kg of batter) was added to the remaining batter and mixed for an additional 3 min. Fibrous sausage casing (5.5-cm diameter; Vista International Packaging Inc., Kenosha, Wis.) was made pliable by soaking in hot water (ca. 50°C for 20 min). Excess water was squeezed manually from the casings, which were then stuffed with batter by using a hand stuffer (F. Dick, Koch Supplies, Inc., Kansas City, Mo.) and tied by hand (ca. 300-g chubs). Sausages were hung in an environmentally controlled room (Biotron Facility, University of Wisconsin—Madison) and processed by heating at 15.6°C for 1 h, at 21.1°C for 1 h, and then at 35.6°C until the product with starter culture reached pH 4.8 (ca. 13 to 14 h). After fermentation, the sausage was tempered to 24 to 32°C. Sausages were held at 12.8°C and 70% relative humidity until the final moisture/protein ratio of the sausage was ≤1.9:1 (ca. 18 to 21 days) (13). Sausages were positioned ≥30 cm apart to allow for proper airflow to prevent mold growth, case shriveling, and case hardening. After drying, sausages were individually vacuum packaged in gas-impermeable Curlon bags (nylon-Saran-polyethylene; O_2 transmission rate, 0.8 to 1.0 cm^3 of O_2 per 645 cm^2 per 24 h at 22.8°C; CO_2 transmission rate, 2.5 to 3.0 cm^3 of CO_2 per 645 cm^2 per 24 h; H_2O transmission rate, 0.5 cm^3 of H_2O per 645 cm^2 per 24 h [all at 37.8°C and 90% relative humidity]; Curwood, Inc., New London, Wis.), using a Multivac AGW vacuum packaging unit (Sepp Haggemuller KG, Wolfertschwenden, Germany) and held at 4°C for 8 weeks.

(iii) Microbiological and chemical analysis of sausage. Three samples of sausage with starter culture and two samples of sausage without starter culture were taken at each sampling time and tested for (i) *E. coli* O157:H7 by direct plating and enrichment, (ii) pH, and (iii) titratable acidity. Sampling times included (i) immediately after stuffing, (ii) at the end of fermentation (when sausage with starter culture reached pH 4.8), (iii) after 10 h of drying, (iv) approximately every 3 days after drying was initiated, and (v) every 2 weeks for up to 8 weeks during storage at 4°C. Sausage casings were wiped with 70% ethanol and cut with a sterile scalpel. The casing and outer 1 cm were removed with a sterile forceps. Meat (25 g) was sampled from different areas within the sausage for microbial analysis. The remainder of the sausage was ground through a 4.76-mm plate of a Hobart grinder (model 84142; Hobart Manufacturing Co., Troy, Ohio) and mixed well for pH, titratable acidity, and other chemical analyses.

Samples were assayed for presence of *E. coli* O157:H7 by being mixed with 225 ml of modified TSB (30 g of Trypticase soy broth [BBL], 1.5 g of bile salts no. 3 [Difco Laboratories, Detroit, Mich.], 1.5 g of K_2PO_4, 10 g of Casamino acids [Difco] [9]) and serially (1:10) diluted in 0.1 M PBS, after which 0.1 ml was plated on MacConkey sorbitol agar with 0.1 g of 4-methylumbelliferyl-β-D-glucuronide (MUG; Sigma Chemical, St. Louis, Mo.) per liter and incubated overnight (16 to 18 h, 37°C). Acriflavin (10 mg/liter) was added to the remaining modified TSB, and the sample was incubated overnight (16 to 18 h) on a shaker (150 rpm) at 37°C for enrichment. Direct plate counts on MacConkey sorbitol agar-MUG (presumptive colonies of *E. coli* O157:H7 were sorbitol negative and MUG fluorescence negative) were screened by the *E. coli* O157:H7 latex agglutination test (Unipath Oxoid US, Columbia, Md.) before streaking onto TSA. Isolates were confirmed as *E. coli* O157:H7 by a direct enzyme-linked immunosorbent assay (10 colonies per sample), using a monoclonal antibody specific for *E. coli* O157:H7 (9), and by the API-20E miniaturized diagnostic test (Analytab, Div. of Sherwood Medical, Plainview, N.Y.). Randomly selected colonies were confirmed serologically with O157 and H7 antisera (Difco). Results reported are averages of duplicate trials.

Titratable acidity and pH were determined according to the procedure described by Sebranek (12). A 25-g sample was macerated by a Stomacher with 225 ml of hot (ca. 70°C) distilled water until well blended. Samples were poured into a 250-ml Erlenmeyer flask and allowed to cool to room temperature, and the fat layer was removed by pipetting before measurements of the pH of the slurry (Orion 8163 combination pH probe with Corning 140 pH meter; Corning Glass Works, Corning, N.Y.). The homogenate was filtered through Whatman no. 1 filter paper, and filtrate (100 ml) was titrated with 1.001 N NaOH to pH 8.1. Titratable acidity (TA) was calculated by using the following equation: %TA = [(N of titrant × ml of titrant × meq wt of acid) × 100]/g of sample (where N is normality). Titratable activity was expressed as percent lactic acid; the milliequivalent weight of lactic acid is 0.09.

The following analyses were done on samples immediately after stuffing and at the end of the drying cycle: (i) moisture (Association of Official Analytical Chemists [AOAC] procedure 950.46 [8]), (ii) salt (AOAC volumetric method 935.47, measuring chlorine as sodium chloride [8]), (iii) fat (AOAC procedure 960.39 [8]), and (iv) protein (semi-microkjeldahl [11]). Other analyses done immediately after stuffing included lactic acid bacteria count (MRS agar, 1-ml pour plate, incubated at 30°C for 48 h) and nitrite (analyzed several hours after addition of cure mixture to batter at the manufacturer; AOAC colorimetric method 973.31 [8]).

RESULTS

Effect of NaCl. *E. coli* O157:H7 is inhibited by NaCl in TSB at concentrations of ≥8.5% (Table 1). Cells grew vigorously in NaCl concentrations of up to 2.5% (generation times ranging from 0.4 to 0.5 h), whereas at 4.5% NaCl, the doubling time was about threefold longer (ca. 1.6 h). *E. coli* grew in 6.5% NaCl, although slowly, with a generation time of 31.7 h after a 36-h lag phase. Cells in 6.5% NaCl grew to a maximum population of ≤7 $\log_{10}$ CFU/ml (from an initial inoculum of 500 CFU/ml) before gradual death occurred. *E. coli* O157:H7 was inactivated (2-$\log_{10}$-CFU/ml reduction within 96 h) in 8.5 and 10.5% NaCl.

Effect of pH. *E. coli* O157:H7 grew well (doubling time, <0.5 h) in TSB acidified with HCl at levels as low as pH 5.0 (Table 2). At pH 4.5, the generation time of the organism

TABLE 1. Growth of *E. coli* O157:H7 in TSB at different NaCl concentrations and incubated at 37°C

Total NaCl (%)	Generation time[a] (h)	Lag time (h)
Control (0.5)	0.4	≤2
1.5	0.4	≤2
2.5	0.5	2
4.5	1.6	7
6.5	31.7[b]	36
8.5	RED_{96h}[c]	ND[d]
10.5	RED_{96h}	ND

[a] Average of three trials.
[b] Maximum population, ≤7 $\log_{10}$ CFU/ml within 21 days.
[c] RED_{96h}, 2-$\log_{10}$-CFU/ml reduction within 96 h.
[d] ND, not determined.

increased to 0.8 h after a lag period of 4.7 h, whereas at pH 4.0 and 3.5, the population was inactivated (4-$\log_{10}$-CFU/ml reduction) within 17 and 10 days, respectively. Lactic acid had a greater inhibitory effect on *E. coli* O157:H7 at an equivalent pH than did HCl. At all corresponding pH values below 6.5, the organism grew consistently more slowly in TSB acidified with lactic acid than in TSB acidified with HCl. TSB adjusted with lactic acid to pH 4.6 supported limited growth (2-$\log_{10}$-CFU/ml increase in 24 h after a 12-h lag phase) in two separate trials (data not shown). At pH 4.5, cells in the presence of lactic acid were inactivated to populations undetectable by direct plating (4-$\log_{10}$-CFU/ml reduction) in ≤14 days. No viable cells were detected within 7 days or 24 h in broth acidified with lactic acid to pH 4.0 or 3.5, respectively.

E. coli O157:H7 grew in alkaline conditions at pH 9.0, which was the highest pH to which the medium was initially adjusted. The generation time at pH 9.0 was 0.5 h, compared with 0.4 h for the control (pH 7.3). Differences in lag time were minor (<1-h difference). The pH of media with the alkaline treatment remained stable at the initial pH until the late lag phase of growth (ca. 10^6 CFU/ml), when acid production reduced the pH by values of 1.0 to 2.0.

Effect of fermentation and drying on *E. coli* O157:H7 in a fermented, dry sausage. Two trials were done to determine the fate of *E. coli* O157:H7 during fermentation, drying, and

TABLE 2. Growth of *E. coli* O157:H7 in TSB at different pH values adjusted with 85% lactic acid or 6 N HCl and incubated at 37°C

pH	Lactic acid		HCl	
	Generation time[a] (h)	Lag time (h)	Generation time (h)	Lag time (h)
Control (7.3)	0.4	≤2	0.4	≤2
7.0	0.4	2	0.4	≤2
6.5	0.4	2	0.4	≤2
6.0	0.5	2	0.4	≤2
5.5	0.6	3	0.5	2.3
5.0	0.6	3	0.5	3.3
4.5	RED_{14}[b]	ND[c]	0.8	4.7
4.0	RED_7[d]	ND	RED_{17}[e]	ND
3.5	RED_{24h}[f]	ND	RED_{10}[g]	ND

[a] Average of three trials.
[b] RED_{14}, 4-$\log_{10}$-CFU/ml reduction within 14 days.
[c] ND, not determined.
[d] RED_7, 4-$\log_{10}$-CFU/ml reduction within 7 days.
[e] RED_{17}, 4-$\log_{10}$-CFU/ml reduction within 17 days.
[f] RED_{24h}, 4-$\log_{10}$-CFU/ml reduction within 24 h.
[g] RED_{10}, 4-$\log_{10}$-CFU/ml reduction within 10 days.

TABLE 3. pH, titratable acidity, and *E. coli* O157:H7 count of fermented, dry sausage[a] during fermentation, drying, and storage at 4°C

Sampling time	pH	Titratable activity (%)	*E. coli* O157:H7 ($\log_{10}$ CFU/g ± SD)
Day 0[b]	6.3	0.4	4.68 ± 0.02
pH 4.8[c]	4.8	0.6	4.36 ± 0.01
Day 1[d]	4.8	0.7	4.32 ± 0.06
Day 4	4.7	0.9	4.07 ± 0.27
Day 7	4.5	1.0	3.93 ± 0.16
Day 10	4.5	1.0	3.85 ± 0.35
Day 13	4.4	1.2	3.93 ± 0.16
Day 16	4.5	1.2	4.04 ± 0.11
End of drying cycle (18–21 days)[e]	4.5	1.5	3.70 ± 0.57
Wk 2[f]	4.4	1.3	3.29 ± 0.95
Wk 4	4.4	1.3	3.36 ± 0.57
Wk 6	4.4	1.4	2.75 ± 1.44
Wk 8	4.4	1.4	2.72 ± 0.69

[a] Average of two trials.
[b] Lactic acid bacteria count, 1.5×10^7 CFU/g; moisture content, 58%; fat content, 21%; sodium chloride concentration, 3.5%; protein content, 15%. All values are averages of three determinations for each of two trials.
[c] End of fermentation; required 13 to 14 h.
[d] Ten hours after start of drying cycle.
[e] Moisture content, 37%; fat content, 31%; sodium chloride concentration, 4.9%; protein content, 21%; moisture/protein ratio, 1.8:1. Values are average of three determinations for each of two trials.
[f] Number of weeks of storage at 4°C.

2 months of storage at 4°C in sausage made with and without starter culture. Results of the chemical composition and fate of *E. coli* O157:H7 in sausage made with starter culture are shown in Table 3.

During active fermentation to pH 4.8, the population of *E. coli* O157:H7 in sausage made with starter culture declined slightly (<0.5-$\log_{10}$-CFU/g reduction). *E. coli* O157:H7 was detected by direct plating (>10^2 CFU/g) throughout drying and storage at 4°C. The *E. coli* O157:H7 population declined from 4.6×10^4 to 2.0×10^3 CFU/g for trial 1 (final moisture/protein ratio of 1.6:1 after 21 days of drying) and from 4.9×10^4 to 1.3×10^4 CFU/g for trial 2 (final moisture/protein ratio of 1.9:1 after 18 days of drying). Storage of sausage at 4°C for 8 weeks resulted in an additional 1-$\log_{10}$-CFU/g decrease to 1.7×10^2 and 1.6×10^3 CFU/g for trials 1 and 2, respectively. Values for pH and titratable acidity were similar for both trials.

Sausage batter before inoculation had 8×10^2 to 2×10^3 lactic acid bacteria per g as indigenous microflora. As a result, fermentation occurred in sausage made without added starter culture, although at a much slower rate than when starter culture was used (results not shown). The pH of sausage without starter culture decreased to pH 5.4 and 4.8 for trials 1 and 2, respectively, by the end of the drying period, whereas levels of *E. coli* O157:H7 declined slightly (1-$\log_{10}$-CFU/g decrease) or remained constant. The population of *E. coli* O157:H7 continued to decline (additional 1-$\log_{10}$-CFU/g decrease) to ca. 1.7×10^3 after storage for 8 weeks at 4°C. At the end of drying and storage, there was little difference in populations of *E. coli* O157:H7 (<0.2 $\log_{10}$ CFU/g) in sausage made with starter culture compared with sausage fermented with indigenous lactic acid bacteria.

DISCUSSION

Studies revealed that *E. coli* O157:H7 could grow in NaCl concentrations as high as 6.5%. The long lag phase at this

NaCl concentration suggests there was selection for a salt-tolerant population, with cells reaching a maximum population of 10^7 CFU/ml before death occurred. Death occurs in broth at 8.5% NaCl. Similar results were obtained by Gibson and Roberts (5) for enteropathogenic *E. coli*, which failed to grow at NaCl concentrations of ≥8% regardless of incubation temperature or pH.

E. coli O157:H7 grew well at all alkaline pH values (to pH 9.0) tested. The pH of the broth decreased only after populations of *E. coli* O157:H7 were $\geq 10^6$ CFU/g, indicating that the organism was able to grow at alkaline conditions of at least pH 9.0 with subsequent acid production. Lactic acid was more inhibitory to *E. coli* O157:H7 at an equivalent pH than was HCl. Results revealed that in TSB adjusted with lactic acid, the organism remained viable at pH 4.6 for at least 14 days, whereas cells were inactivated at pH 4.5. However, when the broth was adjusted with HCl, the minimum pH for growth was between 4.0 and 4.5.

Regardless of whether lactic acid bacteria were added or not, *E. coli* O157:H7 did not grow in fermented, dry sausage during fermentation, drying, or subsequent storage at 4°C for 2 months. *E. coli* O157:H7 populations decreased 1 to 2 $\log_{10}$ CFU/g from an initial inoculum of ca. 5×10^4 CFU/g by the end of the sampling period and were still detectable by direct plating.

Differences (ca. 1 $\log_{10}$ CFU/g) in the final population of *E. coli* O157:H7 between trials 1 and 2 may be attributed to variation in moisture/protein ratio at the end of drying rather than pH and titratable acidity, which were the same for both studies. Although the moisture/protein ratio of trial 2 was at the legal limit, the longer drying conditions given sausage in trial 1 was likely an important factor in killing more *E. coli* O157:H7.

Enteropathogenic *E. coli* will grow in broth with up to 200 ppm of nitrite and 4% NaCl at pH 5.6 (5). *E. coli* O157:H7 populations in fermented sausage containing 3.5% sodium chloride, 69 ppm of sodium nitrite (determination done immediately after stuffing), and pH 4.8 were reduced but not completely killed during the testing period.

Previous studies on the fate of *Listeria monocytogenes* during the manufacture of fermented, dry sausage revealed that greater populations of *Listeria* than of *E. coli* O157:H7 were killed during fermentation and drying (>4-$\log_{10}$-CFU/g decline within 5 days after the start of the drying cycle) (6). Both *E. coli* O157:H7 (this study) and *L. monocytogenes* (3) were inactivated in culture medium acidified with lactic acid to pH ≤4.5; therefore, the difference cannot be attributed solely to pH. Bacteriocins produced by lactic acid bacteria may be a factor. Recent studies in chicken summer sausage (1) compared the antilisterial activity of two lactic starter cultures, i.e., a bacteriocinogenic and a nonbacteriocinogenic strain. Results revealed that substantially more (>2-$\log_{10}$-CFU/g difference) *L. monocytogenes* were killed at pH 4.8 by the bacteriocinogenic strain than by the nonbacteriocinogenic strain. Because bacteriocins offer little protection against gram-negative bacteria, inactivation of *E. coli* O157:H7 in fermented, dry sausage likely would be due principally to acidity and drying. Results of this study indicate that *E. coli* O157:H7, when initially present at 10^4/g in meat, would not likely be killed completely in fermented sausage that does not receive a pasteurization treatment. Hence, it is incumbent on manufacturers of fermented sausage to use raw meat that contains no or very few *E. coli* O157:H7.

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REFERENCES

1. **Baccus-Taylor, G., K. A. Glass, A. J. Maurer, and J. B. Luchansky.** Unpublished data.
2. **Borczyk, A. A., M. A. Karmali, H. Lior, and L. M. C. Duncan.** 1987. Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. Lancet **i**:98.
3. **Conner, D. E., V. N. Scott, and D. T. Bernard.** 1990. Growth, inhibition, and survival of *Listeria monocytogenes* as affected by acidic conditions. J. Food Prot. **53**:652–655.
4. **Doyle, M. P., and J. L. Schoeni.** 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. **48**:855–856.
5. **Gibson, A. M., and T. A. Roberts.** 1986. The effect of pH, water activity, sodium nitrite and storage temperature on the growth of enteropathogenic *Escherichia coli* and salmonellae in a laboratory medium. Int. J. Food Microbiol. **3**:183–194.
6. **Glass, K. A., and M. P. Doyle.** 1989. Fate and thermal inactivation of *Listeria monocytogenes* in beaker sausage and pepperoni. J. Food Prot. **52**:226–231.
7. **Martin, M. L., L. D. Shipman, J. G. Wells, M. E. Potter, K. Hedberg, I. K. Wachsmuth, R. V. Tauxe, J. P. Davis, J. Arnoldi, and J. Tillei.** 1986. Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of haemolytic uraemic syndrome. Lancet **ii**:1043.
8. **McNeal, J. E.** 1990. Meat and meat products, p. 931–938. *In* K. Herlich (ed.), Official methods of analysis, 15th ed. Association of Official Analytical Chemists, Arlington, Va.
9. **Padhye, N. V., and M. P. Doyle.** 1991. Rapid procedure for detecting enterohemorrhagic *Escherichia coli* O157:H7 in food. Appl. Environ. Microbiol. **57**:2693–2698.
10. **Raghubeer, E. V., and J. R. Matches.** 1990. Temperature range for growth of *Escherichia coli* serotype O157:H7 and selected coliforms in *E. coli* medium. J. Clin. Microbiol. **28**:803–805.
11. **Schulte, E. E., J. B. Peters, and P. R. Hodgson (ed.).** 1987. Wisconsin procedures for soil testing: plant analysis and feed and forage analysis, p. 35–38. University of Wisconsin—Extension, Madison.
12. **Sebranek, J.** 1978. Meat science and processing, p. 139–140. Paladin House Publishers, Geneva, Ill.
13. **U.S. Department of Agriculture—Food Safety and Inspection Service, Meat and Poultry and Inspection Services, Standards and Labeling Division.** 1986. Standards and labeling policy book, p. 90–91. U.S. Department of Agriculture—Food Safety and Inspection Service, Washington, D.C.