Thermal Inactivation of *Escherichia coli* O157:H7 in Cow Manure Compost

XIUPING JIANG,† JENNIE MORGAN, AND MICHAEL P. DOYLE*

Center for Food Safety, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223-1797, USA

MS 03-2: Received 10 January 2003/Accepted 7 April 2003

**ABSTRACT**

Rates of inactivation of a five-strain mixture of green fluorescent protein–labeled *Escherichia coli* O157:H7 in autoclaved and unautoclaved commercial cow manure compost with a moisture content of ca. 38\% were determined at temperatures of 50, 55, 60, 65, and 70°C. Trypticase soy agar with ampicillin was determined to be the best medium for the enumeration of heat-injured and uninjured cells of green fluorescent protein–labeled *E. coli* O157:H7. The results obtained in this study revealed that in autoclaved compost, *E. coli* O157:H7 reductions of ca. 4 log CFU/g occurred within 8 h, 3 h, 15 min, 2 min, and <1 min at 50, 55, 60, 65, and 70°C, respectively. At 65 and 70°C, considerably less time was required to kill the pathogen in unautoclaved compost than in autoclaved compost. Decimal reduction times (*D*-values) for autoclaved compost at 50, 55, 60, 65, and 70°C were 137, 50.3, 4.1, 1.8, and 0.93 min, respectively, and *D*-values for unautoclaved compost at 50, 55, and 60°C were 135, 35.4, and 3.9 min, respectively. Considerable tailing was observed for inactivation curves, especially at 60, 65, and 70°C. These results are useful for identifying composting conditions that will reduce the risk of the transmission of *E. coli* O157:H7 to foods produced in the presence of animal fecal waste.

Contamination of food, either directly or indirectly, by animal manure has been identified as a contributing factor in many outbreaks of foodborne illness (12, 18). Cattle are a primary reservoir of *Escherichia coli* O157:H7, with surveys revealing that at least 1 to 5% of cattle shed *E. coli* O157:H7 in feces at levels of <10\(^2\) CFU/g (detectable by enrichment) to 10\(^6\) CFU/g (7, 28). Other reports indicate that the prevalence of fecal shedding of *E. coli* O157:H7 by cattle may be considerably higher, perhaps >25\% during the summer months, when more sensitive detection methods are used (11). Shiga toxin–producing *E. coli* of serogroups O26, O111, and O157 survived in bovine feces from 1 to 18 weeks, depending on inoculation levels and incubation temperatures (12). Our previous studies on the fate of *E. coli* O157:H7 in manure-amended soil revealed that the pathogen survived for up to 77, 226, and 231 days in manure-amended autoclaved soil held at 5, 15, and 21°C (15). These results indicate that the feces of ruminants are a potential source of *E. coli* O157:H7 contamination even after their application to soil.

Food crops are commonly fertilized with animal waste of fecal origin. Uncomposted manure (in the form of slurry and solids) and composted manure are widely used in both organic and traditional farming; hence, there is a risk of contaminating food crops with pathogens and parasites that occur in manure (18). The composting of animal manure has routinely been used by farmers to reduce pathogen contamination as well as to reduce the leakage of nitrogen, phosphorous, or potassium in the form of volatile or leachable nutrients into the environment. Composting is usually an outdoor process, and many variables can affect its outcome. Heat generated during early composting is largely due to the metabolic activity of bacteria and fungi. Generally, heat generated at 50 to 70°C for an extended period can kill microbial pathogens, weed seeds, and fly larvae (20). In a laboratory-scale situation, *E. coli* O157:H7 and *Salmonella Enteritidis* were killed rapidly (within 3 or 2 days, respectively) in cow manure composting at 45°C (17). Studies involving *E. coli* and *Salmonella enterica* serovar Typhimurium revealed that these bacteria could survive for 59 days in an industrial compost and for at least 9 days in laboratory compost at ca. 60°C when ca. 10\(^7\) CFU/g was inoculated into municipal biosolids (8). Other studies have revealed that *Salmonella* can survive the composting process and then grow in compost and stored biosolids when held under favorable conditions (19). In autoclaved and composted biosolids, *Salmonella Typhimurium* and *E. coli* grew rapidly to 10\(^8\) CFU/g after incubation for 30 h (23). However, there is limited information regarding the fate of foodborne pathogens during the composting process, and there are few scientific data regarding the optimal conditions for composting manure to kill pathogens.

Composting ingredients are usually composed of animal fecal waste (the primary ingredient), a carbon source, and a bulking agent. Soon after the ingredients are combined, microbial activity and self-heating begins, provided the carbon-to-nitrogen ratio is in the range of 25:1 to 40:1 and the moisture content is 45 to 60% (21). In order to evaluate the rates of inactivation of *E. coli* O157:H7 in a compost mixture at a specific temperature, a well-controlled
environment is essential. Therefore, commercial cow manure compost was selected for this study because its composition is more uniform and its microbial activity is more stable than that of uncomposted cow manure. The objective of this project was to determine the rates of inactivation of 

\( E. \) coli O157:H7 in commercial cow manure compost at constant elevated temperatures.

**MATERIALS AND METHODS**

Construction of GFP-labeled \( E. \) coli O157:H7. A five-strain mixture of \( E. \) coli O157:H7 (consisting of strains E0143 [meat isolate], C7927 [human isolate], K262 [human isolate], C083 [cattle feces isolate], and E0139 [beef jerky isolate]) was used. In order to facilitate the enumeration of these isolates, the \( E. \) coli O157:H7 strains were labeled with jellyfish green fluorescent protein (GFP) according to the procedure described previously (15). The GFP-labeled strains of \( E. \) coli O157:H7 emitted bright green fluorescence under a handheld UV light. The stability of the GFP label in the \( E. \) coli O157:H7 strains was determined by streaking each isolate on Trypticase soy agar (TSA; Difco Laboratories, Sparks, Md.) containing 100 \( \mu \)g of ampicillin per ml (TSA-A) for several generations.

Preparation of \( E. \) coli O157:H7 cultures. Each strain of GFP-labeled \( E. \) coli O157:H7 was inoculated into 10 ml of Trypticase soy broth (TSB; Difco) containing 100 \( \mu \)g of ampicillin per ml (TSB-A) and incubated at 37°C for 16 to 18 h with agitation (150 rpm). From these cultures, 0.5 ml of each isolate was transferred to separate flasks containing 100 ml of TSB-A and incubated for 16 to 18 h with agitation (150 rpm). The bacteria were sedimented three times by centrifugation (4,000 \( \times \) g for 20 min), and cell pellets were washed in 0.1% peptone water. The cell pellets were suspended in 0.1% peptone water to obtain an optical density at 630 nm (OD\( _{630}\)) of 0.5 (ca. 10\(^8\) CFU/ml). An equal volume of each of the five strains was combined to obtain the five-strain mixture. To determine cell populations of \( E. \) coli O157:H7 in the five-strain mixture, serial dilutions in 0.1% peptone water were plated on TSA-A plates.

Comparison of thermal resistance of GFP-labeled strain with parental strain of \( E. \) coli O157:H7 in nutrient broth. Overnight cultures of both GFP-labeled and parental \( E. \) coli O157:H7 strains K262 and C083 were washed according to the protocol described above and suspended in TSB at a final concentration of ca. 10\(^8\) CFU/ml. Two milliliters of each culture was transferred into a sterile 25-ml screw-cap tube (16 by 150 mm) into which a sterile 40-ml spoon. The inoculated samples were held at 23°C for 20 to 24 h in a closed plastic container with 2.5 cm of tap water on the bottom to enable the inoculated pathogens to acclimatize to the compost environment. For one study, the compost preparation (1 kg) was distributed into two sterile polypropylene trays (26 by 16 by 6.4 cm) at a depth of 4 cm. The compost was held inside a closed plastic container (50 by 40 by 30 cm) and incubated at 50°C in an Innova 4000 incubator containing a pan of saturated K\(_2\)SO\(_4\) solution (water activity = 0.98) (New Brunswick Scientific, Edison, N.J.).

Water baths at 50, 55, 60, 65, and 70°C were used for thermal inactivation studies. The inoculated compost (in 30-g portions), which was prepared as described above including the 20- to 24-h adjustment period at 23°C, was distributed into sterile 250-ml Erlenmeyer flasks; the compost covered the flask bottom at a depth of ca. 0.4 to 0.6 cm. Type T thermocouples were inserted into the compost in each flask, which was sealed with a tight-fitting cap. The heat treatment for compost samples in flasks was carried out with the use of the water bath and involved the heating conditions described above for the inoculated-TSB-in-tubes study. A total of 20 flasks with compost were placed collectively into the water bath. The temperature of compost in each flask was recorded throughout the study, including during cooldown. At predetermined intervals, duplicate flasks were removed from the covered water bath, and the compost was sampled from the center of the bottom of the flask with a sterile spatula for the analysis of \( E. \) coli O157:H7 level, pH, and moisture content. Each heat treatment was repeated twice or three times, and an average of the results for each sampling time was recorded.

Recovery of \( E. \) coli O157:H7 inoculated into commercial cow manure compost. The inoculated compost was sampled periodically, with sampling times being selected on the basis of the estimated rates of inactivation of \( E. \) coli O157:H7. Duplicate samples were obtained at each sampling time, and each study involved at least five sampling times. After quick chilling in an ice bath, each 10-g sample was mixed with 90 ml of 0.1% peptone water in a Whirl-Pak bag and macerated in a stomacher for 30 s at low speed. Serial dilutions (1:10) were prepared with 0.1% peptone water, and 0.1-ml portions of each dilution were spread onto both nonselective- and selective-agar plates. Plates were incubated at 37°C for 2 days, and colonies were examined and counted.

TSA was used as the nonselective medium for the enumeration of \( E. \) coli O157:H7. The selective media included TSA-A, sorbitol MacConkey agar (SMA; Difco). Rainbow agar O157 (Biolog, Inc., Hayward, Calif.) supplemented with 1.0 g of sodium novobiocin per liter, and violet red bile agar (VRBA; Difco). When \( E. \) coli O157:H7 was not detected by the direct plating methods, a selective enrichment method involving the incubation of a 10-g compost sample in 90 ml of selective enrichment broth, i.e., TSB-A, at 37°C for 24 h with agitation (150 rpm) was carried out. Dilutions of cultures were surface plated on TSA-A plates. The colonies were examined under UV light, and those that were green and fluorescent were counted. Representative colonies were selected and confirmed to be \( E. \) coli O157 colonies by an \( E. \) coli O157 immuno latex agglutination test (Oxoid, Hampshire, UK).
**Pulsed-field gel electrophoresis (PFGE) analysis of heat-resistant isolates.** Pulsed-field gel electrophoresis (PFGE) was carried out according to the protocol described by the U.S. Centers for Disease Control and Prevention (3). Colonies of bacteria that survived heat treatments were picked from TSA-A plates, streaked again onto fresh TSA-A plates, and cultured at 37°C for 24 h. Bacteria were harvested into 5 ml of TSB by scraping the surfaces of plates with a sterile swab. The bacteria were sedimented three times by centrifugation at 5,000 × g for 20 min and washed with Tris-EDTA buffer (100 mM Tris and 100 mM EDTA) at pH 7.4 and then resuspended in Tris-EDTA buffer to an OD₆₀₀ of 0.5 (ca. 10⁶ CFU/ml). The bacterial suspension (250 µl) was mixed with 12.5 µl of proteinase K (20 mg of stock per ml) and then with 250 µl of plug agarose solution and dispensed into a disposable plug mold. The agarose plugs were lysed with proteinase K (0.1 mg of proteinase K per ml, 50 mM Tris, 50 mM EDTA, and 1% N-lauroylsarcosine [pH 8.0]) at 54°C for 2 h with agitation (80 rpm). The washed samples were digested with restrictive enzyme XbaI (175 µl of deionized water, 20 µl of REact 2 buffer, and 5 µl of XbaI per slice) at 37°C for 16 h. The DNA samples were then separated by electrophoresis on a 1.2% agarose gel in 0.5× TBE buffer with a contour-clamped homogeneous electric field device (Chef Mapper, Bio-Rad). After electrophoresis for 18 h at 200 V with pulse times of 2.2 to 54.2 s and linear ramping at an electric field angle of 120° at 14°C, the gels were stained with ethidium bromide and the bands were visualized and photographed with UV transillumination.

**Other analyses.** To determine the pH of compost, 5 g of compost was added to 250 ml of distilled water. The suspension was stirred for 5 min and then allowed to settle for 5 min. The pH of the liquid was determined with an Accumet Basic pH meter (Fisher Scientific). The moisture content of compost was determined by drying 5 g of compost at 105°C for 24 h in a Precision oven (Precision Scientific) and then weighing the residual. The water activity of the compost (5 g) was measured at 20°C with a water activity meter (Aqualab, Model CX2, Decagon Devices, Inc., Pullman, Wash.). The temperature of the compost was monitored with type T thermocouples connected to a multiple-channel recorder (HotMux data logger, DCC Corp., Pennsauken, N.J.). The incubator or water bath temperature was also monitored concurrently.

**Statistical analysis.** Data obtained for duplicate or triplicate samples were analyzed by analysis of variance, and means were separated by a least significance difference procedure (22). Decimal reduction times (D-values) in minutes were determined from thermal inactivation curves (D = −1/slope) by linear regression analysis.

**RESULTS**

Isolated colonies of GFP-labeled *E. coli* O157:H7 strains retained the GFP label after repeated streaking on TSA-A plates. In order to determine whether the introduction of the GFP label affected the thermal resistance of *E. coli* O157:H7 strains, both GFP-labeled and parental *E. coli* O157:H7 strains K262 and C083 were subjected to heat treatment at 60°C in TSB for 0, 0.5, 1, 2, 3, 4, and 5 min. The D-values for GFP-labeled and parental *E. coli* O157:H7 strains K262 were 1.65 and 1.45 min, respectively, and those for strain C083 were 1.58 and 1.13 min, respectively. GFP-labeled *E. coli* O157:H7 strains appear to have been slightly more heat resistant than their counterpart parental strains.

The aerobic plate counts for commercial cow manure compost were ca. 10⁶ CFU/g on TSA plates, 10⁴ CFU/g on TSA-A, and 10⁶ CFU/g on both SMA and Rainbow agar plates. However, no colonies typical of *E. coli* O157:H7 (e.g., gray or black colonies on Rainbow agar plates) were observed for compost before inoculation with *E. coli* O157:H7. After the compost was autoclaved for 20 min at 121°C on three successive days, most ampicillin-resistant bacteria were eliminated. The moisture content, water activity, and pH of unautoclaved compost were 38%, 0.997, and 7.7 to 8.4, respectively.

*E. coli* O157 counts increased by ca. 1 log CFU/g during the initial 20-h holding of inoculated autoclaved compost at 23°C. In contrast, *E. coli* O157 counts changed very slightly for inoculated unautoclaved compost under the same incubation conditions. Over the following 2 days of incubation at 23°C, the *E. coli* O157 counts for unautoclaved compost decreased by ca. 0.6 log CFU/g on TSA-A.

Heat treatment of inoculated compost at 50°C was initially carried out with an Innova 4000 incubator shaker. A come-up time of ca. 1.5 h was required for the inoculated compost to reach 50°C. Temperature measurements at different locations of the compost were monitored every 30 s with a Hotmux data logger. The moisture content, water activity, and pH of the compost were measured in duplicate. At 50°C, no measurable change in the water activity of the compost occurred within 24 h of incubation, whereas modest changes in moisture content did occur. Hence, moisture content, rather than water activity, was determined for later experiments. The moisture content of inoculated compost at 23°C remained unchanged (data not shown), whereas that of both autoclaved and unautoclaved inoculated compost at 50°C decreased from ca. 38 to 35% during the first 6 h. Upon further heating at 50°C for 96 h, the moisture contents were reduced to 31.2% for autoclaved compost and to 19.6% for unautoclaved compost after heating. In contrast, the pH of the compost fluctuated in the range of 7.6 to 9.0 for the duration of the heat treatment.

*E. coli* O157 in compost held at 50°C in an Innova 4000 incubator had decreased by ca. 1 log CFU/g after 2 h, by ca. 2 log CFU/g after 4 h, by ca. 3 log CFU/g after 6 h, and by >6 log CFU/g after 24 h. *E. coli* O157 was detected only by enrichment culture for compost held at 50°C for 24 h. No *E. coli* O157 colonies were detected on SMA plates for samples taken at 12 h or on TSA-A plates for samples taken at 24 h. The D-values for *E. coli* O157: H7 at 50°C were 145 and 151 min for autoclaved and unautoclaved compost, respectively. The efficacy levels of six different plating media in the enumeration of *E. coli* O157 cells from heat-treated compost were compared with the Statistical Analysis System for analysis of variance with T grouping (Table 1). Overall, TSA-A and Rainbow agar were significantly (P < 0.05) more effective than TSA, VRBA, and SMA.

Considering that we could not control the loss of moisture from compost during prolonged heating in the incubator, subsequent studies were carried out with a water bath to determine rates of inactivation of *E. coli* O157 in compost at 50, 55, 60, 65, and 70°C. The come-up times required to reach 50 and 55°C for 30 g of compost in a 250-
Enumeration medium | E. coli O157:H7 count (log CFU/g) for heating timea
--- | --- | --- | --- | --- | --- | ---
TSA | 7.591 ± 0.016 AB | 4.690 ± 0.550 AB | 4.046 ± 0.242 ABC | 2.929 ± 0.212 AB | 1.398 ± 0.000 C | 1.398 ± 0.000 B
TSA-A | 7.618 ± 0.022 A | 5.242 ± 0.052 A | 4.355 ± 0.213 AB | 3.669 ± 0.292 A | 2.847 ± 0.275 A | 2.121 ± 0.598 A
Rainbow | 7.761 ± 0.153 A | 5.233 ± 0.169 A | 4.030 ± 0.469 ABC | 3.239 ± 0.088 AB | 2.452 ± 0.213 AB | 2.151 ± 0.213 A
VRBA | 7.415 ± 0.075 bc | 4.246 ± 0.164 B | 3.327 ± 0.462 C | 2.220 ± 0.736 B | 2.000 ± 0.426 BC | 1.398 ± 0.000 B
SMA | 7.337 ± 0.061 c | 4.448 ± 0.071 B | 3.651 ± 0.069 BC | 2.105 ± 1.000 B | 2.000 ± 0.426 BC | 1.398 ± 0.000 B
TSA overlay ND | 4.429 ± 0.213 B | 4.414 ± 0.129 A | 2.980 ± 0.190 AB | 2.753 ± 0.213 A | ND

a Values presented as means ± standard deviations. ND, not detected. Means with different letters in the same column are significantly different (P < 0.05).

for 12 h and at 70°C for 5 min were selected from TSA-A plates. The PFGE patterns for all isolates obtained from autoclaved compost after heating at 70°C matched that for strain C083, whereas those for all isolates from unautoclaved compost matched that for strain E0143 (Table 2). Most isolates obtained from both autoclaved and unautoclaved compost heated at 50°C were of strain E0143.

DISCUSSION

Six types of agar media were evaluated for the enumeration of heat-injured E. coli O157:H7 cells. TSA-A and Rainbow agar O157:H7 enabled the enumeration of significantly (P < 0.05) larger numbers of heat-treated E. coli O157:H7 than did TSA, VRBA, or SMA. Taormina et al. (25) determined E. coli O157:H7 counts for ground beef and obtained higher counts on Rainbow agar O157:H7 than on TSA. SMA is generally regarded as inadequate for the recovery of sublethally injured heat-treated cells owing to the lethal effects of selective ingredients present in this medium (9). Since Rainbow agar is very expensive and was not markedly superior to TSA-A, we selected TSA-A as the medium for the enumeration of GFP-labeled E. coli O157 in subsequent heat treatment studies. Results from this study reveal that GFP-labeled E. coli O157 colonies on TSA-A plates, emitting bright green fluorescence under UV light, were very easily distinguished from those of competing bacteria.

The gfp gene has been used to label a variety of bacterial species, enabling the tracking of targeted bacterial cells in many applications (4, 5, 10, 27). A strain of GFP-labeled E. coli O157:H7 is used as a control according to U.S. Department of Agriculture–Food Safety and Inspection Service guidelines for isolating E. coli O157:H7 from foods (6). Ehrmann et al. (10) evaluated the use of GFP as a reporter under high-pressure conditions and determined that the expression of fluorescent GFP was not influenced by high-pressure treatment. From the results of our study, we determined that GFP-labeled E. coli O157:H7 colonies were easily recognized on agar plates and that larger numbers of E. coli O157:H7 GFP-labeled colonies were counted on TSA-A than on other selective media or nonselective TSA. Furthermore, we determined that there was not a substantial difference between GFP-labeled and parental E. coli...
O157:H7 strains with respect to thermal resistance in nutrient broth.

Recently, Lung et al. (17) reported that the number of a rifampicin-resistant *E. coli* O157:H7 strain was reduced from ca. $10^7$ CFU/g to an undetectable level within 72 h of cow manure composting at 45°C in a 1-liter bench-scale composting system. However, the most desirable temperature range for successful composting is 50 to 70°C (20). For the substantial reduction of pathogen levels in compost, the temperature of the composting material should be maintained at ≥50°C for seven consecutive days with the use of aerated windrows and in-vessel or static-pile composting methods (26). In this study, we determined the thermal inactivation rates for a five-strain mixture of *E. coli* O157:H7 in commercial cow manure compost held at constant temperatures of 50, 55, 60, 65, and 70°C. At 65 and 70°C, the inactivation rates for *E. coli* O157:H7 were considerably greater for unautoclaved compost than for autoclaved
TABLE 2. The most heat-tolerant strains of E. coli O157:H7 in compost, as identified by PFGE

<table>
<thead>
<tr>
<th>Heating medium</th>
<th>Heating temp (°C)</th>
<th>Predominant E. coli O157:H7 strain*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoclaved compost</td>
<td>70</td>
<td>C083 (5/5)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>E0143 (4/5)</td>
</tr>
<tr>
<td>Unautoclaved compost</td>
<td>70</td>
<td>E0143 (5/5)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>E0143 (2/4)</td>
</tr>
</tbody>
</table>

* Values in parentheses are number of colonies that were the predominant strain/total number of colonies selected for assay.

different types of foods (14). These data and results reported by others indicate that E. coli O157:H7 is not unusually heat resistant in high-moisture menstruums (7, 16).

In the present study, the heating menstruum was cow manure compost with a moisture content of ca. 38%. The composition of cow manure compost is considerably different from those of most foods. Kaur et al. (16) reported that a slight reduction in the water activity of the heating menstruum resulted in a marked increase in the heat resistance of E. coli O157:H7. The pathogen survived in sunflower oil even after heating at 60°C for 1 h or at 70°C for 5 min.

The Advisory Committee on the Microbiological Safety of Food stated that a heat treatment of 70°C for 2 min or an equivalent treatment will provide a 6-D reduction in E. coli O157:H7 cells in beef products (1). Results obtained in this study indicate that large populations of E. coli O157:H7 strain C083 cells preconditioned at 23°C for 20 to 24 h in autoclaved cow manure compost can survive at 70°C for several minutes. It is estimated that autoclaved compost must be heated at 70°C for 6 min to reduce the level of strain C083 by 6 log CFU/g. In contrast, most E. coli O157:H7 cells (at ca. 6 log CFU/g) preconditioned at 23°C for 20 to 24 h in unautoclaved cow manure compost were inactivated during the come-up time when compost was heated to 70°C.

Historically, it has been assumed that microorganisms within a population are homogenous, and their rate of thermal inactivation is strain dependent (9). Therefore, in this study, we used a five-strain mixture of E. coli O157:H7 to obtain data encompassing several isolates to address strain variation. In the five-strain mixture, the strains with the highest levels of thermal resistance were identified by PFGE analysis of cells grown from colonies that survived heat treatment for the longest time. Strain C083, which was originally isolated from cattle feces, was the dominant strain identified in the autoclaved compost heated at 70°C. It appears that this strain was exceptionally heat tolerant compared with other strains of E. coli O157:H7.

There is a considerable amount of published information regarding the thermal inactivation of E. coli O157:H7 in foods and culture media (24). Stringer et al. (24) reported that the averages of 72 D-values for E. coli O157:H7 heated at 60°C in broth and buffers, apple juice, meat, and all menstruums were 1.7, 0.8, 1.8, and 1.6 min, respectively, with a range of 0.3 to 10.0 min. Ryu and Beuchat (21) reported that the D-values for the most heat-tolerant isolate of E. coli O157:H7 (strain E0139) evaluated with TSB were 100.2, 28.3, and 6.1 min at 52, 54, and 56°C, respectively, compared with 13.3 to 13.6 min, 6.3 to 9.2 min, and 3.6 to 6.3 min at 52, 54, and 56°C, respectively, for two other E. coli O157:H7 strains. D-values for E. coli O157:H7 at 54 to 64°C range from 39.8 to 0.16 min, respectively, for compost. This difference may be explained in part by the fact that the E. coli O157:H7 level in the autoclaved compost increased by ca. 1 log CFU/g at room temperature during preconditioning, whereas no growth occurred in the unautoclaved compost during preconditioning. However, the difference between autoclaved compost at 65°C and that at 70°C with respect to thermal tolerance may not be accounted for by a larger initial population (1 log CFU/g) of E. coli O157:H7. Preconditioning of E. coli O157:H7 cells by holding inoculated compost at 23°C for 24 h before heat treatment may affect the thermal tolerance of the cells. Factors such as stationary- versus log-phase growth, the nutrient level in the compost, antagonistic interaction with indigenous microbes in the compost, and changes in E. coli O157:H7 cell composition during growth under less than optimal conditions or conditions of stress could contribute to the differences between E. coli O157:H7 cells preconditioned in autoclaved compost and those preconditioned in unautoclaved compost with respect to thermal tolerance.

The ability of E. coli O157:H7 to tolerate heat is strain dependent (9). Therefore, in this study, we used a five-strain mixture of E. coli O157:H7 to obtain data encompassing several isolates to address strain variation. In the five-strain mixture, the strains with the highest levels of thermal resistance were identified by PFGE analysis of cells grown from colonies that survived heat treatment for the longest time. Strain C083, which was originally isolated from cattle feces, was the dominant strain identified in the autoclaved compost heated at 70°C. It appears that this strain was exceptionally heat tolerant compared with other strains of E. coli O157:H7.
claved compost. Results obtained in these studies provide useful information for identifying optimal composting conditions to reduce the risk of the transmission of *E. coli* O157:H7 to foods produced in the presence of animal waste.

ACKNOWLEDGMENTS

This study was funded by a grant from the International Life Sciences Institute–North America and by a grant from the U.S. Department of Agriculture for the Alliance for Food Protection.

REFERENCES