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NOTES

Survival and Growth Characteristics of *Escherichia coli* Associated with Hemorrhagic Colitis

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***Escherichia coli* O157:H7 in ground beef was more sensitive to heat than salmonellae, but survived for 9 months at −20°C with little change in number. The organisms grew well in Trypticase soy broth (BBL Microbiology Systems) between 30 and 42°C, with 37°C being optimal for growth. *E. coli* O157:H7 grew poorly in the temperature range (44 to 45.5°C) generally used for recovery of *E. coli* from foods.**

Escherichia coli O157:H7 has recently been associated with three outbreaks of hemorrhagic colitis (5, 7, 8) and has been linked to cases of hemolytic uremic syndrome (6). Epidemiological data of two outbreaks implicated ground beef sandwiches as the vehicle of transmission (7), and *E. coli* O157:H7 has been isolated from a ground beef patty (7, 10). The purpose of this study was to determine the survival characteristics of the organism in heated and frozen ground beef and its growth response in culture medium at different temperatures.

Survival studies were done in ground beef. To determine rates of thermal inactivation, *E. coli* O157:H7 (strain no. 932; obtained from G. K. Morris, Centers for Disease Control, Atlanta, Ga.) was grown to late stationary phase in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) agitated at 100 gyrations per min at 37°C. Cells were washed, suspended in 0.01 M phosphate-buffered saline (pH 7.5), and inoculated into aseptically prepared ground beef (17 to 20% fat content; aerobic plate count, $\leq 10^4$ CFU/g). Coarsely ground (11/16-in. [ca. 1.74-cm] plate) ground beef was inoculated by adding (dropwise) 1 ml of bacterial culture (ca. 10^9 CFU/ml) to 100 g of meat slowly mixing in a mixer (Hobart Manufacturing Co., Troy, Ohio). After mixing, the meat was put through a 1/8-in. (ca. 0.32-cm) plate (Hobart no. 12 grinder) to assure uniform distribution of the inoculum. Portions (1.0 g) of meat were lightly packed into each of 24 Pyrex test tubes (10 by 75 mm) capped with rubber stoppers. Temperature was monitored by thermocouples placed in the center of selected meat samples. All tubes were submerged in a water bath preadjusted to the appropriate temperature. Once the meat reached the desired temperature, two tubes were immediately removed and cooled in water (ca. 5°C). The number of *E. coli* surviving in these samples was the number present at zero time. Duplicate samples were taken at appropriate intervals and enumerated for *E. coli*.

Surviving *E. coli* were determined by serially diluting (1:10) meat in 0.1% peptone and plating 1.0-ml portions on 5 ml of Trypticase soy agar (BBL). These plates were held at 37°C for 1.5 h and then capped with 10 ml of MacConkey agar and incubated at 37°C for 24 h. Colonies typical of *E. coli* were counted, and isolates from plates were randomly selected and serologically confirmed as *E. coli* O157:H7. Duplicate tests were done for each temperature treatment, and an average of these results is reported as the *D* value.

When different organisms were heated in ground beef at comparable temperatures and under similar experimental conditions, *D* values were less for *E. coli* O157:H7 (Table 1) than for salmonellae (4). For example, the *D* values at 62.8°C for *E. coli* O157:H7 and *Salmonella* spp. were 24 and 36 to 42 s, respectively. Thus, at the same temperature, less time is needed to inactivate *E. coli* O157:H7 than the same number of salmonellae.

Ground beef patties are often stored frozen before use, therefore, a study was done to determine the stability of *E. coli* O157:H7 in ground beef patties held at −20°C for several months. Ground beef was inoculated with *E. coli* O157:H7 (ca. 10^4 CFU/g) according to the procedure described above, formed into 45-g patties with a Hollymatic 200 patty molding machine (Holly Systems Inc., Boca Raton, Fla.), held at −80°C for 30 min, and stored at −20°C until sampled. Three to five patties were sampled on day 0 and at 3-month intervals and enumerated for *E. coli* O157:H7. Each patty (still frozen) was placed into 405 ml of 0.1% peptone (5°C) and blended in a Waring blender for 2 min. The number of surviving *E. coli* O157:H7 was determined according to the procedure described above, and an average of the results is reported.

Results indicate there was little change in the number of *E. coli* O157:H7 from the day patties were inoculated to 9 months of storage (Table 2). Thus, it appears the organism survives well in ground beef during frozen storage.

TABLE 1. Thermal inactivation times of *E. coli* O157:H7 in ground beef[a]

Temp (°C)	*D* value (s)
54.4	2,390
57.2	270
58.9	70
60	45
62.8	24
64.3	9.6

[a] *z* value is 4.1°C.

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TABLE 2. Survival of *E. coli* O157:H7 in ground beef patties held at −20°C

Time after inoculation (months)	No. of *E. coli* O157:H7 per g (±SD)
0[a]	6,700 ± 3,800
3	3,700 ± 2,300
6	6,600 ± 1,700
9	6,200 ± 1,700

[a] Patties were assayed after 30 min at −80°C plus 2 h at −20°C.

TABLE 3. Growth of *E. coli* O157:H7 in Trypticase soy broth at different temperatures

Temp (°C)	Generation time (h)
4	NG[a]
10	NG
25	1.46
30	0.58
37	0.49
40	0.57
42	0.64
44	1.08[b]
44.5	1.26[b]
45	1.21[b]
45.5	NG

[a] NG, No growth.
[b] Less than a 0.5 $\log_{10}$ increase between 8 and 24 h after inoculation; maximum population reached was ca. 10^5 CFU/ml.

In developing an enrichment procedure to detect *E. coli* O157:H7 in food specimens, it is important to identify the growth response of the organism at different temperatures. Generation times were determined by culturing the organism in Trypticase soy broth (50 ml in a 250-ml Erlenmeyer flask) with agitation (100 gyrations per min) and sampling at appropriate intervals. Cultures were diluted in phosphate-buffered saline and plated on Trypticase soy agar and colonies were counted after 24 h at 37°C.

The most rapid growth occurred at 37°C, although prolific growth also occurred within 24 h when cultures were held at 30 to 42°C (Table 3). Growth was slower at 44 to 45°C, and after a population of ca. 10^5 CFU/ml was reached (at 8 h after inoculation from an initial inoculum of ca. 10^3 CFU/ml), there was less than a 0.5 $\log_{10}$ increase thereafter (up to 48 h). The organism did not grow within 48 h at 4, 10, or 45.5°C. The inability of *E. coli* O157:H7 to grow well at 44 to 45.5°C is a significant observation because most procedures used to detect fecal coliforms and subsequently *E. coli* in foods use incubation temperatures in this range (2, 3, 9). This indicates that traditional detection procedures for *E. coli* in foods would not detect *E. coli* O157:H7; therefore, an alternate procedure is needed.

A rapid fluorogenic assay with the compound 4-methylumbelliferone glucuronide (MUG) as an indicator has been developed for *E. coli* (1). MUG is hydrolyzed by glucuronidase, an enzyme present in most *E. coli* strains, to yield a fluorogenic product. We tested five strains of *E. coli* O157:H7 (strain no. 932, CL8, CL40, 40917, and 10547) and three strains of Vero cytotoxin-producing *E. coli* isolated from children with hemolytic uremic syndrome (strain no. CL5 [serotype O26:K60:H11], CL15 [serotype O113:K75:H21], and CL37 [serotype O111:K58:H8]; 6) by the MUG assay. Only strain CL15 produced a fluorescent medium, indicating that seven of the eight strains were negative by the MUG assay.

Results indicate that *E. coli* O157:H7 is in some aspects different from most other *E. coli* strains. Not only does the organism grow poorly, if at all, at temperatures (44 to 45.5°C) often used to detect *E. coli* in foods, but also *E. coli* O157:H7 is not detected by the MUG assay, which is gaining popularity as a rapid test for *E. coli*. Additionally, most *E. coli* strains ferment sorbitol within 24 h; however, *E. coli* O157:H7 does not (5, 10). Fortunately, the organism has no unusual heat resistance, as it is more sensitive to heat than salmonellae; however, *E. coli* O157:H7 can survive in ground beef at −20°C for several months without a major change in numbers.

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