Research Note

Fate of *Escherichia coli* O157:H7 in Coleslaw during Storage

F. M. WU,1,2 L. R. BEUCHAT,1 M. P. DOYLE,1,* V. GARRETT,2 J. G. WELLS,2 AND B. SWAMINATHAN2

1Center for Food Safety and Department of Food Science and Technology, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223-1797; and 2Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention, Public Health Service, U.S. Department of Health and Human Services, 1600 Clifton Road, Atlanta, Georgia 30333, USA

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ABSTRACT

An outbreak of *Escherichia coli* O157:H7 infection associated with the consumption of coleslaw in several units of a restaurant chain prompted a study to determine the fate of the pathogen in two commercial coleslaw preparations (pH 4.3 and 4.5) held at 4, 11, and 21°C for 3 days. At an initial population of 5.3 log10 CFU/g of coleslaw, *E. coli* O157:H7 did not grow in either coleslaw stored at the three temperatures. Rather, the population of *E. coli* O157:H7 decreased by 0.1 to 0.5 log10 CFU/g within 3 days. The greatest reduction (0.4 and 0.5 log10 CFU/g) in population occurred at 21°C, whereas only slight decreases (0.1 to 0.2 log10 CFU/g) occurred at 4 and 11°C. A pH of 4.3 to 4.5 of coleslaw had little effect on reducing *E. coli* O157:H7 populations. Results suggest that the tolerance of *E. coli* O157:H7 to acid pH, not temperature abuse, is a major factor influencing the pathogen’s fate in restaurant-prepared coleslaw.

*Escherichia coli* O157:H7 is more tolerant than most foodborne bacterial pathogens to acid environments (2, 6, 10, 11). Outbreaks of *E. coli* O157:H7 infection have been linked to acidic foods such as apple cider (1), salad dressing containing mayonnaise (8), and salami (4), likely in part because the pathogen is able to survive in acidic environments (5, 7, 11).

In July 1999, an outbreak of *E. coli* O157:H7 infection in Ohio was associated with the consumption of contaminated coleslaw in several units of a restaurant chain. Temperature abuse during the storage of coleslaw was suspected to have promoted the growth of the pathogen. The purpose of this study was to determine the fate of *E. coli* O157:H7 in coleslaw stored under conditions that may occur in food service.

MATERIALS AND METHODS

Bacterial strains and preparation of inoculum. Two clinical isolates of *E. coli* O157:H7 (F6844 and F6845) from a recent outbreak associated with a commercial coleslaw product were used for the study. Each isolate was selected for resistance to nalidixic acid by culturing with increasing concentrations of nalidixic acid until growth occurred in tryptic soy broth (TSB; Difco Laboratories, Detroit, Mich.) supplemented with 50 μg of nalidixic acid per ml (TSBN). Cultures were incubated at 37°C with agitation (150 rpm) for 24 h. Nalidixic acid-resistant cells were used to facilitate the enumeration of *E. coli* O157:H7 in the presence of other microflora in coleslaw. Cultures were transferred at 24-h intervals to TSBN before sedimenting cells by centrifugation (8,000 × g, 1 min); each pellet was then resuspended in 1 ml of sterile 0.1% peptone water. Equal volumes (0.5 ml) of cell suspension were combined to provide a mixture containing approximately equal populations of each strain. The cell suspension (1 ml) was diluted in 9 ml of sterile 0.1% peptone water, and 0.45 ml was added to 200 g of coleslaw in a resealable plastic bag (17 by 20 cm) to provide an inoculum of *E. coli* O157:H7 populations. Results suggest that the tolerance of *E. coli* O157:H7 to acid pH, not temperature abuse, is a major factor influencing the pathogen’s fate in restaurant-prepared coleslaw.

Duplicate 10-g samples of coleslaw stored at 4, 11, and 21°C for up to 3 days were analyzed for populations of *E. coli* O157:H7. Coleslaw (10 g) was combined with 90 ml of sterile 0.01 M phosphate-buffered saline (pH 7.2) in a stomacher bag and suspended at normal speed for 1 min. Samples were serially diluted (1:10) in sterile 1% peptone water and surface plated (0.1 ml in duplicate) on sorbitol MacConkey (SMAC) agar containing 50 μg of nalidixic acid per ml (SMACN). Plates were incubated at 37°C for 18 to 24 h before colonies were counted. Three presumptive colonies with typical *E. coli* O157:H7 colony morphology on SMACN were picked from each plate for confirmation using an *E. coli* O157-specific latex agglutination test reagent (Oxoid Limited, Hampshire, UK). All colonies selected tested positive by the O157 antiserum agglutination assay. The natural microflora of coleslaw did not grow on the SMACN plates because of the selective properties of the plates and the culture medium containing the nalidixic acid. Aerobic plate counts (APC) were determined at days 0 and 3 of storage by surface plating serially diluted samples (0.1 ml in duplicate) on plate count agar (Difco). Plates were incubated at 30°C for 48 h before colonies were counted. The pH of coleslaw stored at 4, 11, or 21°C for 0 and 3 days was determined.

Statistical analysis. All experiments were done in triplicate with duplicate samples analyzed at each sampling time. Data were subjected to analysis of variance and Duncan’s multiple range test.
RESULTS AND DISCUSSION

E. coli O157:H7 did not grow in either coleslaw preparation within 3 days at any of the three storage temperatures. Instead, populations of E. coli O157:H7 decreased (Fig. 1). The largest reduction occurred at 21°C (0.47 and 0.40 log_{10} CFU/g for coleslaw preparations 1 and 2, respectively). There were only slight reductions (0.09 to 0.16 log_{10} CFU/g) in populations in coleslaw held at 4 or 11°C for 3 days.

The acidic pH of the coleslaw was likely the principal factor preventing growth of E. coli O157:H7. The pH decreased at 21°C and did not change significantly at 4 and 11°C (Table 1). The initial APC values in coleslaw preparations 1 and 2 were 5.44 and 6.91 log_{10} CFU/g, respectively. After storage at 4 and 11°C for 3 days, the APC of both coleslaws declined by 0.22 to 0.61 log_{10} CFU/g; however, at 21°C, the APC increased by 2.43 and 1.40 log_{10} CFU/g in coleslaws 1 and 2, respectively, within 3 days. The natural microflora in coleslaw may have affected the survival of E. coli O157:H7; the largest increase of APC and decrease in E. coli O157:H7 populations occurred in coleslaw 1, even though coleslaw 2 had a lower pH (pH 4.09) than coleslaw 1 (pH 4.25) after storage for 3 days at 21°C.

Coleslaw generally has a pH of 4.0 to 4.5, which inhibits or prevents the growth of most foodborne bacterial pathogens (9). The acidic pH of coleslaw is attributed to the acetic acid (0.5 to 1.2%) in mayonnaise used in the dressing. Acetic acid has been reported to be more inhibitory to E. coli O157:H7 than lactic acid, citric acid, or malic acid in tryptic soy broth adjusted to pH 4.0 to 5.5 (6).

Survival characteristics of E. coli O157:H7 in mayonnaise have implications about its fate in coleslaw, since mayonnaise is the major source of acetic acid in coleslaw dressing. Zhao and Doyle (10) reported that E. coli O157:H7 did not grow in mayonnaise (pH 3.6 to 3.9) at 5 or 20°C, but when inoculated at a population of 3.81 log_{10} CFU/g, some cells survived for 34 to 55 days at 5°C and for 8 to 21 days at 20°C. Hathcox et al. (7) studied the behavior of E. coli O157:H7 in real mayonnaise and reduced-calorie mayonnaise. Growth was not observed in either type of mayonnaise stored at 5, 20, or 30°C. Instead, the pathogen died in both products stored at all test temperatures. The rate of death increased with increased storage temperature; viable E. coli O157:H7 cells initially at 6.23 log_{10} CFU/g were not detected in real mayonnaise stored at 20 and 30°C for 21 and 7 days, respectively, or in reduced-calorie mayonnaise dressing stored at 5, 20, and 30°C for 58, 11, and 4 days, respectively.

Mayonnaise usually has a pH of 3.6 to 4.0. When mayonnaise is combined with raw vegetables such as cabbage, the pH can increase to 4.0 to 4.5 (3). Although coleslaw preparations vary in formulation, the results of our study
indicate that coleslaw acidified to pH 4.3 or 4.5 will not likely support the growth of \textit{E. coli} O157:H7. Because of its acid tolerance, however, the pathogen survived with little reduction in population for 3 days at 4, 11, and 21°C. We conclude that the tolerance of \textit{E. coli} O157:H7 to acidic pH, not the growth of this pathogen due to temperature abuse, is a major factor contributing to its persistence in restaurant-prepared coleslaw. Contamination of coleslaw with \textit{E. coli} O157:H7 should be prevented during preparation to ensure the safety of the product.

REFERENCES