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Acid Adaptation of *Escherichia coli* O157:H7 Increases Survival in Acidic Foods

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***Escherichia coli* O157:H7 was adapted to acid by culturing for one to two doublings at pH 5.0. Acid-adapted cells had an increased resistance to lactic acid, survived better than nonadapted cells during a sausage fermentation, and showed enhanced survival in shredded dry salami (pH 5.0) and apple cider (pH 3.4). Acid adaptation is important for the survival of *E. coli* O157:H7 in acidic foods and should be considered a prerequisite for inocula used in food challenge studies.**

Since 1982, food-related diarrheal outbreaks caused by highly virulent strains of enterohemorrhagic *Escherichia coli* O157:H7 have been recognized in the United States (11, 16, 20). Enterohemorrhagic strains of *E. coli* produce Shiga-like toxins, and infections caused by *E. coli* O157:H7 can result in death, particularly in young children and the elderly (11, 20). Various foods have been implicated in outbreaks, including ground beef, raw milk, apple cider (2, 3, 11, 16, 19, 20), and most recently fermented hard salami (4). Since salami and apple cider rely in part on fermentation and acidity for microbial stability and safety, we were interested in determining if adaptation to acid (6, 12) would affect survival in these low-pH foods.

Several investigators have studied acid tolerance and survival of *E. coli* O157:H7 in broth and food systems (1, 15, 21, 22). In these studies, acid tolerance was determined, but cells were not adapted by growing them at low pH as is required for acid adaptation of *Salmonella typhimurium* (6, 7, 9). In *S. typhimurium*, acid adaptation appears to be distinctly different from acid tolerance. However, in *E. coli*, habituation to normal lethal acidity is induced in stationary-phase cells (12) or by prior growth of *E. coli* at a sublethal pH value (9). The survival of stationary-phase cells at extremely low pH is termed acid resistance or acid tolerance. Growth pH does not affect the stationary-phase acid resistance of wild-type cells but instead involves the expression of genes mediated by the alternative sigma factor 38 (12, 17, 18). Sigma 38 is not expressed during adaptation during growth at sublethal pHs. Acid adaptation and tolerance have been studied most extensively in *S. typhimurium* (5, 6, 7, 12), but these characteristics probably also occur in other enteric bacteria, including *E. coli* and *Shigella flexneri* (9, 17, 18).

We previously showed that acid adaptation increased the resistance of *Salmonella* spp. to various organic acids and also greatly increased their survival in certain foods (13). Acid adaptation induced cross-protection against environmental stresses that may be encountered during food processing, including heat, salt, an activated lactoperoxidase system, and surface-active agents (14). We investigated the biochemical mechanisms of acid resistance and found changes in outer membrane structure and possibly in specific porins (14). In view of the recent outbreaks of *E. coli* O157:H7 illnesses from the ingestion of acidic foods, including hard salami and apple cider, we examined in this study whether acid adaptation would affect survival in these foods.

Bacterial strains and growth conditions. Five strains of *E. coli* O157:H7 were used in the study. Strains 932 and 933 were originally obtained from G. K. Morris, Centers for Disease Control and Prevention, Atlanta, Ga. Strain 932 is a clinical isolate, and strain 933 was isolated from ground beef. The three other strains used were 505B, 204P, and C7927. Strain 505B was isolated at the Food Research Institute from ground beef, 204P was isolated from pork, and C7937 is a human isolate from the apple cider outbreak provided by M. P. Doyle, University of Georgia. Cells were routinely grown in tryptic soy broth (Difco Laboratories, Detroit, Mich.) at 37°C and stored on tryptic soy agar at 6°C. For acid adaptation, cells were grown overnight at 37°C in nutrient broth (Difco), pH 7.0, and 100 μl of the overnight culture was inoculated into nutrient broth at pH 7.0 for nonadapted cells or into broth acidified with HCl to a pH of 5.0 for acid-adapted cells (13). The cultures were grown for 4 to 5 h at 37°C, and 1 ml was harvested by centrifugation. The cell pellet was washed once in distilled water and resuspended in 100 μl of distilled water. Strain 933, which had the highest acid adaptation response, was used to determine resistance to acid in apple cider. The five-strain mixture was used for the evaluation of acid resistance in a sausage fermentation and in shredded salami. Three of the five strains were tested individually for the acid adaptation response. In food and in vitro studies, each experiment was repeated at least once.

Acid adaptation enhances resistance to lactic acid. Initially, the acid resistance of nonadapted (grown at pH 7.0) and acid-adapted *E. coli* O157:H7 was determined by adding the cell suspensions to 4 ml of E buffer (13) acidified with 125 mM lactic acid, pH 3.85, to give a cell concentration of $\sim 5 \times 10^7$ CFU/ml. The buffer was incubated statically at 25°C, and cells were enumerated over time by serial dilution in tryptic soy broth and by plating on tryptose-phosphate agar containing 0.1% (wt/vol) sodium pyruvate (TPAP), a medium which improves the recovery of acid-stressed cells (13). Adapted cells showed a marked increase in levels of resistance to lactic acid, but the level of resistance varied among the strains (Fig. 1). Of the three strains tested, 933 showed the greatest resistance to lactic acid after adaptation. For strain 933, the adapted popu-

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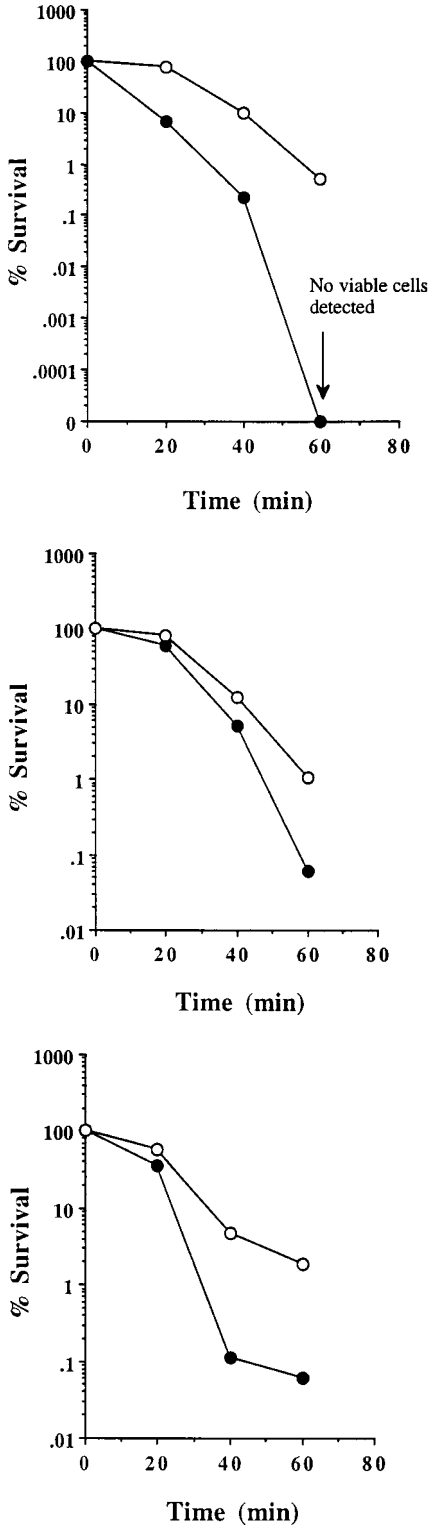

FIG. 1. Survival of acid-adapted (○) and nonadapted (●) *E. coli* O157:H7 during exposure to E buffer acidified to pH 3.85 and containing 125 mM lactic acid. (Top) Strain 933; (middle) strain 932; and (bottom) strain 505B. In this figure and in subsequent figures (2 to 4), each datum point represents the enumeration on three plates of platings conducted in duplicate. The maximum percent differences between replicate points in the panels (top to bottom) were 13.5, 12.26, and 14.3%, respectively.

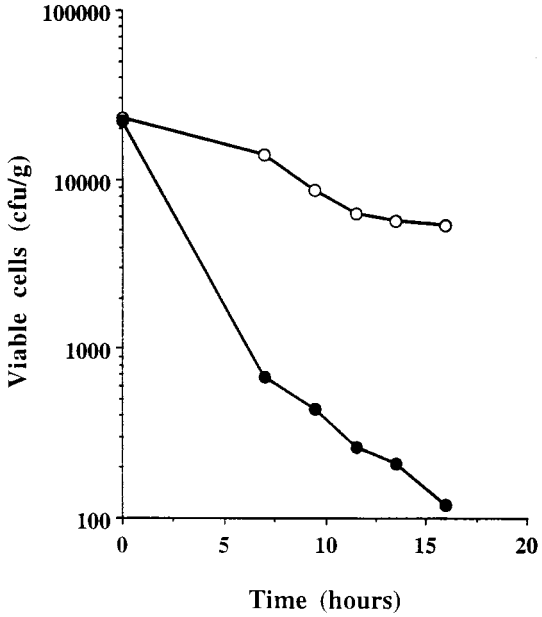

FIG. 2. Survival of *E. coli* O157:H7 during sausage fermentation by two strains of lactobacilli. Symbols: ○, acid adapted cells; ●, nonadapted cells. The pH at the time of the addition of *E. coli* O157:H7 was 5.6, and it further decreased to 4.4 after 14 h and remained at this level until the termination of the fermentation. The maximum percent difference between replicate points was 14.6%.

lation declined 100-fold after 60 min of exposure (Fig. 1). After the same period, nonadapted *E. coli* O157:H7 was not detected, indicating a 10^7-fold loss of viability (Fig. 1). The other strains showed an increased acid resistance after adaptation but not to the same magnitude as strain 933.

Survival of *E. coli* O157:H7 during a meat fermentation. Glass et al. (8) previously showed that *E. coli* O157:H7 survived but did not grow in fermented dry sausage. To determine any potential growth advantage of acid-adapted cells in a meat fermentation, adapted or nonadapted cells of *E. coli* O157:H7 were added to the fermentation when the meat had reached a pH of 5.6. The cells were thoroughly mixed in the sausage by thoroughly massaging the mixture by hand in a Whirlpak bag. Adapted cells were added at this stage to avoid the induction of acid adaptation in the nonadapted population during fermentation. After the addition of the cultures, the fermentation was allowed to proceed at 32°C and the numbers of *E. coli* O157:H7 organisms were determined by plating on Sorbitol-MacConkey agar. Background flora on Sorbitol-MacConkey agar were readily distinguished by colony morphology, and individual colonies were tested for the O157 antigen. In the experiments involving salami, in which the number of background flora was large, *E. coli*-like colonies were confirmed by testing for the O157 antigen by latex agglutination (Unipath, Oxoid U.S.; Diagnostic reagent DR620). The acid-adapted cells showed an increased survival compared with that of the control cells (Fig. 2). About 7 h (pH 5.0) after the addition of the cells, the number of nonadapted cells had dropped from 22,000 to 680 viable cells per g, whereas the number of adapted cells dropped from 23,000 to 14,000 cells per g. The pH continued to drop and was 4.8 at 10 h and 4.6 at 12 h, and then it remained constant at pH 4.4. During this drop, the nonadapted cell population decreased to 120 cells per g, whereas the acid-adapted population decreased only to 5×10^3 cells per g. These results clearly showed that acid adaptation increased the

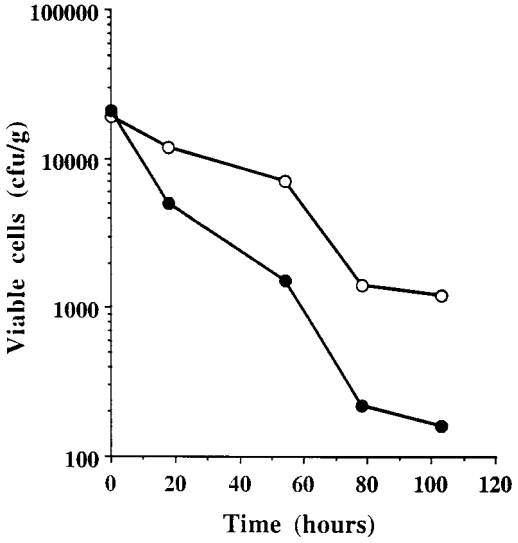

FIG. 3. Survival of *E. coli* O157:H7 in hard salami (pH 5) during storage at 5°C. Symbols: ○, acid-adapted cells; ●, nonadapted cells. The maximum percent difference between replicates was 13.7%.

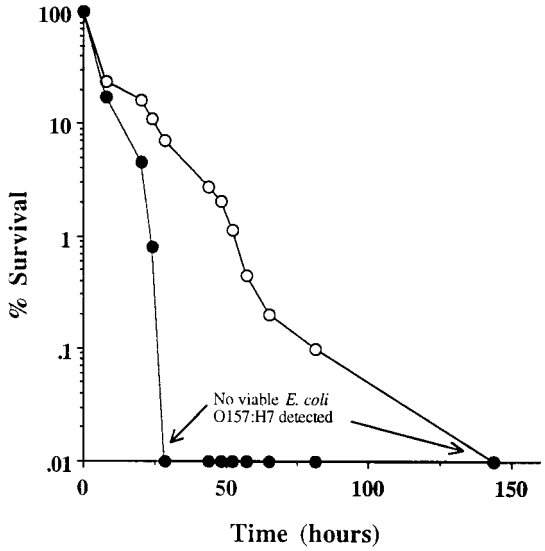

FIG. 4. Survival of *E. coli* O157:H7 in apple cider at pH 3.46. Symbols: ○, acid-adapted cells; ●, nonadapted cells. The maximum percent difference between replicates was 14.3%.

rate of survival during the fermentation, which involves the production of acids by lactic acid bacteria.

Survival of *E. coli* O157:H7 in shredded hard salami. We next evaluated whether acid adaptation would affect the survival of *E. coli* O157:H7 in dry salami. Hard salami was chosen as the substrate, because it had recently been implicated in an outbreak of hemorrhagic colitis and it contains a variety of microbial fermentation products, including organic acids. The salami was shredded in a food processor. A five-strain mixture of *E. coli* O157:H7 was inoculated in ~200 μl of phosphate buffer into 50 g of shredded salami. The cells were mixed by thorough massaging in a sterile Whirlpak bag. Adapted and control cells were inoculated at $\sim 2 \times 10^4$ cells per g into two independent batches and incubated at 5°C. Cells were recovered by extracting the salami with equal volumes of water. Adapted cells survived much better than the nonadapted population (Fig. 3), and 1,200 viable cells per g were detected in the adapted population compared with 160 cells per g in the control at the termination of the incubation. Although the experiment did not exactly simulate the conditions in commercial hard salami, the results indicated that acid-adapted cells may survive better in this food environment.

Acid tolerance and survival in apple cider. Pasteurized apple cider was purchased from a local grocer in Madison, Wis. This cider contained no preservatives and had an initial pH of 3.42. The cider did not yield bacterial colonies when plated on TPAP agar. *E. coli* O157:H7 strain 933 with the highest acid resistance was used and enumerated on TPAP plus pyruvate since this medium is superior for recovering acid-stressed cells (13). Cells were acid adapted as previously described and inoculated into the cider to give an initial cell concentration of $\sim 1 \times 10^5$ CFU/ml. The cider was stored at 6°C, and viable *E. coli* O157:H7 organisms were enumerated over time. Nonadapted *E. coli* O157:H7 933 died off very rapidly in the apple cider, and viable nonadapted cells were not detected after 28 h of incubation (Fig. 4). However, acid-adapted *E. coli* O157:H7 cells survived substantially longer in the cider (Fig. 4). After 28 h, the nonadapted population was completely inactivated but the acid-adapted cell population had decreased about 10-fold and adapted cells were detected at ~60 CFU/ml after 81 h. At 100 h, viable *E. coli* O157:H7 organisms were no longer detected in either population.

The acid adaptation response and its impact on resistance to environmental stresses, especially acid, have been extensively examined for *S. typhimurium*, but comparatively little work has been done with *E. coli*. We have shown here that *E. coli* O157:H7 has an acid-adaptive response, and the expression of this system enhances survival in the presence of lactic acid and in acidified food products such as fermented sausage and apple cider. In this study, nonadapted *E. coli* O157:H7 organisms survived for a shorter time in apple cider than previously reported (15). However, the cider used in this study had a lower pH (3.4) than ciders evaluated previously (pH 3.6 to 4.0). Also, strains of *E. coli* O157:H7 vary widely in their acid tolerance (15, 21, 22), and survival would be expected to differ in studies employing different strains.

Acid tolerance is probably an important component of virulence for *E. coli* O157:H7 and it allows a small number of cells to cause illness by their being protected in the gastric tract. Gorden and Small (10) reported that enteroinvasive and enteropathogenic *E. coli* organisms are significantly more acid tolerant than nonpathogenic strains such as *E. coli* K-12. Results from other laboratories (5, 6, 7, 9, 12) indicate that *E. coli* O157:H7 has several acid tolerance systems that differ from those of *Salmonella* spp. In addition to lowering the pH, acid adaptation may occur after exposure to weak organic acids such as benzoic acid or phentylacetic acid at pHs near neutrality, such as in the rumen or in feces.

The ability of food-borne pathogens to exhibit adaptation responses to stressful conditions has received little attention in food microbiology. We have shown here and in previous studies (13, 14) that adaptation to acid by enteric pathogens can significantly enhance their survival in acidic foods and alter other physiological characteristics of the cell. Our studies support the hypothesis that it is important in laboratory food challenge studies to consider the use of stressed or adapted cells, since the use of healthy exponentially growing cultures may inaccurately represent their survival state in the natural environment.

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