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Effects of pH, Dissolved Oxygen, and Ionic Strength on the Survival of *Escherichia coli* O157:H7 in Organic Acid Solutions†‡

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ABSTRACT

The ability of *Escherichia coli* O157:H7 to survive in acidified vegetable products is of concern because of previously documented outbreaks associated with fruit juices. A study was conducted to determine the survival of *E. coli* O157:H7 in organic acids at pH values typical of acidified vegetable products (pH 3.2 and 3.7) under different dissolved oxygen conditions (≤0.05 and 5 mg/liter) and a range of ionic strengths (0.086 to 1.14). All solutions contained 20 mM gluconic acid, which was used as a noninhibitory low pH buffer to compare the individual acid effect to that of pH alone on the survival of *E. coli* O157:H7. *E. coli* O157:H7 cells challenged in buffered solution with ca. 5-mg/liter dissolved oxygen (present in tap water) over a range of ionic strengths at pH 3.2 exhibited a decrease in survival over 6 h at 30°C as the ionic strength was increased. Cells challenged in 40 mM protonated L-lactic and acetic acid solutions with ionic strength of 0.684 achieved a >4.7-log CFU/ml reduction at pH 3.2. However, under oxygen-limiting conditions in an anaerobic chamber, with ≤0.05-mg/liter oxygen, *E. coli* O157:H7 cells showed ≤1.55-log CFU/ml reduction regardless of pH, acid type, concentration, or ionic strength. Many acid and acidified foods are sold in hermetically sealed containers with oxygen-limiting conditions. Our results demonstrate that *E. coli* O157:H7 may survive better than previously expected from studies with acid solutions containing dissolved oxygen.

Outbreaks of disease from the consumption of acid foods, such as apple cider and apple juice, have raised concerns about the safety of foods that use low pH or organic acids as the main barrier to pathogenic bacteria *(8, 9).* The U.S. Code of Federal Regulations (21 CFR part 114) defines acid and acidified foods as having a pH of 4.6 or lower. Acid foods are foods that naturally have a pH below 4.6. Acidified foods reach their equilibrated pH of 4.6 or lower through the addition of acid or acid food ingredients, such as organic acids.

Organic acids and their salts have been used for many years as food preservatives in sauces, mayonnaises, dressings, drinks, and fruit juices *(23).* These organic acids are thought to enter the bacterial cell in their undissociated form by diffusion across the nonpolar cell membrane *(6).* Studies by Eklund *(17)* demonstrated that the undissociated sorbic acid was 15 to 600 times more effective in inhibiting the growth of *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa,* and *Staphylococcus aureus* than the dissociated form. The acid anion cannot readily diffuse through the cell membrane and may accumulate to molar amounts in the cell cytoplasm, depending on the difference between the internal and external pH *(14).* Organic acids may act on the cell by lowering intracellular pH and inhibiting metabolic reactions. The intracellular accumulation of acid anions may also have deleterious effects *(6).* The antimicrobial activity of an organic acid may be affected by several factors such as the pK_a, temperature, specific type of acid, acid concentration, pH, and ionic strength.

E. coli O157:H7 is a gram-negative, facultative anaerobic bacterium commonly found in the gut of ruminants, specifically cattle *(16).* The bacterium can cause mild diarrhea, bloody diarrhea, life-threatening hemolytic uremic syndrome in children, and thrombotic thrombocytopenic purpura in adults *(16, 26).* In the United States, an estimated 62,458 illnesses, 1,843 hospitalizations, and 52 deaths are caused annually by foodborne infections of *E. coli* O157:H7 *(21).* Studies by Leyer et al. *(20)* demonstrated the ability of *E. coli* O157:H7 to survive in apple cider (pH 3.4) held for 3 days at 6°C. The low infectious dose of *E. coli* O157:H7, estimated to be as few as 10 to 100 cells *(10),* coupled with its ability to survive passage through the human stomach (pH 2; anaerobiosis) *(22)* demonstrates the need to better investigate the survival of this pathogen under conditions typical of acidified vegetable products *(2, 3).*

The objectives of this study were to examine the effects of pH, acid type, ionic strength, and dissolved oxygen on

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the survival of *E. coli* O157:H7 cells. Many acidified vegetable products have brine pH values between 3.0 and 3.3 (condiments) or around pH 3.7 (acidified cucumber pickles). Salt concentrations for acidified vegetables typically range from 2 to 4% NaCl, equivalent to ionic strength values of 0.342 and 0.684, respectively. These products are prepared in hermetically sealed jars, resulting in an anaerobic environment. Our results indicate that *E. coli* O157:H7 may survive better than previously expected from studies in which anaerobic conditions were not considered.

MATERIALS AND METHODS

Bacterial strains. *E. coli* B200 (strain O157:H7, ATCC 43888, human feces isolate), *E. coli* B201 (strain O157:H7, SRCC 1675, apple cider outbreak isolate), *E. coli* B202 (strain O157:H7, SRCC 1486, salami outbreak isolate), *E. coli* B203 (strain O157:H7, SRCC 206, ground beef isolate), and *E. coli* B204 (strain O157:H7, SRCC 1941, pork isolate) were used.

Preparation of inocula for acid challenge. *E. coli* B202 was used in these experiments because it has been shown to be the most acid resistant of the five listed above *(1).* Stock cultures of *E. coli* B202 stored in tryptic soy broth (TSBG; BBL/Difco, Becton Dickinson, Sparks, Md.) supplemented with 1% glucose (Sigma Chemical Co., St. Louis, Mo.) and 16% glycerol at −80°C were streaked by loop inoculum (ca. 10 μl) onto tryptic soy agar (TSAG; BBL/Difco, Becton Dickinson) supplemented with 1% glucose and incubated for 24 h at 30°C (to be consistent with the temperature of acid treatments). Three colonies of strain B202 were then transferred by loop inoculum into 10 ml of TSBG and incubated statically for ca. 18 h at 30°C to induce acid resistance as previously described *(7).* The overnight cultures typically attained 10^9 CFU/ml and a pH of approximately 4.9. Cultures were centrifuged (3,000 × *g*, 10 min, 23 ± 2°C), washed, and resuspended in 0.85% saline solution. To determine initial populations, cells were serially diluted, spiral plated in duplicate (model 4000, Spiral Biotech, Inc., Norwood, Mass.) on TSAG, and incubated at 30°C for 24 h.

Preparation of inocula for acidified cucumber brine. Five strains of *E. coli* O157:H7 were used: *E. coli* B200, *E. coli* B201, *E. coli* B202, *E. coli* B203, and *E. coli* B204. Cultures were prepared as described previously *(2).* Briefly, each strain was grown statically in TSBG for 18 h as described above. The cell suspensions, containing approximately 10^9 CFU/ml, were centrifuged, and the cell pellet was resuspended in 0.1 fraction of the original culture volume by using sterile saline (0.85% NaCl). Equal amounts of each suspension were combined into a cocktail. The initial inoculum in the cucumber brine was approximately 10^8 CFU/ml for the strain mixture. The initial cell concentration was determined by plating as described above.

Preparations of acid solutions. Calculations of pH, ionic strength, and protonated acid concentrations for the buffered acid solutions were made using custom Matlab routines (pH_{tools}, available at: http://www.mathworks.com/matlabcentral) as previously described *(15).* This program also allows for the adjustment of pK_a values based on ionic strength and temperature. L-lactic and acetic acid (Sigma) were tested at concentrations ranging from 0.1 to 40 mM. We used L-lactic acid for our experiments because we have found that D-lactic acid can have a protective effect on the survival of *E. coli* O157:H7 at pH 3.2, compared to the inhibition caused by buffered control solution at the same pH *(2).* All solutions contained gluconic acid (Sigma), which was used as a noninhibitory low pH buffer (20 mM) to determine the inhibitory effects of pH 3.2 and 3.7 in the absence of added organic acids as previously described *(3).* Solutions containing only 20 mM gluconic acid (buffered solution, with no additional organic acid) were used as the controls. The acid solutions were adjusted using HCl or NaOH to reach the desired pH of 3.2 or 3.7, and NaCl was added, as needed, to produce the indicated ionic strength of 0.342 or 0.684. Solutions incubated in the anaerobic chamber (COY, Grass lake, Mich.) were allowed to equilibrate for at least 48 h prior to use. The mixed anaerobic gas atmosphere consisted of 5% CO_2, 10% H_2, and 85% N_2. The dissolved oxygen content of solutions was measured using the CellOx 325 dissolved oxygen sensor (WTW, Weilheim, Germany). The lower limit of detection with this instrument was ca. 0.05 mg/liter.

Acid challenge. The acid solutions (180 μl) were dispensed into wells of a 96-well microtiter plate (Microtest flat bottom plate, BD, Franklin Lakes, N.J.) and allowed to equilibrate at 30°C for 1 h before the inoculum was added. The inoculum (20 μl) resulted in an initial cell suspension of ca. 2×10^8 CFU/ml. The solutions were vortexed for 30 s and incubated statically for 6 h at 30°C. After incubation, 100 μl of the test suspension was diluted in 900 μl of 50 mM MOPS (morpholinepropanesulfonic acid) buffer (Sigma) adjusted to pH 7.2, and the solution was vortexed to facilitate neutralization. Suspensions were serially diluted in 450 μl of saline solutions and plated in duplicate on TSAG. The lower limit for detection of bacterial cells by this method was ca. 4×10^3 CFU/ml. The plates were incubated at 30°C for 24 h to determine the reduction in viable cells.

Acid challenge in acidified cucumber brine. Acidified pickles were prepared as previously described *(4).* Briefly, size 2B cucumbers (ca. 4 cm in diameter) were obtained from a local supplier. Cucumbers (789 ± 2 g) were packed in 1.36-liter (46-oz) glass jars filled with 571 ± 2 ml of brine solution (4% sodium chloride, 0.2% calcium chloride) to equilibrate at 2% sodium chloride, 0.1% calcium chloride, and pH 3.3. Food grade vinegar (20% acetic acid) was used to achieve the target pH (ca. 400 mM acetic acid). After filling and sealing, the jars were heat processed so that the cold point of each jar was held at 74.4°C for 15 min. The jars were stored at 25°C for at least 10 days to allow the equilibration of water-soluble components (e.g., acids, salt, and sugars) between the brine solution and the cucumbers. Brine was removed from the jars and added to double-walled fermentation flasks as previously described *(5).* Remaining jars and flasks were inoculated with a cocktail of five *E. coli* O157:H7 strains prepared as described above to give an initial inoculum of 10^8 CFU/ml. Jars were sampled through rubber septa affixed to the lids as previously described *(4).* Both the jars and flasks were incubated at 30°C, and brine samples (0.5 ml) were taken at the indicated time intervals, serially diluted, and plated as described above.

Statistical analysis. Experiments with buffered acid solutions were replicated three times with two test solutions per replicate, and brine experiments were carried out in triplicate. Log reduction was calculated as the difference between the initial count and the final count ($\log[N_0/N]$). Log numbers were analyzed using the general linear models with Tukey adjustment of the Statistical Analysis Systems version 8.0 (Statistical Analysis System, SAS Institute, Cary, N.C.). For modeling the reduction in CFU per milliliter for brine experiments, a Weibull model was used as previously described *(5).*

RESULTS

Dissolved oxygen. Solutions prepared for use in experiments conducted on the bench top were found to con-

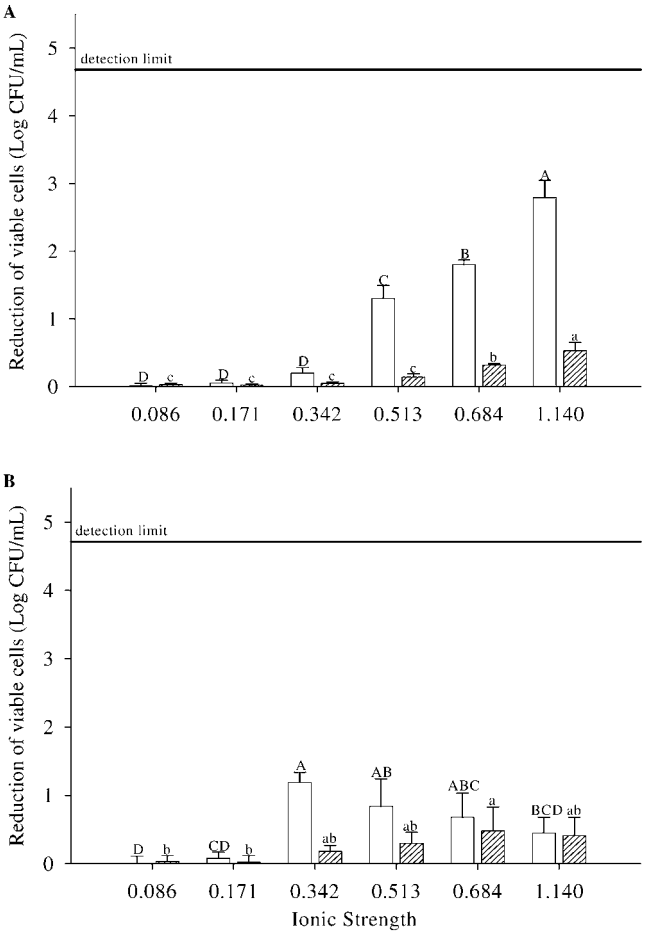

FIGURE 1. *Log reductions of* E. coli *O157:H7 in a range of ionic strengths after 6 h under (A) aerobic and (B) oxygen-limiting conditions at pH 3.2 (empty bars) and 3.7 (striped bars). All solutions were incubated at 30°C for 6 h. Error bars indicate the standard deviation for three trials. Identical letters over error bars indicate that there is no significant difference between treatments (*P > 0.05*); capital letters, pH 3.2; lowercase, pH 3.7.*

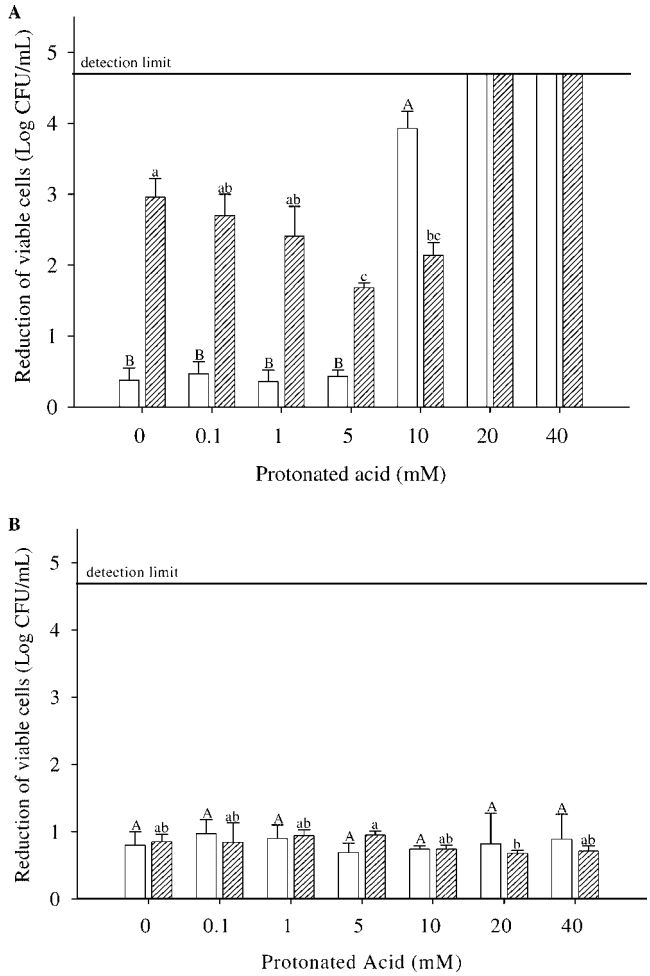

FIGURE 2. *Log reductions of* E. coli *O157:H7 in concentrations of lactic acid after 6 h under (A) aerobic and (B) oxygen-limiting conditions with ionic strength (μ) of 0.342 (empty bars) and 0.684 (striped bars) at pH 3.2. All solutions were incubated at 30°C for 6 h. Error bars indicate the standard deviation for three trials. Identical letters over error bars indicate that there is no significant difference between treatments (*P > 0.05*); capital letters, μ = 0.342; lowercase, μ = 0.684.*

tain 5.3 ± 0.2 mg of dissolved oxygen per liter at 25 ± 2°C and were designated "aerobic." The maximum dissolved oxygen concentration of distilled water at sea level (37°C) is approximately 6.7 mg/liter (0.21 mM). The solutions in the anaerobic chamber equilibrated for up to 1 week had oxygen-limiting conditions and retained 0.05 mg of dissolved oxygen per liter or less and were designated "anaerobic."

Ionic strength. The log reductions of *E. coli* O157:H7 cells recovered from solutions with various ionic strengths (μ) buffered at pH 3.2 and 3.7 (adjusted with HCl) are shown in Figure 1A and 1B. There were no significant differences ($P > 0.05$) in cell survival between μ of 0.086 and μ of 0.342 under aerobic conditions, regardless of pH. However, there was a gradual decrease in the survival as the ionic strength increased up to 1.14 at pH 3.2 and 3.7 (Fig. 1A). Under anaerobic conditions (≤0.05 mg/liter, anaerobic), cells treated with μ of 0.342 were reduced by 1.19 log CFU/ml compared with only a 0.45-log CFU/ml reduction with μ of 1.140 at pH 3.2 (Fig. 1B).

Lactic acid. The log reductions of *E. coli* O157:H7 cells recovered from solutions containing 0 to 40 mM protonated lactic acid at pH 3.2 are shown in Figure 2A and 2B. There was no significant difference ($P > 0.05$) in the survival of *E. coli* O157:H7 cells recovered from the control solution and solutions containing up to 5 mM protonated lactic acid (μ, 0.342) under aerobic conditions (Fig. 2A). There was a 1.67-log CFU/ml reduction in cells treated with 5 mM protonated lactic acid (μ, 0.684) compared with a 2.96-log CFU/ml reduction in cells treated with the control solution. An increase in survival at 5 mM lactic acid (pH 3.2; μ, 0.60 to 0.68; 25°C) was also reported by Bjornsdottir et al. *(2).* Under anaerobic conditions, there was no significant difference ($P > 0.05$) in the reduction of *E. coli* O157:H7 cells treated with the control solution (0.85 log CFU/ml) compared with 40 mM lactic acid (0.71 log CFU/ml; μ, 0.684) (Fig. 2B). Survival levels of cells treated with solutions with μ of 0.342 and 0.684 were not

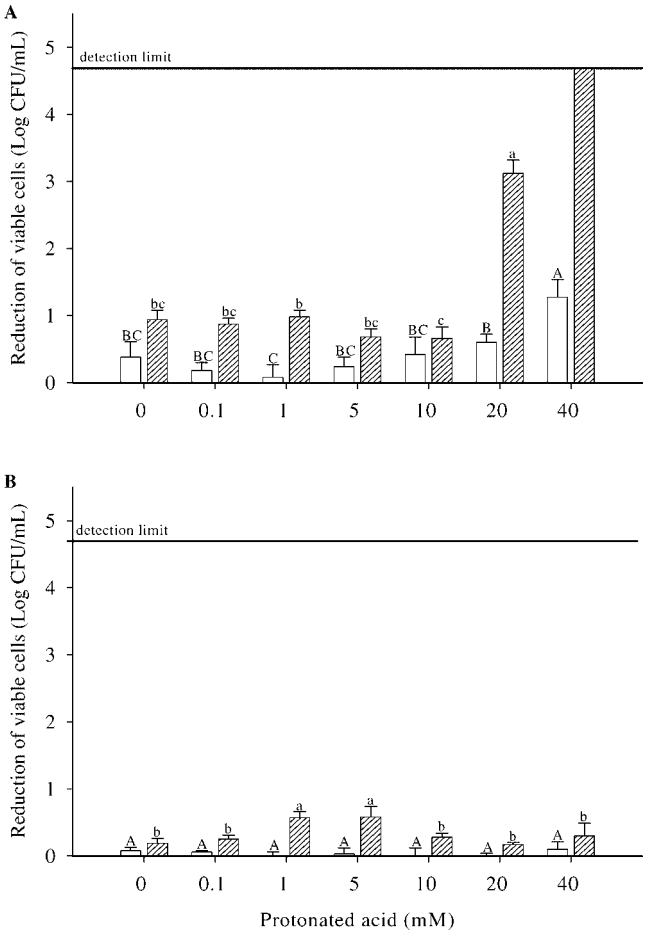

FIGURE 3. *Log reductions of* E. coli *O157:H7 in concentrations of lactic acid after 6 h under (A) aerobic and (B) oxygen-limiting conditions with μ of 0.342 (empty bars) and μ of 0.684 (striped bars) at pH 3.7. All solutions were incubated at 30°C for 6 h. Error bars indicate the standard deviation for three trials. Identical letters over error bars indicate that there is no significant difference between treatments (*P > 0.05*); capital letters, μ = 0.342; lowercase, μ = 0.684.*

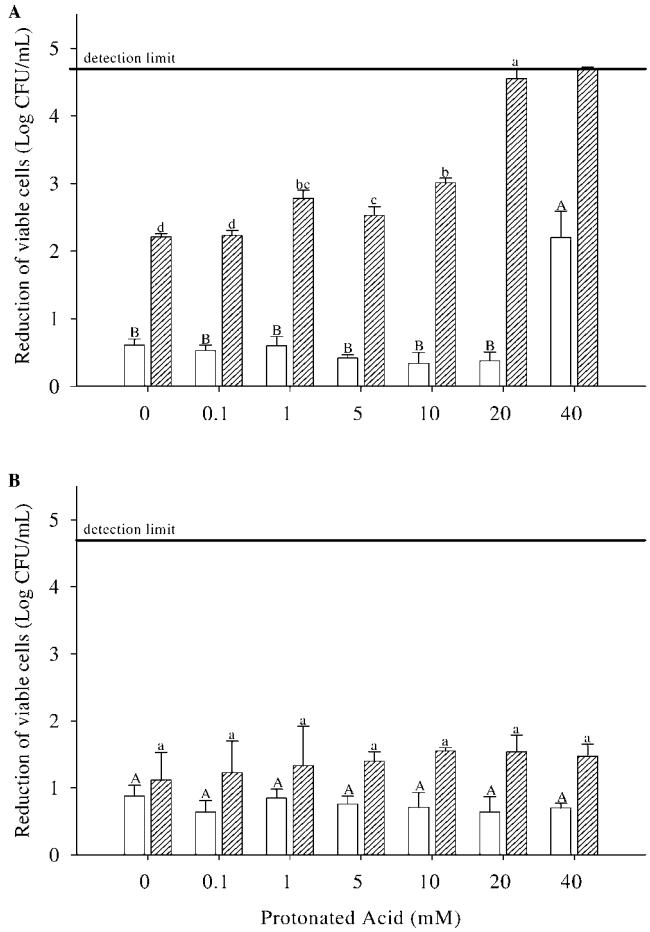

FIGURE 4. *Log reductions of* E. coli *O157:H7 in concentrations of acetic acid after 6 h under (A) aerobic and (B) oxygen-limiting conditions with μ of 0.342 (empty bars) and μ of 0.684 (striped bars) at pH 3.2. All solutions were incubated at 30°C for 6 h. Error bars indicate the standard deviation for three trials. Identical letters over error bars indicate that there is no significant difference between treatments (*P > 0.05*); capital letters, μ = 0.342; lowercase, μ = 0.684.*

significantly different ($P > 0.05$) under anaerobic conditions.

The log reductions of *E. coli* O157:H7 cells recovered from solutions of protonated lactic acid (0 to 40 mM) at pH 3.7 are shown in Figure 3A and 3B. *E. coli* O157:H7 cells treated with 40 mM protonated lactic acid (μ, 0.342) were reduced in number by 1.27 log CFU/ml, while cells in 40 mM protonated lactic acid (μ, 0.684) were reduced by >4.7 log CFU/ml. There was no significant difference ($P > 0.05$) in the survival of *E. coli* O157:H7 cells recovered from any test solutions with μ of 0.342 under anaerobic conditions (Fig. 3B), and all anaerobic test solutions resulted in a reduction of ≤0.58 log CFU/ml regardless of ionic strength.

Acetic acid. The log reduction in *E. coli* O157:H7 cells recovered from solutions of protonated acetic acid (0 to 40 mM) at pH 3.2 are shown in Figure 4A and 4B. There was no significant difference ($P > 0.05$) in the reduction of *E. coli* O157:H7 cells treated with acid solutions ranging from 0 to 20 mM protonated acetic acid at μ of 0.342. For all concentrations of protonated acetic acid tested, we observed a significant decrease in survival of *E. coli* O157:H7 cells treated with solutions of μ of 0.684 compared to μ of 0.342. *E. coli* O157:H7 cells treated with solutions with μ of 0.684 decreased in survival as the concentration of acetic acid was increased up to 40 mM. Cells treated with solutions containing 40 mM protonated acetic acid were reduced by 2.20 log CFU/ml at μ of 0.342 and by >4.7 log CFU/ml at μ of 0.684. Under anaerobic conditions, there was no significant difference in the reduction of *E. coli* O157:H7 cells treated with solutions containing up to 40 mM acetic acid, regardless of ionic strength.

Cells treated with 40 mM protonated acetic acid (pH 3.7) at μ of 0.342 and 0.684 were reduced by 0.62 and 1.26 log CFU/ml, respectively (data not shown). Solutions containing protonated acetic acid (up to 40 mM) were not significantly different ($P \geq 0.05$) in reducing *E. coli* O157:H7 cells under anaerobic conditions (≤0.80 log CFU/ml), regardless of ionic strength (data not shown).

Cucumber brine. Survival of *E. coli* O157:H7 in acidified cucumber brine was measured in sealed jars, as well

as with the equilibrated brine removed from the jars. Immediately upon opening, the dissolved oxygen measured in the jars was 0.5 mg/liter; however, similar measurements were obtained with solutions having 0.05 mg/liter oxygen or less that were measured immediately upon removal from the anaerobic chamber (data not shown). With our apparatus, it was not possible to directly measure the dissolved oxygen in the sealed jars. The bacterial cell counts from the brine removed from the jars were below the limit of detection within 12 h. More than 60 h was required for the detection limit to be reached with cells inoculated and sampled through the septa in the jars (Figure 5). Because the brine composition, pH, and ionic strength were the same for all samples, the data indicate that reduced oxygen content of the brine in the jars was responsible for the 48-h difference in the time needed to achieve a 5-log reduction in *E. coli* O157:H7 cell numbers.

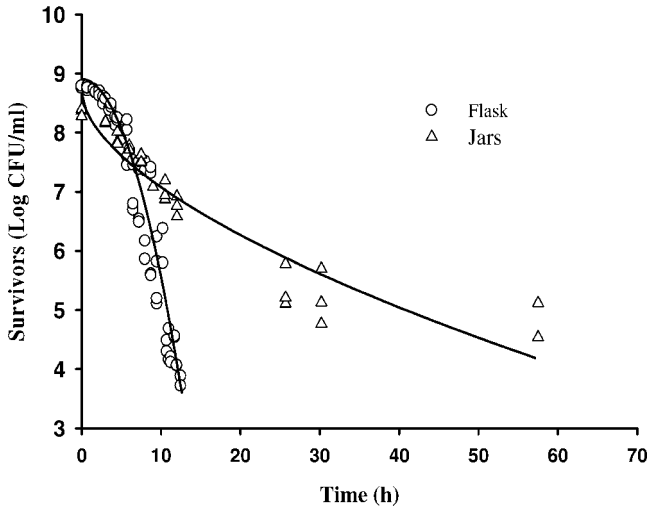

FIGURE 5. *Survival of* E. coli *O157:H7 in acidified pickle brines. Bacteria cell counts were obtained from sealed jars through rubber septa (triangles) or in brine removed from jars and exposed to air (circles). The lines represent the Weibull models of each data point with three replications.*

DISCUSSION

We investigated the ability of *E. coli* O157:H7 to survive in acid solutions under anaerobic conditions. Acidified vegetable products such as pickles, banana peppers, and olives are typically prepared in hermetically sealed jars (anaerobic conditions). These types of products are protected through a hurdle strategy using a combination of pasteurization, salt (2 to 4%), and low pH (typically pH 3 to 4) via addition of organic acids. However, some acidified vegetable products cannot be pasteurized and maintain acceptable sensory properties. Although there have not been any known outbreaks of disease from acid-resistant vegetative pathogens in acidified vegetables, recent research *(3, 4)* has shown that *E. coli* O157:H7 can survive in these products.

Salt has long been used to preserve and flavor foods. Studies by Entani et al. *(19)* showed that NaCl worked synergistically with acetic acid to increase its bactericidal action against *E. coli* O157:H7. Clavero and Beuchat *(11)* demonstrated that the combination of decreased pH (pH 4.8) and water activity (a_w 0.90, adjusted by NaCl) increased the inactivation or inhibition of *E. coli* O157:H7 in TSB at 30°C over 48 h. Our results show that the addition of NaCl to the organic acid solutions under aerobic, but not anaerobic, conditions correlated with a decrease in the survival of *E. coli* O157:H7 for the conditions tested. Additional studies with greater acid concentrations are needed to further investigate this effect.

Lactic acid is a common fermentation product and is used to lower the pH of foods *(18, 25).* Acetic acid is used in the food industry as an acidulant and flavoring agent in food items. Organic acid toxicity for bacterial cells is attributed to lowering cytoplasmic pH and intracellular accumulation of acid anion *(14).* A >4.7-log CFU/ml reduction was achieved after 6 h when cells were treated with 40 mM protonated lactic or acetic acid at pH 3.2 (μ, 0.684), regardless of ionic strength. Under anaerobic conditions, there was ≤1.55-log CFU/ml reduction in the populations of *E. coli* O157:H7 incubated in these solutions. Cleary and McFeeters *(12)* found the dissolved oxygen content of commercial acidified cucumber pickles to be ca. 0.45 ± 0.9 mg/liter when measurements were taken upon opening the jars. Due to rapid equilibration, it is likely that the dissolved oxygen content was <0.45 mg/liter. As shown in Figure 5, once brine is exposed to dissolved oxygen, the survival of *E. coli* O157:H7 is significantly reduced. Further research is being carried out to investigate and exploit this phenomenon.

Previous experiments conducted on the inhibition of acid resistant pathogenic bacteria by organic acids have been confounded by differences in experimental design and methods for calculating acid effects. For example, data from Young and Foegeding *(27)* can be used to show that citric acid was more inhibitory to *Listeria monocytogenes* than lactic and acetic acids, based on protonated acid concentrations, but for total acid concentrations (acid anion plus the protonated acid), the order is reversed. Ryu et al. *(24)* and Deng et al. *(13)* found that acetic acid was the most inhibitory to *E. coli* O157:H7 when added to media to reach the desired pH. By controlling pH, ionic strength, and temperature, Bjornsdottir et al. *(2)* found that protonated lactic acid was the most inhibitory to *E. coli* O157:H7. However, these reports on the inhibitory effects of organic acids on *E. coli* O157:H7 do not take into consideration the effects of dissolved oxygen in the acid solutions.

We have demonstrated that an *E. coli* O157:H7 strain can survive significantly better in acid solutions under anaerobic conditions than under aerobic conditions. For the acid solutions tested in the absence of oxygen, *E. coli* O157:H7 was reduced only by ≤1.55 log CFU/ml for all conditions tested. We found that increasing the incubation time from 6 h up to 24 h did not enhance the reduction of *E. coli* O157:H7 cells under anaerobic conditions *(1).* Under aerobic conditions, a >4.7-log or greater reduction was observed. Results from previous research carried out with oxygen-containing bench-top solutions may not be representative of acid or acidified food products in hermetically sealed containers or anaerobic digestive organs of animals. Further characterization of the effect of oxygen limitation

on the survival of *E. coli* and other acid-resistant pathogens in acid solutions may help ensure the safety of acid and acidified foods.

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REFERENCES

1. Bjornsdottir, K. 2005. Effects of organic acids on *Escherichia coli* O157:H7 independent of pH under acidified food conditions. M.S. thesis. North Carolina State University, Raleigh.
2. Bjornsdottir, K., F. Breidt, Jr., and R. F. McFeeters. 2006. Protective effects of organic acids on survival of *Escherichia coli* O157:H7 in acidic environments. *Appl. Environ. Microbiol.* 72:660–664.
3. Breidt, F., Jr., J. S. Hayes, and R. F. McFeeters. 2004. Independent effects of acetic acid and pH on survival of *Escherichia coli* in simulated acidified pickle products. *J. Food Prot.* 67:12–18.
4. Breidt, F., Jr., J. Hayes, and R. F. McFeeters. 2007. Determination of 5-log reduction times for food pathogens in acidified cucumbers during storage at 10 and 25°C. *J. Food Prot.* 70:2638–2641.
5. Breidt, F., Jr., J. S. Hayes, J. A. Osborne, and R. F. McFeeters. 2005. Determination of 5-log pathogen reduction times for heat-processed, acidified vegetable brines. *J. Food Prot.* 68:305–310.
6. Brul, S., and P. Coote. 1999. Preservative agents in foods: mode of action and microbial resistance mechanisms. *Int. J. Food Microbiol.* 50:1–17.
7. Buchanan, R., and S. Edelson. 1996. Culturing enterohemorrhagic *Escherichia coli* in the presence and absence of glucose as a simple means of evaluating the acid tolerance of stationary-phase cells. *Appl. Environ. Microbiol.* 62:4009–4013.
8. Centers for Disease Control and Prevention. 1996. Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice—British Columbia, California, Colorado, and Washington, October 1996. *Morb. Mortal. Wkly. Rep.* 45: 975.
9. Centers for Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider—Connecticut and New York, October 1996. *Morb. Mortal. Wkly. Rep.* 46:4–8.
10. Chart, H. 2000. VTEC enteropathogenicity. *J. Appl. Microbiol.* 88: 12S–23S.
11. Clavero, M., and L. Beuchat. 1996. Survival of *Escherichia coli* O157:H7 in broth and processed salami as influenced by pH, water activity, and temperature and suitability of media for its recovery. *Appl. Environ. Microbiol.* 62:2735–2740.
12. Cleary, K., and R. F. McFeeters. 2006. Effects of oxygen and turmeric on the formation of oxidative aldehydes in fresh-pack dill pickles. *J. Agric. Food Chem.* 54:3421–3427.
13. Deng, Y., J.-H. Ryu, and L. R. Beuchat. 1999. Tolerance of acid-adapted and non-adapted *Escherichia coli* O157:H7 cells to reduced pH as affected by type of acidulant. *J. Appl. Microbiol.* 86:203–210.
14. Diez-Gonzalez, F., and J. Russell. 1997. The ability of *Escherichia coli* O157:H7 to decrease its intracellular pH and resist the toxicity of acetic acid. *Microbiology* 143:1175–1180.
15. Dougherty, D. P., E. R. Da Conceicao Neta, R. F. McFeeters, S. R. Lubkin, and F. Breidt. 2006. Semi-mechanistic partial buffer approach to modeling pH, the buffer properties, and the distribution of ionic species in complex solutions. *J. Agric. Food Chem.* 54:6021–6029.
16. Doyle, M. P. 1991. *Escherichia coli* O157: H7 and its significance in foods. *Int. J. Food Microbiol.* 12:289–301.
17. Eklund, T. 1983. The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. *J. Appl. Bacteriol.* 54: 383–389.
18. Eklund, T. 1989. Organic acid and esters, p. 161–200. *In* G. W. Gould (ed.), Mechanisms of action of food preservation procedures. Elsevier Applied Science, New York.
19. Entani, E., M. Asai, S. Tsujihata, Y. Tsukamoto, and M. Ohta. 1998. Antibacterial action of vinegar against food-borne pathogenic bacteria including *Escherichia coli* O157:H7. *J. Food Prot.* 61:953–959.
20. Leyer, G., L. Wang, and E. Johnson. 1995. Acid adaptation of *Escherichia coli* O157:H7 increases survival in acidic foods. *Appl. Environ. Microbiol.* 61:3752–3755.
21. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607–625.
22. Naim, F., S. Messier, L. Saucier, and G. Piette. 2004. Postprocessing in vitro digestion challenge to evaluate survival of *Escherichia coli* O157:H7 in fermented dry sausages. *Appl. Environ. Microbiol.* 70: 6637–6642.
23. Russell, N. J., and G. W. Gould. 2003. Major preservation technologies, p. 14–24. *In* N. J. Russell and G. W. Gould (ed.), Food preservatives, 2nd ed. Kluwer Academic/Plenum Publishers, New York.
24. Ryu, J.-H., Y. Deng, and L. R. Beuchat. 1999. Behavior of acid-adapted and unadapted *Escherichia coli* O157:H7 when exposed to reduced pH achieved with various organic acids. *J. Food Prot.* 62: 451–455.
25. Shelef, L. A. 1994. Antimicrobial effects of lactates: a review. *J. Food Prot.* 57:445–450.
26. Tarr, P. I. 1995. *Escherichia coli* O157:H7: clinical, diagnostic, and epidemiological aspects of human infection. *Clin. Infect. Dis.* 20:1–10.
27. Young, K., and P. Foegeding. 1993. Acetic, lactic, and citric acids and pH inhibition of *Listeria monocytogenes* Scott A and the effect on intracellular pH. *J. Appl. Bacteriol.* 74:515–520.